Application Note

Food & Beverage

The finest HPAEC-PAD Applications for Carbohydrate analysis

Food and Beverage
Mono– and disaccharides
Sugars in meat & fish
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Carbohydrates in instant coffee
Carbohydrates in honey

Prebiotics Food additives
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TGOS in food products
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Lactose Free Products
Lactose in dairy & meat
Lactose in lactose-free products

Artificial Sweeteners
Sugar alcohols

Glycans & Glycoproteins
Monosaccharides in glycoproteins
Analysis of *N*-glycans

Analysis of Maltodextrin in Syrups

- **ALEXYS™ Carbohydrate Analyzer**
- **SweetSep™ AEX200 anion-exchange column**
- **High-resolution separation for maltodextrin profiling**
- **Glucose syrup and high maltose corn syrup samples**

Summary

Maltodextrin is a carbohydrate mixtures, consisting of mono-, di-, and oligosaccharides. Maltodextrin is produced from starch using either chemical or enzymatical hydrolysis [1, 2]. Due to a large number of oligosaccharides, maltodextrin is often used as a nutritious additive in many food products, such as in dried instant food formulations, confectionary, cereals, snacks, syrups, and beverages [3]. However, maltodextrin has a high glycemic index, leading to concerns about increased blood glucose levels particularly for individuals with diabetes [4, 5]. Therefore, there is a demand for an analytical method that provides insights into maltodextrin profile and allows for sensitive quantification.

In this application note a method is presented for the analysis of maltodextrin using the ALEXYS™ Carbohydrate Analyzer. High-performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is the method of choice for selective and sensitive analysis of maltodextrin. High-resolution separation of maltodextrin up to DP_{40} were achieved using a strong anion-exchange column SweetSep™ AEX200. Pulsed amperometric detection enables sensitive detection with low detection limit. Two syrup samples were analyzed to demonstrate the applicability of the method.

Introduction

Maltodextrins are mixtures of carbohydrates obtained by partial hydrolysis of starch. As a product of partial hydrolysis of starch, maltodextrin consists of mainly oligosaccharides from a degree of polymerization (DP) of 3 up to 40, and a low amount of monosaccharides and disaccharides [1, 2]. The composition of maltodextrin is attributed to its low sweetness and nutritious value, which makes maltodextrin attractive to be used as an additive to food products [2, 6].

Despite the GRAS (Generally Recognized As Safe) status [1], maltodextrin is a substance with a glycemic index higher than table sugar [4, 5]. Consumption of food products modified with maltodextrin can therefore increase blood glucose, which is not preferred for people with diabetes. The sudden increase in blood glucose level is closely correlated to the mono-saccharides and disaccharides amount in maltodextrin. Therefore, there is a need for an analytical method that can provide insights into the maltodextrin profile and sensitive quantification of maltodextrin.

A number of analytical methods can be used for the analysis of maltodextrin, such as using titration, or thin-layer chromatography [7—9]. However, these methods only produce qualitative results. In order to have reliable quantitative methods for the analysis of maltodextrin, high-performance liquid chromatography with refractive index (RI) detection [9], or evaporative light scattering detection (ELSD) has been developed [10]. However, these methods are lacking of sufficient selectivity and sensitivity.

Figure 1: ALEXYS Carbohydrate Analyzer consisting of the ET210 eluent tray, P6.1L LPG quaternary pump, AS6.1L autosampler, CT2.1 column thermostat, and DECADE Elite electrochemical detector in combination with the SenCell™ 2 mm Au WE and HyREF (Pd/H_2) REF.

High-performance anion exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is the preferred method for maltodextrin analysis [11—13]. It combines superior selectivity with sensitive detection. The hydroxyl groups on carbohydrates can be oxidized, therefore, carbohydrates can be detected without derivatization using pulsed amperometric detection. In this application note, a method is presented based on a new type of anion-exchange column, the Antec Scientific SweetSep™ AEX200 with highly monodisperse 5 µm particles for the selective and sensitive analysis of maltodextrin.

