Application Note

Food & Beverage

The finest LC-EC applications for Food & Beverage analysis

Phenols
Bisphenol A
Catechins
Flavonoids
Phenols
Antioxidants
Resveratrol
Epicatechin
Quercetin
Other polyphenols

Carbohydrates
Sugar alcohols
Monosaccharides
Disaccharides
Lactose
Galactooligosaccharides
Other oligo- and polysaccharides

Vitamins, minerals etc.
A, C, D, E, and K
Iodide
Q10, ubiquinols

Sugar alcohols

- **ALEXYS Carbohydrate Analyzer**
- **SenCell™ with Au working electrode**
- **High-resolution isocratic HPAEC-PAD analysis**
- **Separation of 7 sugar alcohols within 15 min**
- **Chewing gum, energy drink and pastilles**

Summary

Diabetes is a chronic disease characterized by elevated blood glucose that affects about 10% of total human population [1]. To prevent such conditions, a large number of food products with reduced or limited amount of sweetener has been introduced to the market. In these products, sugar alcohols (also called polyols or alditols) are commonly used as the artificial sweetener due to their low glycemic index properties. In addition, sugar alcohols possess some benefits such as non-cariogenic, lower calorie level, and water holding properties beneficial to the colon. Unfortunately, excess consumption of sugar alcohols may cause laxative effects. In European Union, products containing more than 10% polyols should have a warning label in regards to their laxative effects.

In this application note a method is presented for the analysis of sugar alcohols using the ALEXYS Carbohydrate Analyzer with DECADE Elite detector in combination with the SenCell. The method is based on separation and detection by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using a high-resolution high-capacity IC column. The use of a narrow-bore (2 mm ID) IC column with 4 µm particle size allowed the separation of the 7 sugar alcohols of interest in less than 15 minutes with a two-fold reduction of mobile phase usage ,compared to the conventional 4 mm ID IC column typically used for this application. A variety of sugar-free product (chewing gum, pastilles and energy drink) were analyzed to demonstrate the performance and versatility of the method.

Introduction

Sugar alcohols (also called polyols or alditols) are organic compounds, typically derived from sugars, containing one hydroxyl group (–OH) attached to each carbon atom. Sugar alcohols are used widely in the food industry as thickeners and artificial sweeteners [2]. Sugar alcohols are non-cariogenic or, in case of xylitol, even anti-cariogenic, have a low glycemic index and insulinemic index (useful in obesity and diabetes), are digested more slowly and also have osmotic properties showing water holding properties beneficial to the colon [3]. Due to their laxative effects the approval is restricted and the products with more than 10% added polyols must bear the words "excessive consumption may produce laxative effects" [2].

There are various analysis methods for sugar alcohols, including Gas Chromatography (GC), Capillary Electrophoresis (CE), colorimetry, and High Performance Liquid Chromatography (HPLC) using UV or RI detectors [4, 5, 6]. However, some of these methods require derivatization or are limited in sensitivity. Mass spectrometry (MS) can also be utilize to quantify sugar alcohols without derivatization and with superior sensitivity compared to the previously mentioned methods but it requires more expensive measurement equipment [6, 7]. High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) is an attractive and cost-effective alternative. It offers high-resolution separations of a large variety of carbohydrates combined with sensitive detection down to femtomole levels without derivatization.

Figure 1: ALEXYS Carbohydrates analyzer consisting of the ET 210 eluent tray, P6.1L analytical pump, AS6.1L autosampler, CT2.1 column thermostat and DECADE Elite electrochemical detector equipped with SenCell.

HPAEC-PAD can be used as a tool for the compositional analysis of sugar alcohols [6, 8]. In this application note a method is presented for the separation and quantification of 7 sugar alcohols commonly found in food and processed food products using the ALEXYS Carbohydrates Analyzer. The method is based on the separation of the sugar alcohols on a high-resolution high-capacity anion-exchange column with a small particle size of 4 µm and column diameter of 2 mm. To demonstrate the performance and versatility of the method a variety of sugar-free products (chewing gum, pastilles and energy drink) were analyzed as an example.

Method

The HPAEC-PAD analysis of sugar alcohols is performed using the ALEXYS Carbohydrates Analyzer (figure 1) with the LC conditions specified in table 1. The ALEXYS analyzer was controlled via a PC using Clarity™ CDS Version 8.7 software.

