



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

## Sugars in beer

- **ALEXYS™ Carbohydrate Analyzer**
- **SweetSep™ AEX200 anion-exchange column**
- **Comprehensive analysis of fermentable sugars**
- **Alcoholic and non-alcoholic beers**

### Summary

Beer is the most consumed alcoholic beverages in the world, with global consumption reaching approximately 192 billion liter in 2022 [1]. According to the Brewers Association, there are approximately 160 types of beer, each distinguished based on the flavor, bitterness, taste intensity, color, as well as the geographical origin [2]. One key factor influencing beer flavor is the fermentation process. During the fermentation process, several sugars are converted into alcohol. The residual sugar content after fermentation process plays a crucial role in determining the flavor profile and the quality of the beer. Therefore, determining the amount of the sugars in the beer is of importance, for assessing the nutritional value and ensuring quality control.

Carbohydrates can be separated and detected without derivatization using High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD). In this application note a HPAEC-PAD method is presented for the analysis of fermentable sugars and two additional non-fermentable sugars in beer samples. The method utilizes the ALEXYS™ Carbohydrate Analyzer and the new SweetSep™ AEX200 column, a strong anion exchange column with highly monodisperse 5 µm particles for separation of the sugars. Several beer samples were analyzed to demonstrate the applicability of the method.



### Introduction

Beer is one of the oldest and the most popular alcoholic beverages in the world. Beer is produced through the fermentation process of starches from grains, most commonly from barley and wheat. The brewing process typically involves the extraction and enzymatic breakdown of the carbohydrates from the grains, which results in various carbohydrates ranging from mono- to polysaccharides. Yeast is added after the extraction process to ferment sugars into alcohols. However, not all sugars are fermentable. Only glucose, sucrose, fructose, maltose and maltotriose are commonly converted into alcohol [3, 4]. These fermentable sugars are critical to the strength, flavor, color, and aroma of the beer [4, 5]. Therefore, monitoring the fermentable sugar content in beer is essential for both quality control process and for evaluating the nutritional value of the beer product.

The analysis of the fermentable sugars in beer is challenging due to the presence of other non-fermentable sugars, as well as other byproducts of fermentation. Additionally, beer contains phenolic acids and other organic compounds extracted from the grains [6]. Several analytical techniques have been reported for the analysis of sugars in alcoholic beverages, such as gas chromatography and liquid chromatography [7, 8, 9]. Some of these techniques usually require derivatization of the sugars for the detection. One of the liquid chromatography methods that does not require derivatization while maintaining high sensitivity for sugars is high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) [9, 10]. Using HPAEC-PAD, typically carbohydrates can be detected without derivatization up to pico- and femtomol sensitivity [11].

In this note, a HPAEC-PAD method is presented for the separation and quantification of fermentable sugars (glucose, sucrose, fructose, maltose, and maltotriose) and two other non-fermentable sugars (galactose and lactose) in beer. The new SweetSep™ AEX200 column with 5 µm particle size is used for the separation of these sugars. To demonstrate the performance and the applicability of the method, several beer samples (3 alcoholic beer samples and 1 non-alcoholic beer sample) were analyzed.

### Method

The analysis was performed using the ALEXYS™ Carbohydrate Analyzer (Figure 1) equipped with the DECADE Elite

electrochemical detector. The SenCell™ with Au working electrode and HyREF (Pd/H<sub>2</sub>) reference electrode was selected for sensitive detection of the sugars.



Figure 1. ALEXYS Carbohydrate Analyzer consisting of the ET210 eluent tray (for N<sub>2</sub> blanketing), a P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, and the DECADE Elite electrochemical detector.

### Separation

Carbohydrates are weak acids with pK<sub>a</sub> values ranging between 12 and 14. At high pH they will be either completely or partially ionized depending on their pK<sub>a</sub> value. Therefore, under alkaline conditions (pH > 12) carbohydrates can be separated by means of HPAEC. 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 pK<sub>a</sub> value and increases significantly with molecular weight.

The separation of sugars in beer was carried out using SweetSep™ AEX200 columns (4 × 200 mm analytical column and 4 × 50 mm precolumn), which are strong anion-exchange columns containing highly monodisperse 5 µm resin coated with quaternary amine functionalized nanoparticles. In addition, a borate ion trap was installed between the pump and the injector.