Method

The method for the analysis of maltodextrin was evaluated using the ALEXYS™ Carbohydrate Analyzer (Figure 1). This dedicated HPAEC-PAD system consists of a P6.1L LPG quaternary pump, an AS6.1L autosampler, a CT2.1 column thermostat, an ET210 eluent tray for nitrogen blanketing, and the DECADE Elite electrochemical detector. Take into account

Table 1

Conditions	
HPLC system	ALEXYS™ Carbohydrate Analyzer
Detector	DECADE Elite electrochemical detector
Columns	SweetSep™ AEX200, 4 × 200 mm column, 5 µm SweetSep™ BIT, 4 × 50 mm borate ion trap All columns: Antec Scientific
Mobile phase	A: 100 mM NaOH B: 100 mM NaOH, 500 mM NaOAc Eluents prepared & blanketed with Nitrogen 5.0
Flow rate	0.7 mL/min
System backpressure	About 195 bar at the start of run, about 215 bar at the end of the NaOAc gradient (t=35 min)
Injection	10 µL
Temperature	30°C for separation, 35°C for detection
Flow cell	SenCell with 2 mm Au WE, stainless steel AE, and HyREF 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 0.3-0.4 µA
ADF	0.5 Hz
Range	5 µA/V; 500 nA/V for LOD determination

that the selection of a third-party quaternary HPLC systems may influence the separation performance, and may require some small adjustment of the conditions to achieve the results outlined in this application note. For sensitive detection of the carbohydrates, the SenCell™ with Au working electrode and HyREF (Pd/H_2) reference electrode was selected.

Separation

Carbohydrates are weak acids with pKa values ranging between 12 and 14. At high pH, carbohydrates will be either completely or partially ionized depending on their pKa value. Therefore, under alkaline conditions (pH > 12) carbohydrates can be separated using HPAEC. Only polymeric anion-exchange columns are suitable for carbohydrate separation at such alkaline conditions. The retention time of carbohydrates is inversely correlated with the pKa value and increases significantly with molecular weight. Maltodextrin is a breakdown product of starch consisting of unbranched linear glucose oligomer and polymer chains. Consequently, separation of such maltodextrin samples can be done based on their degree of polymerization. A high-resolution anion-exchange column with a 5 µm particle size, SweetSep™ AEX200, was chosen for the separation of carbohydrates in maltodextrin. The morphology and exchange capacity of this new AEX200 resin were specifically optimized to enable the analysis of a wide variety of carbohydrate samples, ranging from monosaccharides present in food, plants, and glycoproteins, up to polysaccharides such as in maltodextrin. The use of a pre-column filter is advised when using samples which might contain particulate matter.

The separation was performed at 30°C using the gradient profile shown in Table 2. The starting condition of the run is 100 mM NaOH with 40 mM sodium acetate. All carbohydrates were eluted in 30 minutes using a linear gradient from 40 mM up to 450 mM sodium acetate in 100 mM NaOH. The column was subsequently cleaned for 5 minutes using 450 mM sodium acetate in 100 mM NaOH, followed by equilibration to the starting conditions for 15 minutes. The addition of a wash and an equilibration step resulted in a total run time of 50 minutes.

Table 2

Gradient program

Time (min)	*Mobile phase*	*Description*
0	100 mM NaOH + 40 mM NaOAc	Elution & detection
30	100 mM NaOH + 450 mM NaOAc	
30-35	100 mM NaOH + 450 mM NaOAc	Column clean-up and regeneration
35-50	100 mM NaOH + 40 mM NaOAc	Equilibration, starting conditions

Detection

For the pulsed amperometric detection, a Sencell™ electrochemical flow cell is used [14]. This flow cell has a confined wall-jet design and consists of a Au working electrode (WE), a HyREF (Pd/H_2) reference electrode (RE), and a stainless steel auxiliary electrode (AE). A 4-step potential waveform was applied as described in Table 1. The detection temperature was set to 35°C. The cell current was typically about 0.3-0.4 µA under the specified conditions. This particular 4-step waveform has the following benefits (1): a consistent long-term peak area response and (2) minimal electrode wear [15], resulting in less flow cell maintenance and system down time.

Preparation of standards and samples

Standards: 50000 ppm stock standards of the individual sugars, glucose (DP_1), maltose (DP_2), and maltotriose (DP_3), and maltodextrin (DE* 4-7 and DE 10-15) were prepared in 95/5 (v/v%) water/acetonitrile. Acetonitrile was added to suppress bacterial and fungal growth and to prevent fast degradation. The stock standards under these conditions are stable for more than a month in the fridge at 4°C. A working standard mix of DP_1, DP_2, and DP_3, in the concentration ranges from 0.1 ppm to 10 ppm was prepared by serial dilution of the stock standards with DI water. The working standard of the maltodextrin (DE 4-7 and DE 10-15) was prepared by serial dilution of the stock standards with DI water, with a final concentration of 500 ppm. The concentration of the maltodextrin working standard is significantly larger than the DP_1—DP_3 mix due to the smaller response factor of higher DPs present in the maltodextrin compared to the DP_1—DP_3.