Carbonate ions

Carbonate (CO_3^{2-}) ions, which can be formed from CO_2 dissolved in the mobile phase at high pH, can interfere with carbohydrate retention on anion exchangers due to its strong binding properties as a divalent ion. This can lead to shortened retention times, decreased column selectivity, and a loss in resolution of the separation. To minimize the introduction of carbonate ions in the mobile phase the eluents were carefully prepared manually using a commercially available carbonate-free 50% w/w NaOH solution. The diluent was DI water (resistivity >18 MΩ.cm), which was sparged with Nitrogen 5.0 using the sparging function of the ET 210 Eluent tray. During analysis the eluent tray is used to pressurize the head space above the mobile phase with Nitrogen gas (0.2- 0.4 bar N_2 overpressure).

Borate ions

Borate ions (BO_3^{-3}) can pair with the vicinal hydroxyls present on some carbohydrates. This can lead to peak tailing and loss of peak symmetry of the affected carbohydrate even when borate is present at low ppb concentrations in the mobile phase. Especially, fructose, mannose and sugar alcohols are susceptible to peak tailing due to borate ions. Possible sources of borate contaminants entering the mobile phase are via (1) the DI water system, borate is one of the first ions released when the filters loosing their capacity or (2) it can leach from glass bottles. To eliminate the presence of borate contaminants in the mobile phase, a trap column was installed in the solvent line between pump and autosampler.

Table 1

LC-EC conditions	
HPLC system	ALEXYS Carbohydrates Analyzer
Detector	DECADE Elite electrochemical detector
Columns	Thermo Scientific™ Dionex™ CarboPac™ PA300, 250 x 2.0 mm ID + 50 x 2.0 mm ID Thermo Scientific™ Dionex™ BorateTrap™ Inline Trap Column, 50 x 4.0 mm ID, 20 µm
Mobile phase (MP)	A: 10 mM NaOH B: 200 mM NaOH Eluents prepared & blanketed with Nitrogen 5.0
Flow rate	0.22 mL/min
Back pressure	about 270 bar
Injection	5 µL (partial loop fill)
Temperature	23 °C for separation, 35 °C for detection
Flow cell	SenCell with 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 0.2 µA
ADF	0.5 Hz
Range	2 µA/V
Software	Clarity™ CDS Version 8.7.1.19

Table 2

Step-gradient program		
Time (min)	*Mobile phase*	*Description*
0 - 15	10 mM NaOH	Isocratic elution & detection
15 – 27	200 mM NaOH	Column clean-up and regeneration
27 - 52	10 mM NaOH	Equilibration to starting conditions

Separation

The analysis of the sugar alcohols is based on isocratic elution using 10 mM NaOH followed by a column clean-up step (200 mM NaOH) and re-equilibration to starting conditions at t = 15 minutes and t = 27 min, respectively (see table 2). During the clean-up and regeneration step all late eluting interferences and carbonate ion build-up will be removed from the column, ensuring reproducible analysis. The separation was performed at 23°C using the CT2.1 column thermostat. The total run-time of the analysis is 52 minutes. Note, that it usually takes a few gradient runs to equilibrate the HPAEC-PAD system and get stabile retention times.

Detection

For the pulsed amperometric detection of simple sugars the Antec SenCell electrochemical flow cell is used. This flow cell [8] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF (Pd-Hydrogen) reference electrode (RE) and stainless steel auxiliary electrode (AE). For detection, a 4-step potential waveform was applied. The choice of the 4-step potential waveform was proven to result in an excellent reproducibility and minimal electrode wear [9]; i.e. resulting in less flow cell maintenance and system down time. The pulsed amperometric detection was performed at 35°C. Under the specified conditions the cell current was typical about 0.2 µA.

Preparation of standards and reagents

Standards: 20 mM stock standards of individual sugar alcohols (Erythritol, xylitol, mannitol, sorbitol, myo-inositol, isomalt, and maltitol) were prepared in 95/5 (v/v %) water/acetonitrile. Small amount of acetonitrile was added to prevent fast degradation and minimize bacterial or fungal growth. The stock standards were stored in the fridge at 4°C and stable for more than a month. Working standards in the concentration range of 100 nM— 60 µM were prepared by serial dilution of the stock standards with DI water. Note that isomalt is a mixture of 2 isomers which can be chromatographically separated using this method. Based on the assumption that isomalt contains an equimolar amount of both isomers, the working standards were prepared with a twice higher concentration of isomalt compared to the other sugar alcohols to get a comparable molar concentration and peak response for the individual isomers.