For the separation of the sugars, a gradient profile in Table 2 was employed. The method started with isocratic elution at 9 mM NaOH from t=0 min to t=15 min, followed by a linear gradient to 100 mM NaOH within 10 minutes to speed up the elution of maltose, which is more strongly retained on the



Table 1

## HPAEC-ECD Conditions

|                             |   |
|-----------------------------|---|
| HPLC system                 | ALEXYS™ Carbohydrate Analyzer   |
| Detector                    | DECADE™ Elite electrochemical detector  |
| Columns                     | SweetSep™ AEX200, 4 x 200 mm analytical column, 5 µm<br>SweetSep™ AEX200, 4 x 50 mm precolumn, 5 µm<br>Borate ion trap, 4 x 50 mm column, 10 µm<br>(Antec Scientific) |
| Mobile phase (MP)           | A: DI Water (resistivity > 18 MΩ.cm and TOC<5ppb)<br>B: 100 mM NaOH<br>C: 100 mM NaOH + 100 mM NaOAc<br>Eluents blanketed with Nitrogen 5.0                           |
| Flow rate                   | 0.7 mL/min  |
| Back pressure               | about 250 bar   |
| Injection                   | 10 µL   |
| Temperature                 | 30 °C for separation, 35 °C for detection   |
| Flow cell                   | SenCell with Au WE, stainless steel AE and HyREF (Pd/H <sub>2</sub> ) RE, AST 2   |
| Potential waveform (4-step) | E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V<br>ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s  |
| I-cell                      | about 0.2— 0.4 µA   |
| ADF                         | 0.1 Hz  |
| Range                       | 10 µA/V   |

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 and electrochemical grade sodium acetate salt (all commercially available). The diluent was DI 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 also blanketed with Nitrogen gas (0.2—0.4 bar N<sub>2</sub> overpressure). The inert gas atmosphere will minimize the introduction of CO<sub>2</sub> in the mobile phase and the subsequent formation of CO<sub>3</sub><sup>2-</sup> ions, ensuring reproducible analysis.

## Detection

For the pulsed amperometric detection, the Antec SenCell™ electrochemical flow cell is used. This flow cell [12] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF (Pd/H<sub>2</sub>) reference electrode (RE) and stainless steel auxiliary electrode (AE). A 4-step potential waveform was applied as described in Table 1. The temperature for detection was set to 35°C. The cell current was typically about 0.2—0.4 µA using these PAD settings under the specified conditions. This particular 4-step waveform with a pulse duration of 500 ms has been claimed to have benefits: (1) a consistent long-term peak area response and (2) minimal electrode wear [13], resulting in less flow cell maintenance and system down time.

## Preparation of standards, reagents and samples

**Standards:** 10 mM 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 0.1 - 80 µM were prepared by serial dilution of the stock standards with DI water.

**Samples:** Four commercially available beer samples were purchased from the supermarkets in the Netherlands and in Spain. The following products were purchased and analyzed:

- Alhambra Tradicional Pale Lager beer (4.6% alc. vol.)
- San Miguel Especial Pale Lager beer (5.4% alc. vol.)
- Affligem Belgian White 0.0 (alcohol free)
- Corona Extra (4.5% alc. vol.)

The samples were prepared as follows: 100 mL of beer was transferred to a beaker, and carefully sonicated for 20 minutes

column. Maltotriose is a trisaccharides retained even stronger than maltose. Therefore another linear gradient from t=25 to t=40 min with a strong modifier 100 mM sodium acetate (NaOAc) is needed to elute maltotriose faster. The composition of the mobile phase is kept at 100 mM NaOH and 100 mM NaOAc until t=45 min in every run to elute late eluting components and to remove carbonate ions (CO<sub>3</sub><sup>2-</sup>) build up on the column. After the clean-up step the column is equilibrated for 35 minutes to the starting conditions, resulting in a total run time of 80 minutes. The temperature for separation was set at 30°C.

