Application Note

Food & Beverage

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

Fructans in infant formula

- **ALEXYS™ Carbohydrate Analyzer**
- **AOAC 2016.14/ISO 22579:2020**
- **High-resolution isocratic HPAEC-PAD separation**
- **SweetSep™ AEX200 anion exchange column**

Summary

Fructans are naturally occurring carbohydrates found in many fruits, vegetables, and legume. They are polymers predominantly composed of fructose monomers with a length of 10-60; Fructans with a shorter chain length are known as fructooligosaccharides (FOS). Fructans pass the stomach and small intestine unchanged and are therefore and source of water-soluble dietary fiber with a prebiotic function; They help maintain a healthy and balanced gut microflora by selectively stimulating the growth of one or a limited number of beneficial bacteria in the colon [1]. However, the intake of fructans can also have adverse effects, as the breakdown of fructooligosaccharides by bacterial fermentation could lead to symptoms similar to those of irritable bowel syndrome [2].

The AOAC method 2016.4 and ISO 22579:2020 were recently developed for the quantification of fructans in infant formula and adult/pediatric nutritional formula [3, 4]. This method is based on enzymatic hydrolysis of the fructans and subsequent analysis of the reaction products (fructose & glucose) with High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD). The use of the DECADE Elite electrochemical detector and SenCell in combination with the SweetSep AEX200 anion exchange column resulted in an excellent sensitivity and dynamic range. Therefore, this method allows for direct analysis of the fructan constituents without the need for post-column addition of NaOH as described in both AOAC and ISO methods. Four infant formula samples are analyzed as an example to demonstrate the performance of this method using the ALEXYS Carbohydrates Analyzer.

Introduction

Fructans are naturally occurring carbohydrates found in many fruits, vegetables, and legumes. They are classified into two groups, inulin-type and levan-type, which are composed of fructosyl units linked together via β(2–1) and β(2–6) glycosidic bonds, respectively, with an optional glucose residue linked via α(1–2) glycosidic bond at the reducing end [1]. The β(2–1), and β(2–6) glycosidic bonds prevent fructans from being digested like a typical carbohydrate; they reach the gut intact and are therefore available as a nutrient for the beneficial bacteria in the local microflora [2].

FOS as a functional food ingredient has been gaining significant interest due to its desirable organoleptic and prebiotic properties. Fructans can be as a food additive in processed products, for example it can be used as a low-caloric sweetener or a texture-improving ingredient of low-fat foods [5]. In infant formula and adult nutritionals FOS and other oligosaccharides, such as galactooligosaccharides, are often added for their prebiotic effect.

A method for the determination of fructans in infant formula and adult/pediatric nutritional formula was recently adopted as an AOAC final action method as well as an ISO 22579:2020 method [3, 4]. In fact, both documents describe exactly the same methodology to determine the total fructan content, i.e. the total fructan content is determined indirectly based on the quantification of the monosaccharides fructose and glucose released after enzymatic treatment. The sample preparation consists of three steps:

(1) In the first step all carbohydrates containing fructose and glucose that are not fructans (i.e. sucrose and α-glucooligosaccharides) are completely hydrolyzed.

(2) Subsequently, the remaining oligosaccharides, including the fructans, are separated from the monosaccharides using solid phase extraction.

(3) In the last step the isolated fructans are hydrolyzed with a mix of fructanase into glucose and fructose.

The released monosaccharides glucose and fructose are then analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

HPAEC-PAD is the method of choice for the analysis of monosaccharides as it combines high selectivity with high sensitivity and a large dynamic range. The AOAC 2016.14 and ISO 22579:2020 method utilize post-column addition of NaOH to further extend the linear detection range. However, the use of post-column addition requires additional hardware and chemicals. In this application note it is demonstrated that, with the use of the ALEXYS carbohydrates analyzer, accurate and sensitive quantification of fructans is also possible without the use of post-column addition.

Figure 1. ALEXYS Carbohydrates Analyzer consisting of the ET210 eluent tray, P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, and the DECADE Elite electrochemical detector.

Method

The adapted method from both AOAC 2016.14 and ISO 22579:2020 for total fructan analysis was evaluated using the ALEXYS Carbohydrates Analyzer as shown in Figure 1. The HPAEC-PAD system consists of a ET210 eluent tray for inert gas blanketing (using N_2), P6.1L quaternary LPG pump capable of running step gradients, AS6.1L autosampler, and the DECADE Elite electrochemical detector. The SenCell with Au working electrode and HyREF reference electrode was selected for sensitive detection of the sugars.

Requirements

The main method performance requirements for the determination of fructans in infant formula, as defined the AOAC Working Group for Fructans, are summarized in Table 3 [6]. In this application note a small set of samples was analyzed to demonstrate that this method meets the key requirements.