A total of five commercial products (three chewing gums, one pastilles, and one energy drink) containing sugar alcohols were purchased from the supermarkets in The Netherlands. The samples containing sugar alcohols were prepared based on the published procedure with slight modifications [10].

Chewing gum samples

1. The chewing gums were frozen at -30°C for several hours and then crushed/ground to small pieces.
2. 0.25 gram of the crushed chewing gum samples were weighed accurately and transferred to a centrifuge tube, followed by addition of 25 mL DI water.
3. The centrifuge tube was sonicated for 30 minutes to help the dissolution process.
4. The solution along with the leftover sticky materials were stirred in a 50°C water bath for 1.5 hours.
5. Subsequently, the solution was stirred for 1 hour at room temperature.
6. 6 aliquots of 1 mL were centrifuged in Eppendorf vials for 5 minutes at 6000 rpm.
7. The supernatant was collected and filtered twice over Whatman 589/1 filter paper.
8. The filtrate was diluted to the desired concentration (in the linear response range of calibration curves).

9. The diluted samples were filtered using a 0.22 µm PES (Polyethersulfone)syringe filter (GVS) directly into the 1.5 mL autosampler vials used for analysis.

Pastilles sample

Unlike chewing gums, pastilles are easily soluble in water and thus sample preparation was done in a simpler manner.

1. 0.25 gram of (crushed) pastilles samples were weighed accurately and dissolved in 25 mL DI water.

Subsequently, step 5 - 9 from the procedure described for chewing gums were followed.

Energy drink sample

In this application note, the energy drink sample was carbonated and sample preparation followed the procedure below.

1. 25 mL energy drink was transferred to a centrifuge tube and carefully sonicated to remove the dissolved gas for 30 minutes.

Subsequently, step 5 - 9 from the procedure described for chewing gums were followed.

Results

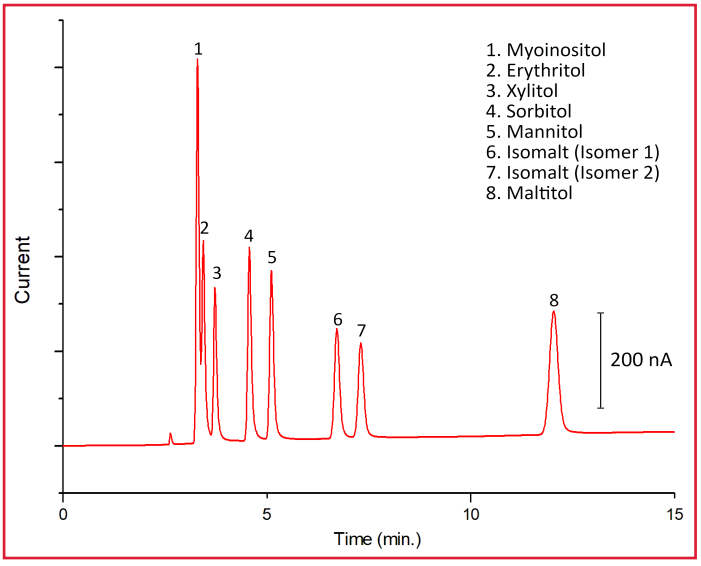

Figure 2: Chromatogram obtained with a 5 µL injection of a 10 µM sugar alcohols standard mix in DI water.