Table 2

## Gradient program

| Time (min) | Mobile phase                 | %A | %B  | %C  | Description                        |
|------------|------------------------------|----|-----|-----|------------------------------------|
| 0 - 15     | 9 mM NaOH                    | 91 | 9   | 0   | Elution & detection                |
| 25         | 100 mM NaOH                  | 0  | 100 | 0   |                                    |
| 40         | 100 mM NaOH,<br>100 mM NaOAc | 0  | 0   | 100 |                                    |
| 40 - 45    | 100 mM NaOH,<br>100 mM NaOAc | 0  | 0   | 100 | Column clean-up and regeneration   |
| 45 - 80    | 9 mM NaOH                    | 91 | 9   | 0   | Equilibration, starting conditions |



## Sugars in beer

to remove the dissolved gas and foam. After sonication, the sample was filtered with 0.22  $\mu\text{m}$  PES syringe filter into a clean volumetric flask. Six aliquots of 1.5 mL of samples were transferred into Eppendorf vials and centrifuged for 5 minutes at 6000 rpm. The supernatant was collected and diluted with DI water to a desired dilution factor before injection. Note that the dilution factor of the sample depends on the amount of the sugars in the sample. Alcohol free beer has more sugars compared to the alcoholic beer, and therefore required higher dilution factor. The spiked samples were prepared at the last step of dilution with the final concentration of 10  $\mu\text{M}$  of standard mix.

### Results

A chromatogram obtained with the 10  $\mu\text{L}$  injection of a 10  $\mu\text{M}$  standard mix is depicted in Figure 2. The chromatogram shows baseline separation of all 7 sugars, 5 of which eluted within 15 minutes. Maltose eluted at around 26 minute, maltotriose at about 34 minutes. Note, that the use of a NaOH and acetate gradient results in baseline drift (onset at about 18 min), caused by the change in pH and mobile phase composition.

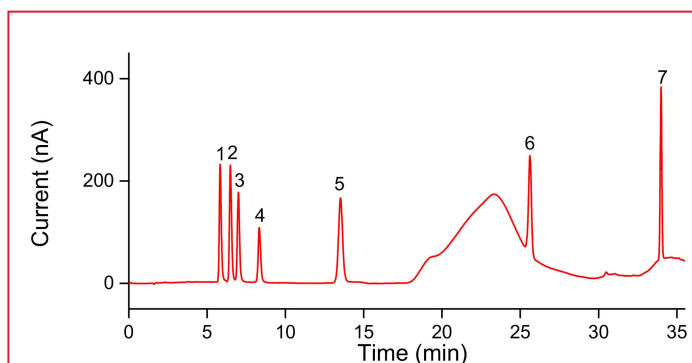


Figure 2. The chromatogram obtained from a 10  $\mu\text{L}$  injection of the 10  $\mu\text{M}$  sugar standard mix in DI water. Peak labels: (1) Galactose\*, (2) Glucose, (3) Sucrose, (4) Fructose, (5) Lactose\*, (6) Maltose, and (7) Maltotriose.

\*Galactose and Lactose are non-fermentable sugars.

### Linearity

The linearity was investigated with standards mix in the concentration range of 0.1 - 80  $\mu\text{M}$ . Excellent linearity was achieved with correlation coefficients based on the peak area better than 0.999 for all 7 sugars (Table 3).

### Repeatability

The relative standard deviations (RSDs) of the retention time and peak area were determined for 10 repetitive injections of the 10  $\mu\text{M}$  and 1  $\mu\text{M}$  standard mix in DI water. The results are listed in Table 4. The retention times were stable, with RSD values < 0.40% for all sugars in both 10 and 1  $\mu\text{M}$  standard mix. The RSD for peak areas for all sugars was < 0.40% for the 10  $\mu\text{M}$

**Table 3**

Linearity of 10  $\mu\text{L}$  injections of standards (0.1—80  $\mu\text{M}$ )

| Compound    | Correlation coefficient (r) |
|-------------|-----------------------------|
| Galactose   | 0.9999                      |
| Glucose     | 0.9999                      |
| Sucrose     | 0.9997                      |
| Fructose    | 0.9998                      |
| Lactose     | 0.9998                      |
| Maltose     | 0.9999                      |
| Maltotriose | 0.9999                      |