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 (MP)	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

Table 2

Step-gradient program

Time (min)	*Mobile phase*	*%A*	*%B*	*%C*	*Description*
0 – 8	25 mM NaOH	75	25	0	Elution & detection
8 –13	100 mM NaOH, 100 mM NaOAc	0	0	100	Column clean-up and regeneration
13 - 33	25 mM NaOH	75	25	0	Equilibration, starting conditions

Separation

Under alkaline conditions (pH > 12) carbohydrates can be separated by means of HPAEC. Carbohydrates are weak acids with pKa values ranging between 12 and 14. At high pH they will be either completely or partially ionized depending on their pKa value. Due to the extreme alkaline conditions only polymeric anion-exchange columns are suitable for carbohydrate separation.

In the AOAC 2016.14 and ISO 22579:2020 methods, two sets of chromatographic conditions are described for an anion-exchange column with a 10 µm and 6.5 µm particle size, respectively. In the method described in this application note method (Table 1) a similar type of IC column (SweetSep™ AEX200) was used. The SweetSep™ AEX200 column is a strong anion-exchange column containing highly monodisperse 5 µm ethylvinylbenzene-divinylbenzene copolymer (80% crosslinked)

Table 3

Method performance requirements

Parameter	*Value*
Analytical range (g/100g)	0.03 - 5.0
LOQ (g/100g)	≤ 0.03
Repeatability (RSD, %)	< 6
Reproducibility (RSD, %)	< 12
Recovery (%)	90 - 110

coated with functionalized nanoparticles with quaternary amine. The use of this specific column enables high-resolution separation with a short analysis time. An inline trap was used to trap borate ions to reduce tailing of the fructose peak. The temperature for the separation was set to 35°C. All analytes of interest (glucose, fructose and the internal standard (IS) N,N'diacetyl-chitobiose) are separated under isocratic elution at 25 mM NaOH (Table 2). A strong column clean-up and regeneration step is executed at t =13 min to elute strongly retaining components and to remove carbonate ion (CO_3^{2-}) build up on the column. After the cleaning step the column is equilibrated for 20 minutes at the starting conditions, resulting in a total run time of 33 minutes.

Detection

For the sensitive detection of the saccharides the SenCell™ electrochemical flow cell was used in Pulsed Amperometric Detection (PAD) mode. This flow cell [7] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF palladium reference electrode (RE) and stainless steel auxiliary electrode (AE). The temperature for detection was set to 45°C. The cell current was typical about 0.2 µA using these PAD settings under the specified conditions.

Preparation of reagents, standards and samples

Buffers: Sodium acetate buffer (100 mM, pH 4.5) was prepared by diluting 2.9 mL glacial acetic in 450 mL of water, adjusting the PH to 4.5 with a 2M NaOH solution, and diluting it to a final volume 500 mL with water. Sodium maleate buffer (100 mM, pH 6.5) was prepared by dissolving 5.8 g maleic acid in 450 mL of water, adjusting the PH to 6.5 with a 2M NaOH solution, and diluting it to a final volume 500 mL with water. Both buffers were kept at 4°C until use.

Enzyme solution: Sucrase/b-amylase/pullulanase/maltase solution and fructanase solution from the Fructan Assay Kit (K-FRUC, Megazyme International Ireland Ltd) were prepared according to the instructions of the manufacturer. The Sucrase/

Table 4

Sugar concentration in sugar standards

	Sugar standard concentration [µg/mL]		*Injection solution concentration [µg/mL]*	
Standard	*Fructose*	*Glucose*	*Fructose*	*Glucose*
Level 1	20	2	1.4	0.14
Level 2	200	50	14	3.5
Level 3	400	100	28	7
Level 4	600	150	42	10.5
Level 5	800	200	56	14
Level 6	1000	250	70	17.5

b-amylase/pullulanase/maltase solution was prepared by dissolving the freeze-dried enzymes in 22 mL sodium maleate buffer. The fructanase solution was prepared by dissolving the freeze-dried powdered enzymes in 22 mL sodium acetate buffer. Both enzyme solutions were aliquoted and stored at -20°C until use.

SPE reagents: The prewash solution for the SPE columns (80% ACN, 0.1% TFA) was prepared by diluting 80 mL acetonitrile and 100 µL TFA to a total volume of 100 ml with DI water. The wash solution (1 M NaCl) was prepared by dissolving 5.8 g NaCl in water and diluting it to an end volume of 100 mL with DI water. The SPE elution solution (25% ACN, 0.1% TFA) was prepared by diluting 25 mL of acetonitrile and 50 µL of TFA to 100 mL with DI water.

Standards: 10 g/L fructose stock solution, 5 g/L glucose stock solution, and 600 mg/L N,N'diacetylchitobiose (Megazyme International Ireland Ltd) internal standard solution were prepared in DI water and stored at -20°C until further use. Working standards were prepared by dilution of the stock solution with DI water according to the dilution scheme in AOAC method 2016.14. To prepare the injection solution for the calibration curve the sugar standards were diluted in exactly the same way as the samples during the enzymatic treatment and SPE extraction. In short, 140 µL of sugar standard was added to 70 µL internal standard, 140 µL DI water, 1050 µl SPE elution solution, 600 µl sodium acetate buffer and mixed well. As a result, the actual sugar concentration of the injection solution is lower than the calibration standards it represents, the actual concentration are shown in Table 4.