An example chromatogram is shown in figure 2 of a 7 sugar alcohols standard mix, consisting of 10 µM erythritol, xylitol, mannitol, sorbitol, myo-inositol, isomalt, and maltitol dissolved in DI water. The complete separation of all sugar alcohols can be performed within 15 minutes, which is at least twice as fast compared to the other conventional HPAEC columns [10, 11],

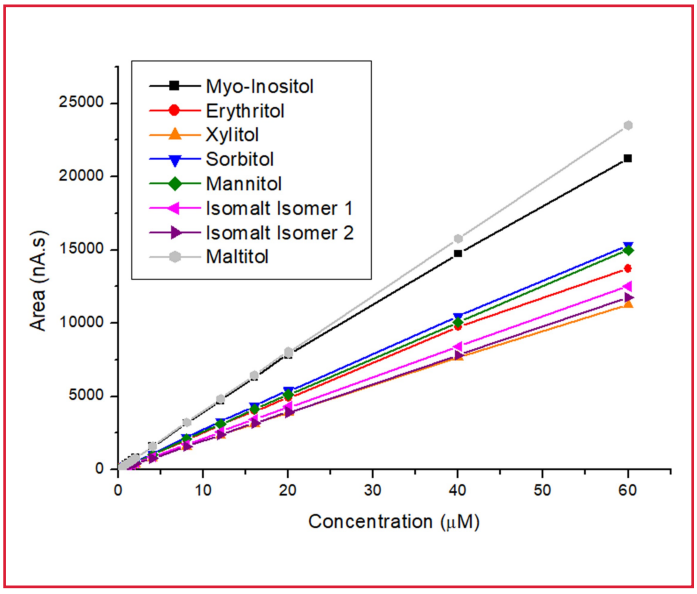

Figure 3: Calibration curves of all sugar alcohols in the concentration range of 0.4 - 60 µM. The fitted lines are extrapolated to the origin.

which enables a higher sample throughput. Under these LC conditions most of the sugar alcohols are baseline-separated with a resolution higher than 1.5. Except for myo-inositol and erythritol, which are separated with a resolution of 0.95. Isomalt is a mixture of two isomers: α-D-glucopyranosyl–1-6-mannitol (GPM) and α-D-glucopyranosyl–1-6-sorbitol (GPS). It is remarkable that the two isomers in isomalt can be separated using this method, hence the two isomalt peaks. Peak identification of the isomers was not possible due to the lack of the individual isomer standards. However, it is estimated that the peaks designated to Isomer 1 and 2 corresponds to GPS and GPM, respectively [12]. So GPS and GPM follow the elution order of the alditols sorbitol and mannitol. The peak efficiencies range from 26000 to 46000 theoretical plates/meter for all sugar alcohols in this standard mix. Most peak asymmetry factors range from 1.1 to 1.5 except for erythritol and xylitol, which show peak tailing (asymmetry factor of 1.9 and 1.6, respectively).

Linearity

Linearity was investigated in the concentration range of 0.4 µM to 60 µM. Calibration curves of all sugar alcohols are shown in the Figure 3. A linear fitting was done to assess the linearity for all sugar alcohols, and the fitted lines are extrapolated to the origin. The linearity is excellent in this concentration range with correlation coefficients for peak area better than 0.999 for all 7 sugars.

Repeatability

The relative standard deviations (RSDs) of the retention time peak height and peak area were determined for 7 repetitive injections of the 10 µM standard mix in DI water. In table 3 the results are listed. Retention times were stable, with RSD values below < 0.1% for all analytes. The RSDs for peak height and area for all sugars were < 1 % and < 1.2%, respectively. These data demonstrate that with this method reproducible analysis of all the analytes of interest can be achieved using the ALEXYS Carbohydrates Analyzer.

Table 3

Repeatability of 5 µL injections of a 10 µM sugar alcohols standard mix in DI water (n=7)

Compound	*RSDs (%)*		
	Ret. Time	Peak Height	Peak Area
Myo-inositol	0.00	0.52	0.61
Erythritol	0.00	0.71	1.01
Xylitol	0.11	0.94	1.20
Sorbitol	0.00	0.72	0.76
Mannitol	0.09	0.40	0.51
Isomalt (Isomer 1)	0.06	0.53	0.53
Isomalt (Isomer 2)	0.06	0.41	0.38
Maltitol	0.06	0.75	0.77

Table 4

Calculated Limit of Detection (LOD) and Limit of Quantification (LOQ) (n=7)

Compound	*LOD*		*LOQ*
	nmol/L	µg/L (ppb)	µg/L (ppb)
Myo-inositol	4	0.7	2.2
Erythritol	7	0.9	3.0
Xylitol	9	1.4	4.6
Sorbitol	7	1.3	4.4
Mannitol	8	1.5	4.9
Isomalt (Isomer 1)	13	4.6	15.7
Isomalt (Isomer 2)	17	5.8	19.4
Maltitol	12	4.2	13.9