**Table 4**

Repeatability of 10  $\mu\text{L}$  injections of a 10 and 1  $\mu\text{M}$  standard mix in DI water (n=10)

| Compound    | RSD's (%)<br>10 $\mu\text{M}$ |      | RSD's (%)<br>1 $\mu\text{M}$ |      |
|-------------|-------------------------------|------|------------------------------|------|
|             | $t_R$                         | Area | $t_R$                        | Area |
| Galactose   | 0.11                          | 0.20 | 0.37                         | 0.56 |
| Glucose     | 0.13                          | 0.30 | 0.33                         | 0.47 |
| Sucrose     | 0.09                          | 0.23 | 0.25                         | 0.65 |
| Fructose    | 0.13                          | 0.28 | 0.31                         | 0.78 |
| Lactose     | 0.10                          | 0.11 | 0.17                         | 0.65 |
| Maltose     | 0.10                          | 0.33 | 0.08                         | 0.54 |
| Maltotriose | 0.02                          | 0.24 | 0.01                         | 0.59 |

**Table 5**

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

| Compound    | Limit of detection |                 | Limit of Quantification |                 |
|-------------|--------------------|-----------------|-------------------------|-----------------|
|             | nM                 | $\mu\text{g/L}$ | nM                      | $\mu\text{g/L}$ |
| Galactose   | 20                 | 3.6             | 67                      | 12.2            |
| Glucose     | 21                 | 3.7             | 69                      | 12.4            |
| Sucrose     | 26                 | 9.0             | 88                      | 30.1            |
| Fructose    | 42                 | 7.6             | 141                     | 25.4            |
| Lactose     | 29                 | 9.8             | 95                      | 32.5            |
| Maltose     | 26                 | 8.7             | 85                      | 29.1            |
| Maltotriose | 14                 | 7.2             | 47                      | 24.0            |

standard mix and < 0.80% for the 1  $\mu\text{M}$  standard mix. These data demonstrate that with this method reproducible analysis of all the analytes of interest can be achieved.