Samples: two types of syrup were obtained from a food ingredient company. One of the samples is a low sugar glucose syrup (GS), while the other sample is a high maltose corn syrup (HMCS). The stock solutions of the samples were prepared by weighing an appropriate amount of the syrups (GS = 208.1 mg, HMCS = 210.6 mg) and dissolving the syrups in 95/5 (v/v%) water/acetonitrile with a final concentration of 50000 ppm. Samples with a final concentration of 50 ppm were prepared by serial dilution of the stock solutions with DI water (dilution factor = 1000×).

*) DE = Dextrose Equivalent is a measure of the amount of reducing sugars present in a sugar product, expressed as a percentage on a dry basis relative to dextrose. The dextrose equivalent gives an indication of the average degree of polymerisation (DP) for starch sugars.

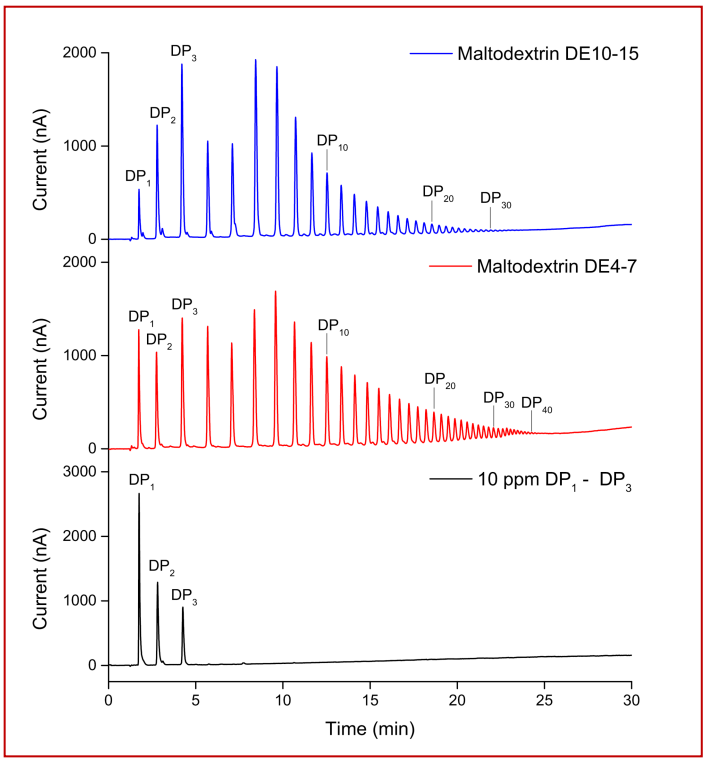

Figure 2: Overlay chromatograms of the 10 µL injections of the DP1—DP3 mix (black), maltodextrin DE4—7 (red), and maltodextrin DE 10—15 (blue).

Results

An overlay chromatogram of the 10 µL injections of the DP_1—DP_3 mix, maltodextrin DE 4-7, and maltodextrin DE 10-15 is shown in Figure 2. DP_1—DP_3 are baseline separated (resolution > 1.5) and eluted within 5 minutes under this condition. It should be noted that the response factor is inversely proportional to the molecular weight, i.e. the higher the DP, the smaller the response factor.

Separations of the oligo– and polysaccharides in maltodextrin standards are excellent. In maltodextrin DE 4-7, a high-resolution separation of each DP up to DP_{40} was observed within 25 minutes. In maltodextrin DE 10-15, the separation was achieved up to DP_{30}. Maltodextrin DE 4-7 theoretically consists of a larger proportion of the larger DPs (DP_{14}—DP_{25}) compared to Maltodextrin DE 10-15 (DP_6—DP_{10}). Therefore, it is obvious that the larger DPs in maltodextrin DE 4-7 have significantly higher responses compared to the maltodextrin DE 10-15.