Detection limit

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for all sugar alcohols were determined using a method that was described in the ICH guidelines [13]. The LODs were calculated as the analyte response corresponding to 3× the ASTM noise (average peak - to - peak baseline noise of 6 segments of 0.5 min). The noise was calculated based on a 3 minute section of baseline at 8.1 up to 11.1 minutes. The average responses of 7 replicate injections obtained with a 100 nM standard mix in 500 nA/V range were used to calculate the LOD's for all sugar alcohols. The LODs and LOQs are shown in Table 4 in µg/L (ppb) and molar concentrations. From Table 4, it is evident that the method has a high sensitivity. The LODs for most of the sugar alcohols are below 10 nmol/L, except for isomalt (two isomers) and the late eluting maltitol (larger peak width) the LODs are slightly higher. The LOQs were calculated in a similar way to LODs, with 10× S/N ratio instead of 3×. The calculated LOQs varies, from 2 ppb for myo-inositol to 20 ppb for isomalt (isomer 2). Again, it should be stressed that the mentioned concentrations for the isomalt isomers are estimates based on the assumption that isomalt consists of equimolar amounts of the two isomers (actual concentration ratio unknown). To sum up, the presented method has an excellent sensitivity for the detection and quantification of sugar alcohols.

Sample analysis

Five commercial products were purchased from supermarkets in the Netherlands for the compositional analysis of sugar alcohols using the beforementioned method. Among five, three

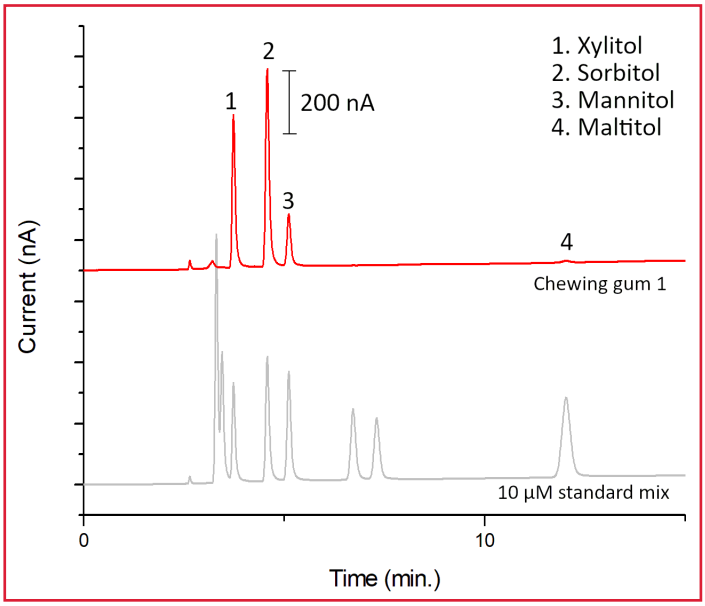

Figure 4: Overlay of chromatograms of: chewing gum sample 1 (red) and a 10 µM standard mix in DI water (grey).

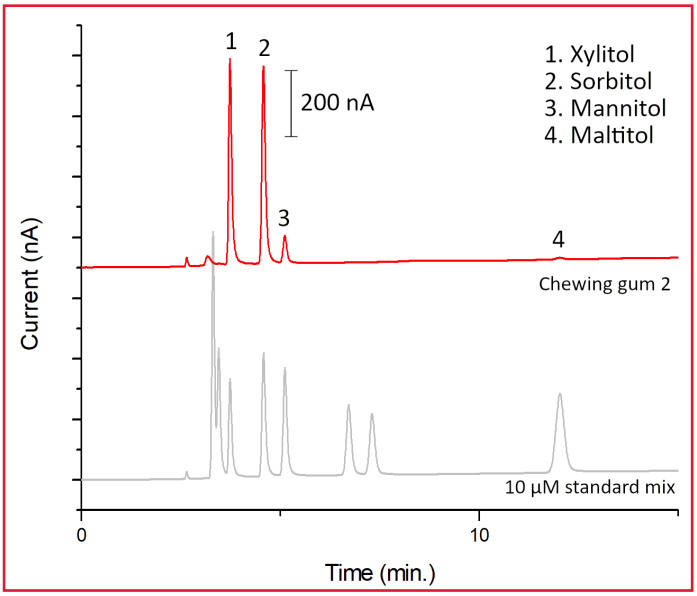

Figure 5: Overlay of chromatograms of: chewing gum sample 2 (red) and a 10 µM standard mix in DI water (grey).