Sample preparation:

Two infant formula products were purchased from a local supermarket for this study. One store-brand infant formula without any fructo-oligosaccharides (Infant formula A) and one name-brand infant formula containing 0.8 mg FOS per 100 mL reconstituted product (Infant formula B). To simulate a high-level FOS product, infant formula A was enriched with Orafti®Synergy1 Inulin-FOS powder. Additionally, the FOS concentration of Infant formula B was increased with the Inulin -FOS powder to simulate a medium-level FOS product.

In total four samples were prepared according to the AOAC method 2016.14. An extensive description of the sample preparation can be found in the method [3]. The optional Carrez clarification was not performed. A flow-chart of the sample preparation is shown in figure 2.

- Infant formula A
- Infant formula A - enriched with FOS powder
- Infant formula B
- Infant formula B - enriched with FOS powder

Reconstitution and dilution: The infant formula powder was reconstituted in DI water according to instructions on the package and well homogenized. 9 grams of reconstituted product was added to 30 mL of water and the pH of the diluted product was confirmed to be between 5 – 9. The solution was heated using a water bath at 80°C with constant agitation for 20 minutes and cooled down to room temperature. The solution was diluted with DI water to and end volume of 50 mL and subsequently further diluted based on the expected fructan content according to the dilution scheme described in the AOAC method.

Hydrolysis of sucrose and α-glucans: 420 µL of N, N'-diacetyl-chitobiose internal standard solution was added to 840 µL of the diluted sample. 840 µL of buffered Sucrase/b-amylase/ pullulanase/maltase solution was added and the solution was incubated at 40°C for 90 min.

Removal of monosaccharides: Graphitized carbon SPE columns (Supelclean™ ENVI-Carb™ SPE Tube 0.5g/6mL, Sigma Aldrich St. Louis, USA) were used for the removal of monosaccharides. These SPE columns have a higher bed weight than the SPE column in the original AOAC method, therefore all the working volumes were increases five-fold. The SPE columns were flushed 3x with 2 mL prewash solution followed by 3x 2 mL water. Subsequently, 2 mL of enzyme treated solution was loaded onto the SPE column followed by a washing step of 2x 5 mL of the NaCl wash solution and 4x 5 mL water. Finally, the trapped fructans were eluted using 4x 2 mL SPE elution solution. The eluate fractions were collected in one test tube and thoroughly mixed.

Hydrolysis of fructans: 200 µl of the sodium acetate buffer and 100 µl fructanase enzyme mixture was added to 700 µL of the eluate and incubated for 40 minutes at 40°C. Blank samples were prepared by mixing 300 µL of the sodium acetate buffer with 700 µL of the eluate and incubating for 40 minutes at 40°C. All samples were filtered over a 0.22 µm Polyethersulfone (PES) syringe filter (13 mm Ø FFL/MLS) prior to injection.

Calculations

The amount of glucose released from the constituted product (C_G) can be calculated by multiplying the measured glucose concentration (C_{GB}) with the dilution factor (D) and the dilution factor during extraction (total extraction volume V_A divided by sample mass m_A) and multiplied with 0.0001 (factor to convert analyte concentration in solution (in mg/mL) to analyte concentration in sample (in g/100 g)), as follows:

$$C_G = C_{GB} \times D \times \left(\frac{V_A}{m_A}\right) \times 0.0001$$

The amount of fructose released from the constituted product (C_F) can be calculated in a similar way, using the following formula:

$$C_F = C_{FB} \times D \times \left(\frac{V_A}{m_A}\right) \times 0.0001$$

The total fructan content (TF) can be calculated by adding the amount of released fructose (C_F), multiplied by 0.9 to correct for the water uptake during hydrolysis, to the amount of released glucose (C_G):

$$TF = (C_F \times 0.9) + C_G$$

The formula can be adapted to include a correction based on a blank measurement. In that case, first the concentration found in the blank (C_0) is subtracted from the concentration in the treated sample (C_B), as follows:

$$C_G = (C_{GB} - C_{G0}) \times D \times \left(\frac{V_A}{m_A}\right) \times 0.0001$$

And:

$$C_F = (C_{FB} - C_{F0}) \times D \times \left(\frac{V_A}{m_A}\right) \times 0.0001$$