### Limit of detection (LOD)

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

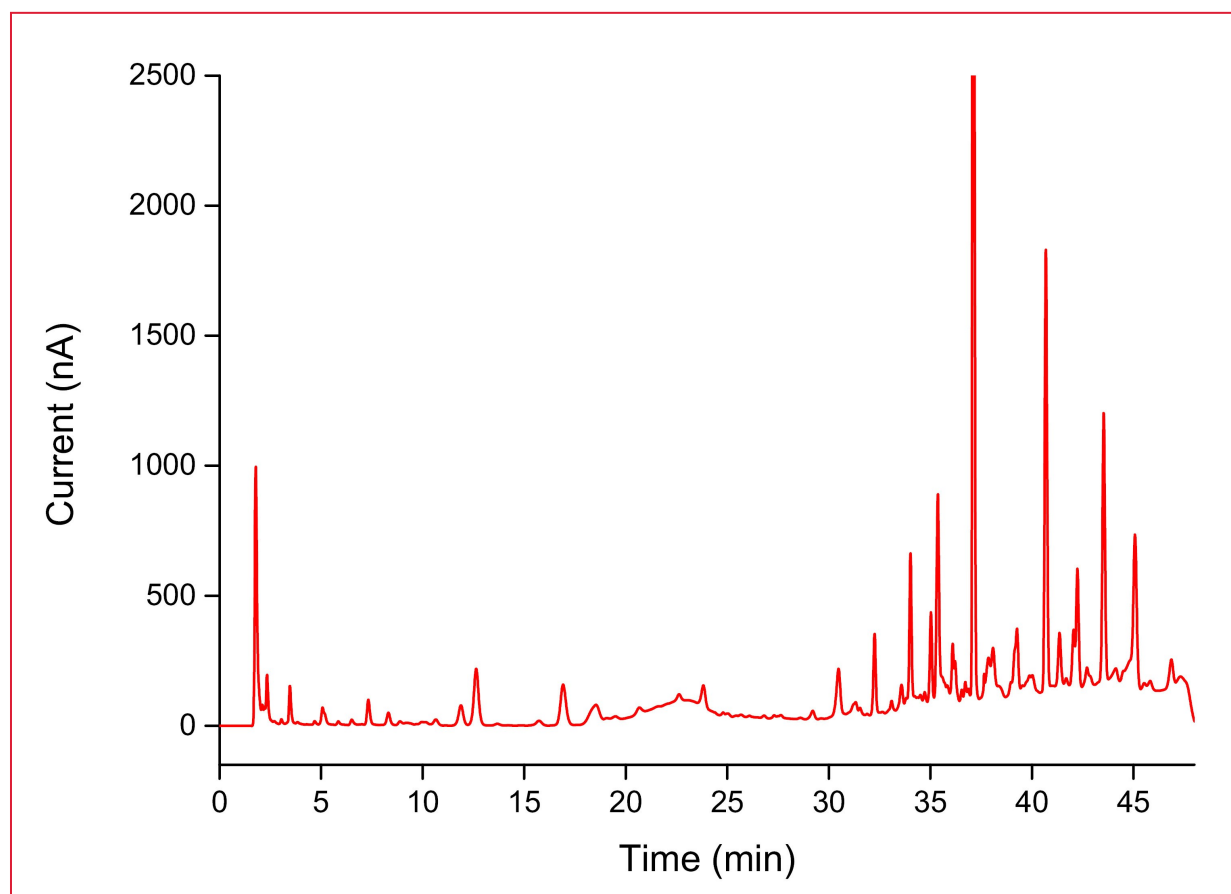


Figure 3. Chromatograms obtained from a 10  $\mu$ L injection of the 100 $\times$  diluted Corona beer sample.

for all sugars are shown in Table 5. The LODs were calculated as the analyte response corresponding to 3 $\times$  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 from  $t = 75$  minutes to  $t = 80$  minutes. The average response based on 10 replicate injections obtained with the 1  $\mu$ M standard mix was used to calculate the LOD and LOQ. The LOQs were calculated in a similar way to LODs, with 10 $\times$  S/N ratio instead of 3 $\times$ . The calculated LODs ranging from 3 to 10 ppb ( $\mu$ g/L) demonstrate the excellent sensitivity of the method.

### Sample analysis

A total of four beer samples were analyzed, one of which is a non-alcoholic beer sample. The beer samples were diluted 100 $\times$  before injection, except for the non-alcoholic beer sample, which was diluted 1000 $\times$  before injection. To ensure correct peak identification, the samples were spiked with the standard mix with a final concentration of 10  $\mu$ M for the spiking solution.

An example chromatogram of a beer sample (100 $\times$  diluted Corona beer) is shown in Figure 3. In this chromatogram,

alcohol, sugar alcohol, mono- and disaccharides elute before 25 minutes. Larger carbohydrates such as oligo- and polysaccharides elute after 25 minutes. For clarity, detailed chromatograms of all beer samples are shown in Figure 4—7.

The sugar content in all samples (Table 6) were calculated based on the calibration curves of the standard (0.1—80  $\mu$ M). In all beer samples, galactose, glucose, sucrose, fructose, maltose, and maltotriose were found. Lactose was not detected in the beers in a quantifiable amount. Lactose is known to be added to some beers like stouts, to add sweetness and improved mouthfeel. In all alcoholic beer samples tested, maltotriose is the most dominant sugars detected, followed by fructose. In contrast with the alcoholic beer, the non-alcoholic beer Affligem 0.0 has a substantially higher amount of fermentable sugars, one possible reason for this might be the addition of sugars after the alcohol removal process to improve flavor. The content of sugars quantified with HPAEC-PAD in Affligem 0.0 was 3.4 g/100mL, which is close to the content stated on the product label (3.7 g/100 mL). Another notable difference in the chromatograms is the presence of the early eluting peaks at about 2—3 minutes in the alcoholic beer samples. These peaks may correspond to alcohol content which clearly is absent in the non-alcoholic beer.