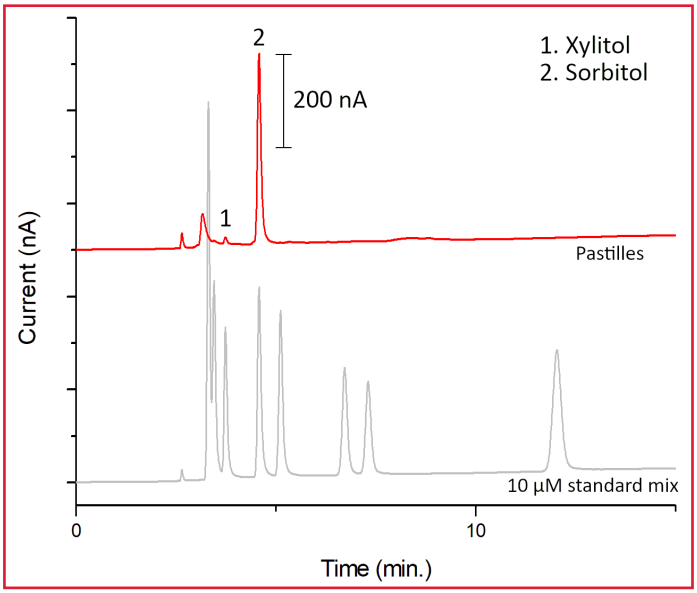

Figure 7: Overlay of chromatograms of: pastilles sample (red) and a 10 µM standard mix in DI water (grey).

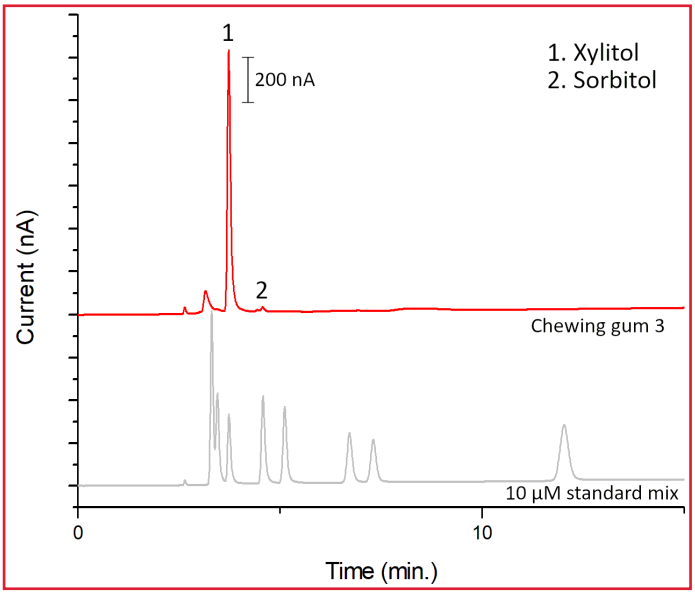

Figure 6: Overlay of chromatograms of: chewing gum sample 3 (red) and a 10 µM standard mix in DI water (grey).