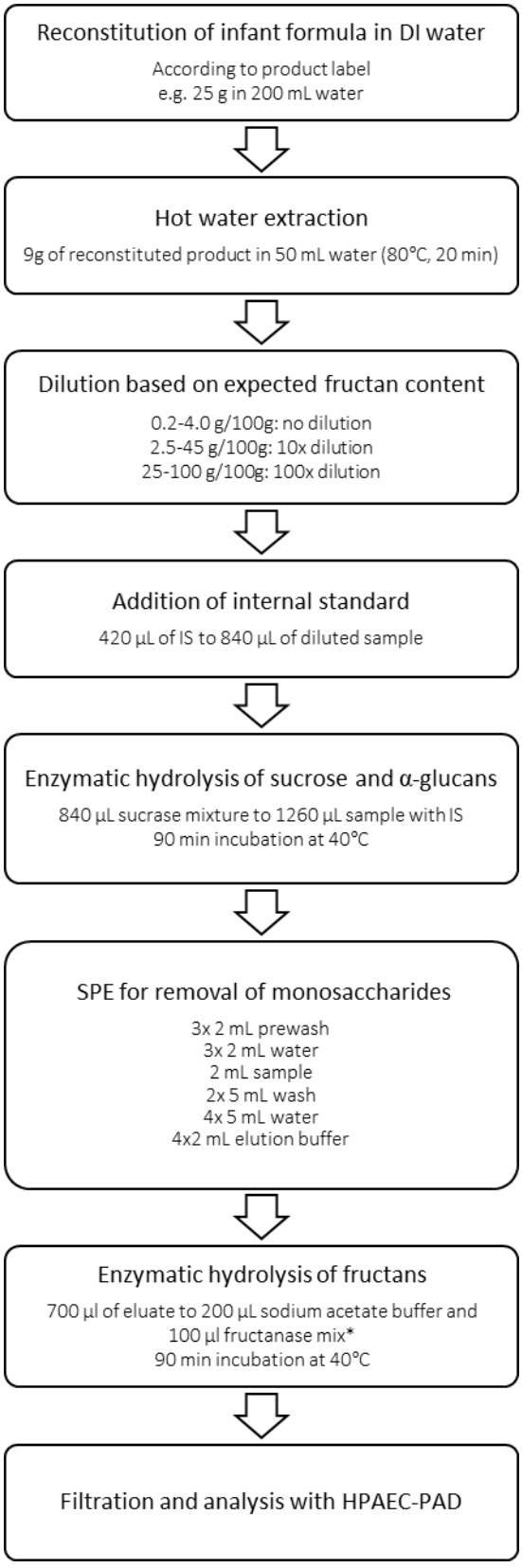

Figure 2: Flow chart of the sample preparation. The hydrolysis of sucrose/α-glucans and SPE were performed with 5x larger volumes than the original AOAC method to accommodate for the higher bed weights of the SPE-columns. *) for the blank measurement the 100 µL of fructanase mix is replaced by 100 µL of sodium acetate buffer.

Results

In Figure 3 an example of a typical chromatogram of a calibration standard is shown. The injection solution consist of the sugar standard containing 600 µg/mL fructose and 150 µg/mL glucose diluted with internal standard solution, the SPE elution solution and the sodium acetate buffer. The actual sugar concentration in the injection solution are 42 µg/mL fructose, 10.5 µg/mL glucose, and 83.9 µg/mL N,N'-diacetylchitobiose. All analytes of interest elute within 7 minutes, which is at least 2× faster than the examples described in the AOAC 2016.14 and ISO 22579:2020 [3, 4]. The total analysis time for each sample is 33 min, which includes a 5 min post-run step gradient for column clean-up and 20 min re-equilibration time. The peak efficiencies found for the sugars are in the range of 45 000 theoretical plates/meter. All analytes of interest has a tailing factor of around 1.2.

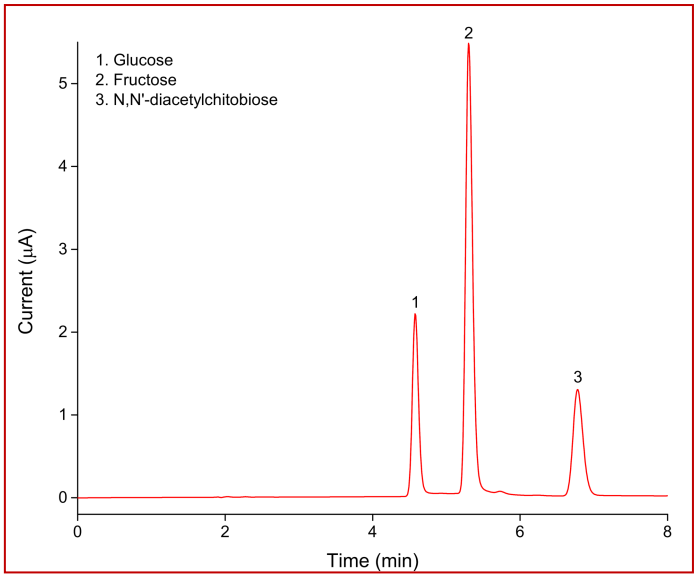

Figure 3: Chromatogram obtained with a 2.5 µL injection of the level 4 calibration standard containing glucose (1), fructose (2) and N,N'-diacetylchitobiose (3).

The injected organic solvents from the SPE elution buffer cause a small disturbance in the baseline after N,N'-diacetyl-chitobiose was eluted. Initially, this small peak coeluted with the internal standard when using a separation temperature of 20 or 30°C as described in the AOAC and ISO methods. Therefore, the temperature setting was optimized to improve separation. At 35°C the small peak was well separated (RT of about 8.5 min) from the internal standard and didn't interfere with the quantification of the saccharides.