Linearity

The linearity of the method was investigated for DP_1—DP_3. For higher DPs no linearity was measured due to the lack of commercial standards. The calibration curves for DP_1—DP_3 in the concentration range of 0.1—10 ppm are shown in figure 3. In this concentration range, the linearity is excellent, with

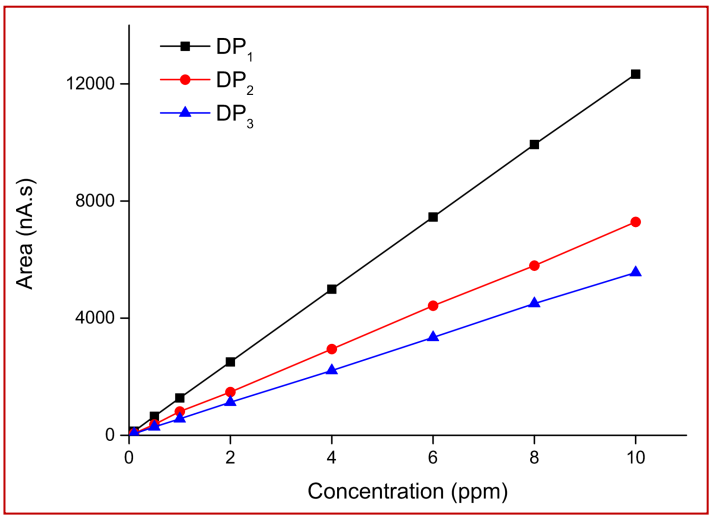

Figure 3: Calibration curves of DP_1, DP_2, and DP_3 in the concentration range of 0.1—10 ppm.

correlation coefficients larger than 0.9999 for DP_1, DP_2, and DP_3.

Repeatability

The repeatability of the method was evaluated by repetitive injections of a 0.1, 1 and 10 ppm DP_1 - DP_3 standard mix in DI water. The relative standard deviations (RSDs) for retention time and peak area for the three concentrations of the DP_1—DP_3 standards are listed in Table 3. Good repeatability of the method is evident from the low RSD values obtained. RSDs for retention time were < 0.25%.

Table 3

Repeatability of 10 µL injections of a 10 ppm and 1 ppm of DP_1—DP_3 mix in water (n=10)						
Compound	*RSDs (%) 10 ppm*		*RSDs (%) 1 ppm*		*RSDs (%)* 0.1 ppm*	
	t_R	*Area*	t_R	*Area*	t_R	*Area*
DP_1	0.18	0.13	0.14	1.41	0.18	2.27
DP_2	0.21	0.35	0.19	1.08	0.19	4.63
DP_3	0.13	0.36	0.09	2.23	0.15	2.79

***)** n=5 for the repeatability test with 0.1 ppm standard.

For peak area, the RSDs were < 0.40% for all components in the 10 ppm standard and < 2.5% for all components in the 1 ppm standard. The RSDs of all components in the 0.1 ppm standard were < 5% because this concentration is close to the limit of detection (LOD). Nevertheless, the presented data demonstrate that a repeatable analysis of DP_1—DP_3 can be achieved.

LOD & LOQ

The limit of detection (LOD) and limit of quantification (LOQ) for DP_1—DP_3 in ppm level are shown in Table 4. 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 calculated based on a 5-minute section of the baseline. LOQs were calculated as the analyte response corresponding to 10× the ASTM noise. The average responses of 5 replicate injections obtained with a 0.1 ppm standard in the 500 nA/V range were used to calculate the LODs and LOQs for DP_1, DP_2, and DP_3. The sensitivity of the method is evident from Table 4. Higher DP maltodextrins have lower response factors. Therefore, DP_3 has the highest concentration detection limit (0.055 ppm) followed by DP_2 (0.040 ppm) and DP_1 (0.018 ppm), respectively.

Table 4

Limit of Detections (LODs) and Limit of Quantifications (LOQs)		
Compound	*LODs (ppm)*	*LOQs (ppm)*
DP_1	0.018	0.058
DP_2	0.040	0.132
DP_3	0.055	0.184

Sample analysis

Two syrup samples labelled low sugar glucose syrup (GS) and high-maltose corn syrup (HMCS) were analyzed using the presented method. The analyses were performed to obtain the maltodextrin profile of the syrups and to quantify the amount of DP_1, DP_2, DP_3, DP_4 and above in the syrups. The chromatograms of GS and HMCS are depicted in Figure 4 and Figure 5.