## Sugars in beer

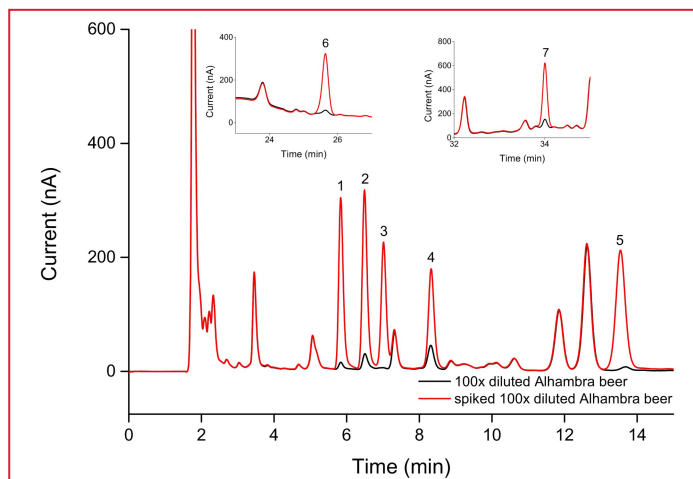


Figure 4. Chromatograms obtained from a 10  $\mu$ L injection of the 100 $\times$  diluted Alhambra beer (black trace) and spiked 100 $\times$  diluted Alhambra beer (red trace). Peak labels: (1) Galactose, (2) Glucose, (3) Sucrose, (4) Fructose, (5) Lactose, (6) Maltose, and (7) Maltotriose.

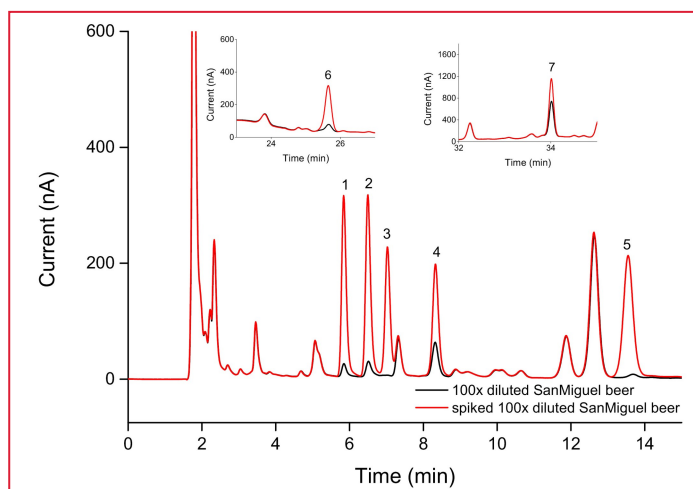


Figure 5. Chromatograms obtained from a 10  $\mu$ L injection of the 100 $\times$  diluted San Miguel beer (black trace) and spiked 100 $\times$  diluted San Miguel beer (red trace). Peak labels: (1) Galactose, (2) Glucose, (3) Sucrose, (4) Fructose, (5) Lactose, (6) Maltose, and (7) Maltotriose.

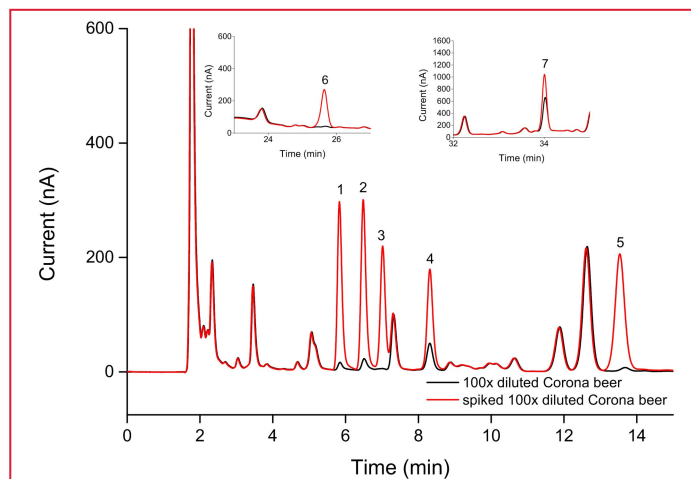


Figure 6. Chromatograms obtained from a 10  $\mu$ L injection of the 100 $\times$  diluted Corona beer (black trace) and spiked 100 $\times$  diluted Corona beer (red trace). Peak labels: (1) Galactose, (2) Glucose, (3) Sucrose, (4) Fructose, (5) Lactose, (6) Maltose, and (7) Maltotriose.