Calibration

Electrochemical detection of saccharides has been shown to have a large linear dynamic range, however at high analyte concentrations, calibration curves of amperometric detectors may deviate from linearity, therefore a quadratic fit was used in AOAC method 2016.14 and ISO 22579:2020. Eleven series of calibration standards were prepared and the quadratic fit of the curve was investigated by determination of the residual error for every calibration level. The standard curve of fructose and glucose using N,N'-diacetylchitobiose as an internal standard are shown in Figure 4 and 5, respectively.

The relative residual errors for the calibration curves for fructose and glucose are shown in the figure 6 and 7, respectively. The predicted concentration and the actual concentration of the standards correspond well with most of

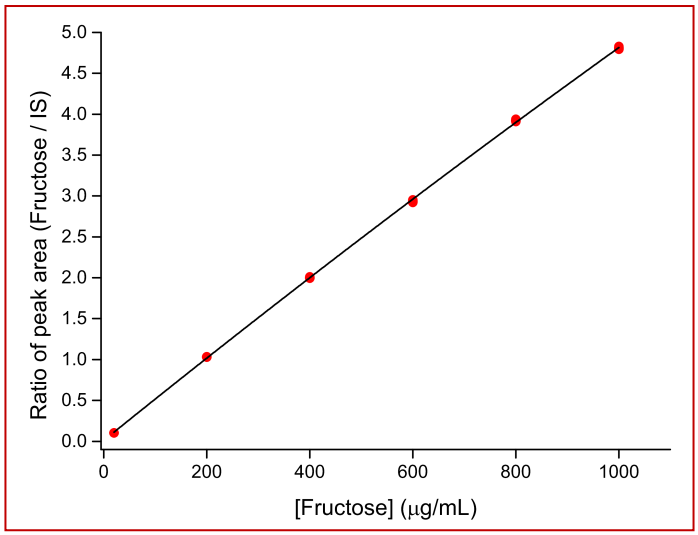

Fig. 4: Calibration curve of fructose using chitobiose as an internal std.

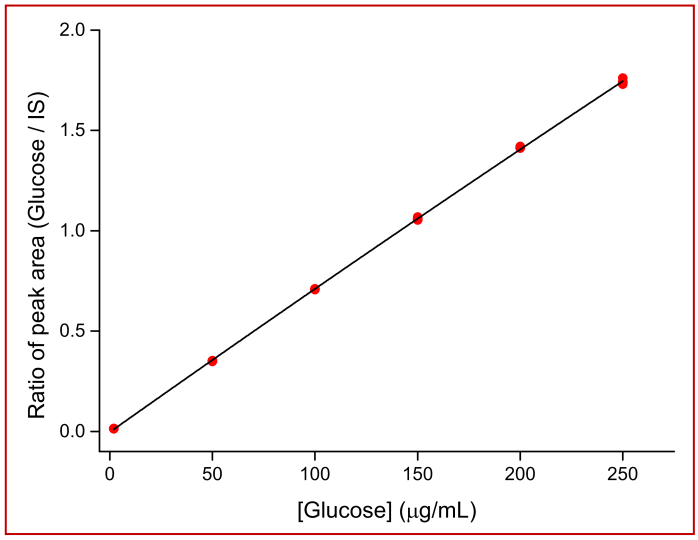

Fig. 5: Calibration curve of glucose using chitobiose as an internal std.

the residual errors below ± 3%. The generally accepted criteria for a good calibration model is that the lack-of-fit for the standards should be less than 5%, with the exception of the lowest standard, which may be higher, below < 10% [8]. The relative residual errors are small over the whole concentration range and fall well within the acceptance criteria. It is evident that a good quadratic fit can be obtained using this method without post-column addition of sodium hydroxide.

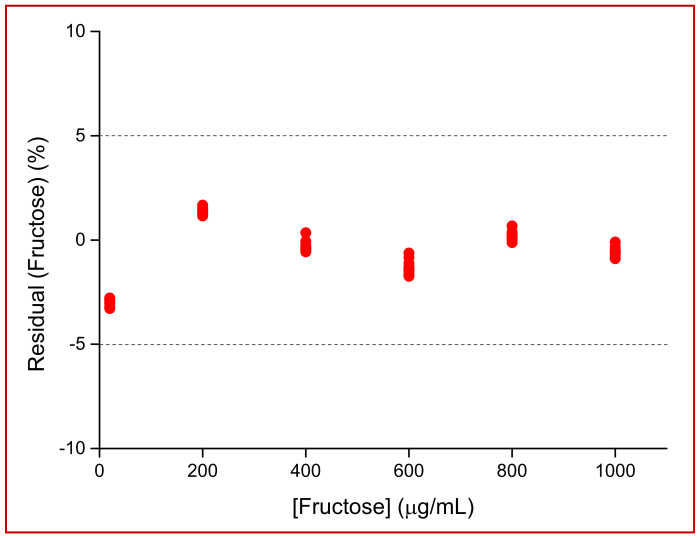

Fig. 6: Relative residual errors of the fructose calibration curve.