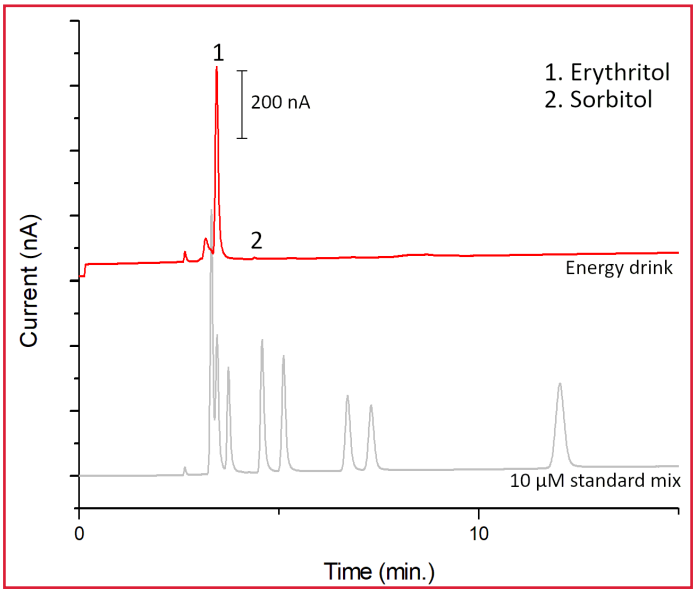

Figure 8: Overlay of chromatograms of: energy drink sample (red) and a 10 µM standard mix in DI water (grey).

are chewing gums products, one pastilles product, and one energy drink product. On the product labels of all tested samples the total contents of polyols is listed, but the labels do not specify the quantity of the individual sugar alcohols which might be present. The content of sugar alcohols in the samples were determined using a calibration curve based on the standards in the concentration range of 4 - 20 µM. The representative chromatograms are shown below in figure 4 - 8.

As can be seen from the chromatograms, the analysis of sugar alcohols in commercial samples is quite straightforward with minimal to no interference. For all samples the sugar alcohols that were listed on the product label are identified in the chromatograms.

Table 5

Sugar alcohol contents (g / 100 g product)*

Product	*Xylitol (g/100g)*	*Sorbitol (g/100g)*	*Mannitol (g/100g)*	*Maltitol (g/100g)*	*Erythritol (g/100g)*
Chewing gum 1	22.6	24.2	7.8	0.3	-
Chewing gum 2	30.8	24.5	4.3	0.3	-
Chewing gum 3	56.5	1.0	-	-	-
Pastilles	3.0	79.6	-	-	-
Energy drink#	-	0.007	-	-	0.6

*) Average of n=5 repetitive injections, #) amount in mg / 100 mL product

In table 5 the amounts of the individual sugar alcohols quantified in the samples are listed (g / 100 g) product. In case of liquid samples such as energy drink the contents is specified in g /100 mL of product. The total amount of sugar alcohols quantified in the products was in agreement with the information on the product label. However, in all samples the total amount of sugar alcohols was slightly lower than the amount of polyols stated on the product labels. One of the possible reasons for that could be the presence of glycerol, also a polyol. Glycerol is used in the food processing industry as stabilizer, sweetener, filler or in case of chewing gum to increase flexibility of the gum. Glycerol is not quantified with the presented method.

To eliminate faulty / incomplete extraction process, recovery experiment was done for energy drink sample due to low complexity of the sample matrix. The recovery experiment was done with spiking the initial energy drink sample before dilution with known concentration of erythritol and sorbitol. The recovery can be calculated using the formula below.

$$\text{Recovery (\%)} = 100\% \times \frac{\text{Area}_{\text{spiked}} - \text{Area}_{\text{non-spiked}}}{\text{Area}_{\text{standard}}}$$

It is found that the average recovery percentage (n=5) for erythritol and sorbitol in the energy drink are 103.2% and 107.8%, respectively. These values fall in acceptable range according to the standard guidelines [13].

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Conclusion

The ALEXYS Carbohydrate Analyzer with DECADE Elite detector and SenCell provides a sensitive and user -friendly HPAEC-PAD analysis solution for the quantification of sugar alcohols in various types of samples. An optimized method is presented using a high-resolution and high-capacity anion-exchange column with 4 µm particle size. The column enables fast isocratic separation of the 7 sugar alcohols of interest within 15 minutes. Furthermore, the smaller column ID (2 mm) and lower flow rate, compared to the conventional anion-exchange column (4 mm ID) used for this application, led to 50% less mobile phase consumption. It is demonstrated that the presented method was applicable to a range of commercial products (chewing gum, pastilles and energy drink) with fairly simple sample pretreatment without derivatization of sugar alcohols.

Ordering information

System	
180.0055W	ALEXYS Carbohydrates Analyzer, isocratic
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT 2.1 Column thermostat
Software*	
195.0035	Clarity CDS single instr. incl LC, AS module

*) The ALEXYS Carbohydrates Analyzer can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. 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. Specifications mentioned in this application note are subject to change without further notice.

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