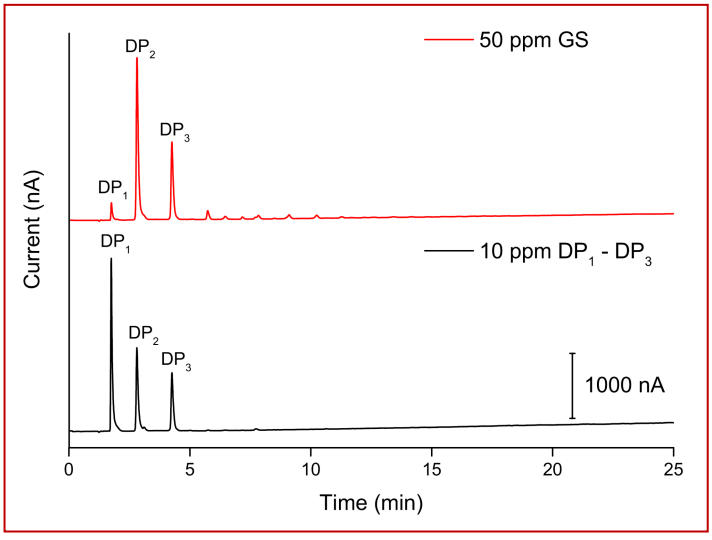

Figure 4: Overlay chromatograms of 10 µL injections of 10 ppm DP_1—DP_3 mix (black), and 50 ppm GS sample (red).

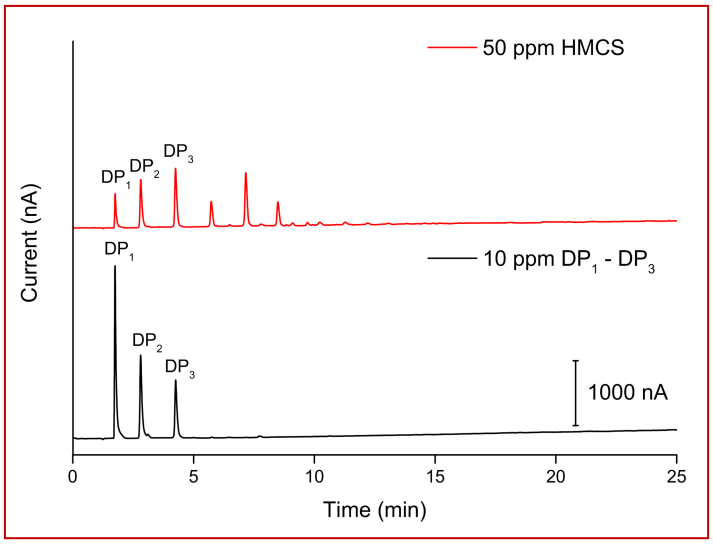

Figure 5: Overlay chromatograms of 10 µL injections of 10 ppm DP_1—DP_3 mix (black), and 50 ppm HMCS sample (red).

The amounts of DP_1—DP_3 were quantified using the calibration curve shown in figure 2, while DP_4 and above were quantified using the equation below. The average amounts based on triplicate measurements are shown in Table 5.

DP_4 and above = TC — (DP_1 + DP_2 + DP_3)

TC = Total carbohydrate content on the label.

Table 5

Average calculated amount of each DPs in the stock solutions of the sample, n = 3		
Compound	*GS (mg)*	*HMCS (mg)*
DP_1	4.29	8.20
DP_2	89.67	23.54
DP_3	57.08	41.50
DP_4 and higher	16.21	98.36

The chromatogram of GS in figure 3 along with table 5 shows that GS primarily consists of DP_1—DP_3, with DP_2 being the most abundant component in the sample. In HMCS the DP_1 content is almost twice higher as in the GS sample. The DP_2 and DP_3 content in HMCS is significantly smaller compared to GS. DP_4 and higher DPs are the dominant components in the HMCS sample. The amount of DP4 and higher DPs in HMCS is a factor of about 6× larger compared to the GS sample.

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Conclusion

The ALEXYS™ Carbohydrate Analyzer in combination with SweetSep™ AEX200 anion-exchange column demonstrates a high-resolution separation of maltodextrins, offering insights into its composition up to DP_{40}. Demonstrated by the excellent linearity, repeatability, and sensitivity, the method provides a valuable tool for quantification of components in maltodextrins. The maltodextrin profile and the amount of DP_1, DP_2, DP_3, DP_4 and above in two syrup samples were assessed. In summary, the presented HPAEC-PAD method is a suitable method for the analysis of maltodextrins in food products.

Ordering information

Detector only	
176.0035B	DECADE Elite SCC electrochemical detector
116.4321	SenCell 2 mm Au HyREF
Recommended ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer—gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Column	
260.0010	SweetSep™ AEX200, 4 × 200 mm column, 5 µm
260.0030	SweetSep™ BIT, 4 × 50 mm borate ion trap
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.

*) The ALEXYS Carbohydrate Analyzer can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. For the DECADE Elite electrochemical detector only, control drivers are also available for Waters Empower™, Agilent OpenLab CDS, and Agilent OpenLab CDS Chemstation Edition. Please Contact Antec for more details.

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 and may be adjusted accordingly. Specifications mentioned in this application note are subject to change without further notice.

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