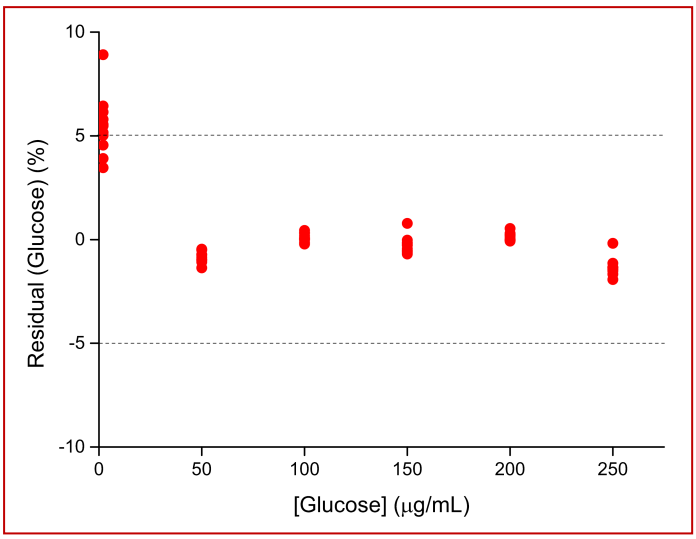

Fig. 7: Relative residual errors of the glucose calibration curve.

Repeatability

The relative standard deviation (RSD) of the retention time and peak area were determined for 10 repetitive injections with the level 1 (1.4 µg/mL fructose and 0.14 µg/mL glucose) and level 2 (14 µg/mL fructose and 3.5 µg/mL glucose) calibration standards (table 5). The RSD's for peak area and retention time for all sugars were below 1% and 0.2%, respectively. These data are well below the minimal required repeatability (< 6%, Table 3) and demonstrate that with this method reproducible analysis of all the analytes of interest can be achieved.

Table 5

Repeatability of 2.5 µL injections of a level 2 and level 1 calibration standard (n=10)

	RSD's (%) L2 standard		*RSD's (%) L1 standard*	
Compound	t_R	*Area*	t_R	*Area*
Glucose	0.10	0.22	0.13	0.91
Fructose	0.08	0.12	0.16	0.17
IS	0.10	0.16	0.15	0.24

Limit of quantification

The excellent sensitivity is evident from the S/N ratios of the lowest level calibration standard, as shown in Table 6. The noise was calculated based on a 5-minute section of the baseline from t = 25 min to t = 30 min (ASTM noise, average peak-to-peak baseline noise of 10 segments of 0.5 min). The resulting S/N ratios are calculated based on the average of 10 repetitive injection of the lowest level calibration standard. The S/N ratios of the individual sugars are a least a factor 16 higher than minimally required for quantification (LOQ, S/N of 10). The sum of glucose and fructose in the level 1 calibration standard corresponds roughly to the equivalent of 0.011 g /100 g fructan content. The exact equivalent depends on the ratio fructose/glucose in the fructans, but these data already demonstrate that fructan contents down to 0.01 g per 100 g product can be reliably quantified.

Table 6

Signal to noise ratio

Sample	*S/N Glucose*	*S/N Fructose*
L1 calibration standard	168	1264
Infant formula B	245	4356

Sample analysis

The four samples (2 consumer products and 2 enriched consumer products) were prepared and analyzed using the presented method. The obtained chromatograms are shown in Figure 8 to 11. The samples and the sample blank are shown in an overlay in red and black, respectively. For infant formula A (figure 8), the infant formula without any fructans, the sample

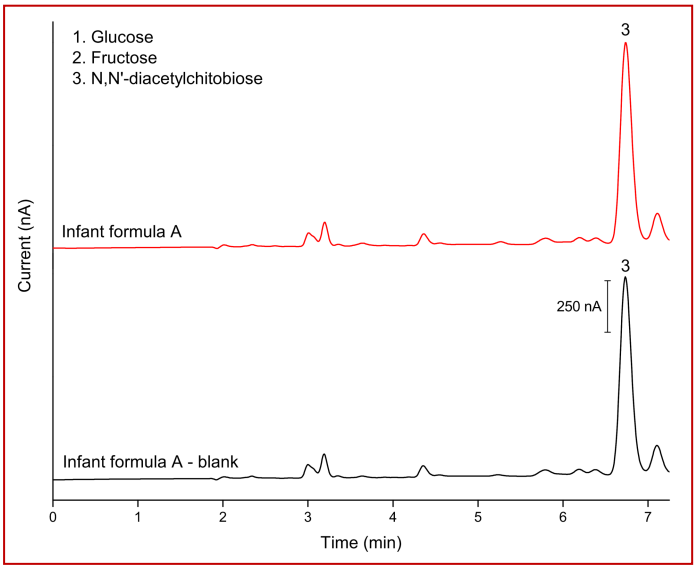

Figure 8: Overlay of the chromatograms obtained with the non hydrolyzed solutions (blank) of infant formula A (black) and infant formula A (red).

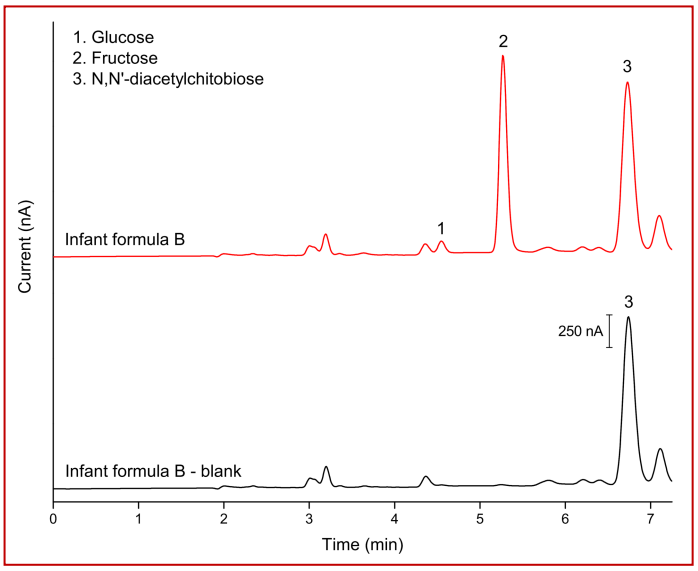

Figure 10: Overlay of the chromatograms obtained with the non hydrolyzed test solutions (blank) of infant formula B (black) and infant formula B (red).

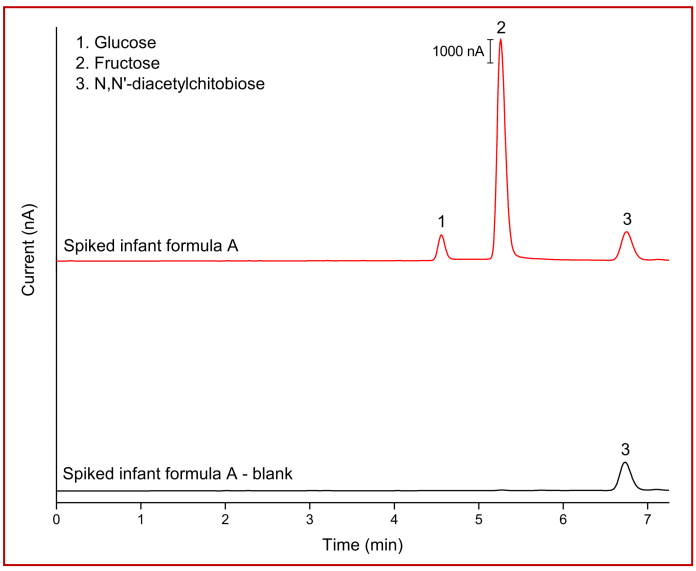

Figure 9: Overlay of the chromatograms obtained with the non hydrolyzed solutions (blank) of the spiked infant formula A (black) and spiked infant formula A (red).

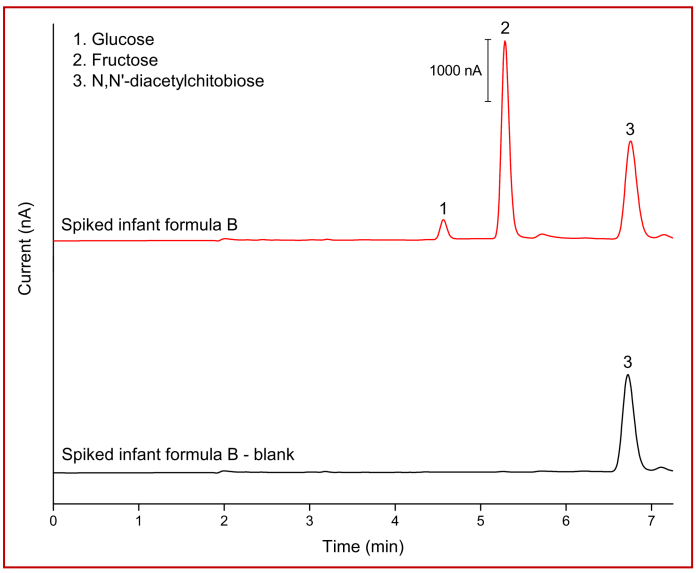

Figure 11: Overlay of the chromatograms obtained with the non hydrolyzed solutions (blank) of the spiked infant formula B (black) and spiked infant formula B (red).

and the sample blank correspond well. No fructose or glucose was detected after the second hydrolysis step with the fructanase mixture, which confirms no fructans were present in this product as stated on the label.

As infant formula A was confirmed to be free of fructans, it could be spiked with a high level of fructan (10 g fructan per 100 g product). Using a dilution factor of 10×, the chromatogram of the spiked sample was recorded and is shown in Figure 9.

The chromatogram of infant formula B, which contained Fructans in a level near LOQ as described in Table 3, is shown in Figure 10. Fructose and sucrose are observed after hydrolysis with fructanase, which confirms that fructans are present in this sample. The S/N of glucose and fructose are 245 or higher (see Table 6), indicating that this method is suitable for the analysis of infant formula with low concentration levels of fructans. Infant formula B was also spiked with additional fructans to simulate a product with a higher fructan content. The spiked sample is shown in Figure 11. All peaks other than glucose and fructose are greatly reduced in response, since this sample was additionally diluted based on its expected fructan

content, as described in the official AOAC and ISO method.

For all samples the total fructan (TF) content was calculated and the recovery for the spiked samples was determined. The recovery was calculated by subtracting the total fructan content from the infant formula (TF_{sample}) from the total fructan content from the spiked infant formula ($TF_{spiked\ sample}$), and subsequently dividing it by the total fructan added (TF_{spiked}), as described in the following formula:

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

The results of the determination of the total fructan content and recovery are summarized in Table 7. The found values correspond well with the expected fructan content. The fructan content found in infant formula B is in accordance with the content specification on the product label. Infant formula A was confirmed to be free of fructans. The recoveries, 98.3% and 100.2% for the spiked infant formula, fall well within the required range (90% - 110%).

Table 7

Total fructan content

Sample	g/100 g reconstituted product	Recovery
Infant formula A	0.00	-
spiked infant formula A	4.80	98.3%
Infant formula B	0.07	-
spiked infant formula B	1.47	100.2%

For new type of products it should be verified that any interferences of the sample matrices, if present, have a neglectable impact on the quantification. Therefore the measurements were also performed with a blank correction included as described in the calculation section. These results are summarized in Table 8.

The results are near identical, only the recovery of the spiked infant formula A is slightly lower and the recovery of the spiked infant formula B is slightly increased. The blank correction had no significant impact on the quantified (absolute) amounts of fructan in the sample. From this data it is evident that no blank measurement and subsequent correction is required for these specific infant formula products.

Table 8

Total fructan content after blank correction

Sample	g/100 g reconstituted product	Recovery
Infant formula A	0.00	-
spiked infant formula A	4.80	98.1%
Infant formula B	0.07	-
spiked infant formula B	1.47	100.3%

References

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Conclusion

The ALEXYS™ Carbohydrate Analyzer provides a solution for the determination of fructans in infant formula and adult nutritionals adapting the AOAC 2016.14 and the ISO 22579:2020. An optimized method is presented which meets all the key performance requirements for the analysis of fructans as defined by the AOAC Working Group for Fructans. The use of a SweetSep™ AEX200 column enables fast elution of the analytes of interest within 7 minutes. Overall, the presented method enables fast, sensitive, and accurate quantification of fructans over a wide concentration range, without the need for post-column addition of sodium hydroxide. Moreover, the use of 2.1 mm ID columns also contributes to a greener HPLC method due to the reduction in mobile phase consumption.

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
Columns	
260.0020	SweetSep™ AEX200, 2.1×200 column, 5 µm
260.0025	SweetSep™ AEX200, 2.1×50 precolumn, 5 µm
260.0031	Borate ion trap, 2.1×200 column, 10 µm
260.0100*	Pre-column filter PEEK, 0.5 µm
Software#	
195.0035	Clarity CDS single instr. incl LC, AS module

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

#) Antec ECD drivers are available for Chromeleon CDS, OpenLAB CDS and Empower CDS. The ALEXYS Carbohydrates Analyzer (full system) can also be controlled under ThermoFisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

Reagents, standards and sample prep accessories

Chemicals	
50% (w/w) NaOH, carbonate-free	Fisher Scientific, pn SS254-500
Sodium acetate trihydrate, HPLC grade	Fisher Scientific, pn 10122400
Fructose	Sigma, pn Car-11 (Carbohydrate kit)
N,N'-diacetylchitobiose, >95%	Megazyme, pn 700004938
Glucose	Sigma, pn Car-11 (Carbohydrate kit)
Glacial acetic acid, 99.8%	Acros, pn 222140010
Maleic acid, 99.7%	Sigma, pn 125231000
Fructan Assay Kit (sucrase and fructanase)	Megazyme, pn K-Fruc, SKU: 700004285
Acetonitrile, 99.99% LC-MS grade	Fisher Scientific, pn A955-212
Trifluoroacetic acid, >99%	Fisher Scientific, pn 293812500
Sodium chloride	Baker, pn 0277
Hydrochloric acid, 36-38%	Baker, pn 6012
Deionized water, > 18 MΩ-cm, TOC < 10 ppb	Barnstead, Easy pure II
Supelclean™ ENVI-Carb™ SPE Tube, 0.5g / 6mL	Merck, pn 57094
Choice™ PES (Polyethersulfone) Syringe Filters, 0.22 µm, 13 mm Ø FFL/MLS	ThermoFisher Scientific, pn CH2213-PES

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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