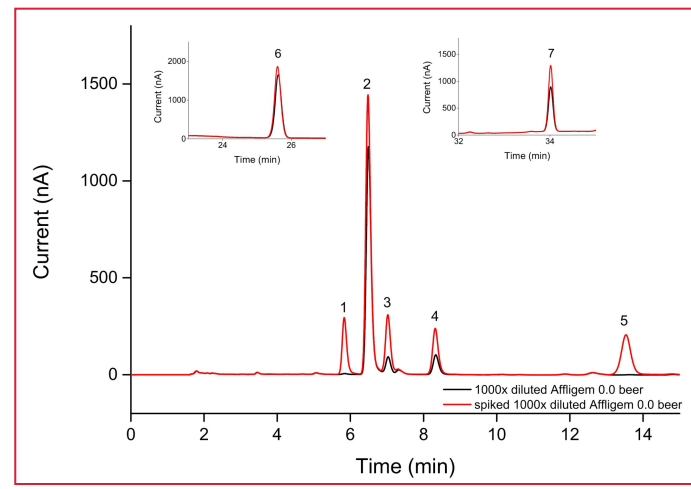


Figure 7. Chromatograms obtained from a 10  $\mu$ L injection of the 1000 $\times$  diluted non-alcoholic Affligem 0.0 beer (black trace) and spiked 1000 $\times$  diluted non-alcoholic Affligem 0.0 beer (red trace). Peak labels: (1) Galactose, (2) Glucose, (3) Sucrose, (4) Fructose, (5) Lactose, (6) Maltose, and (7) Maltotriose.

**Table 6**

Sugar content in beer samples [mg/L product]

| Compound    | Alhambra | San Miguel | Corona | Affligem 0.0 |
|-------------|----------|------------|--------|--------------|
| Galactose   | 8.3      | 14.7       | 9.58   | 26.3         |
| Glucose     | 18.5     | 18.4       | 13.46  | 7673.3       |
| Sucrose     | 2.6      | 1.7        | 2.66   | 1138.9       |
| Fructose    | 60.3     | 88.4       | 70.18  | 1395.2       |
| Lactose     | n.d.     | n.d.       | n.d.   | n.d.         |
| Maltose     | 35.0     | 67.8       | 15.66  | 23473.2      |
| Maltotriose | 72.3     | 744.9      | 655.91 | 10324.6      |

\*n.d. = not detected

**Table 7**

Recovery (%) of sugars in beer samples

| Compound    | Alhambra | San Miguel | Corona | Affligem 0.0 |
|-------------|----------|------------|--------|--------------|
| Galactose   | 102.2    | 102.5      | 99.2   | 97.7         |
| Glucose     | 100.8    | 101.3      | 97.8   | 94.0         |
| Sucrose     | 102.4    | 102.4      | 99.4   | 98.6         |
| Fructose    | 101.7    | 103.3      | 98.8   | 104.7        |
| Lactose     | 107.9    | 104.9      | 102.3  | 102.8        |
| Maltose     | 110.0    | 99.7       | 95.1   | 96.7         |
| Maltotriose | 109.8    | 103.3      | 92.9   | 98.6         |





The sample recovery can be calculated based on the average amount of the analytes in the sample, spiked sample, and 10 µM standard mix.

$$\text{Recovery (\%)} = 100\% * \frac{\text{Amount}_{\text{spiked sample}} - \text{Amount}_{\text{sample}}}{\text{Amount}_{\text{standard}}}$$

The sample recovery are listed in the Table 7. The sample recovery found for all sugars ranged between 90% - 110%.

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## Conclusion

HPAEC-PAD analysis using the ALEXYS™ Carbohydrate Analyzer in combination with the SweetSep™ AEX200 offers a selective separation and sensitive detection of sugars in beer samples. All sugars of interest are baseline separated and eluted within 35 minutes. The method shows excellent linearity, good repeatability and high detection sensitivity, as demonstrated by the low LOD's. The method applicability was further validated by successful analysis of different beer samples, including a non-alcoholic beer. Overall, HPAEC-PAD offers the best solution for sugar analysis in beers.



## Sugars in beer

### Ordering information

|                                    |   |
|------------------------------------|---|
| <b>Detector only</b>               |   |
| 176.0035B                          | DECADE Elite SCC electrochemical detector                 |
| 116.4321                           | SenCell 2 mm Au HyREF                                     |
| <b>Recommended ALEXYS analyzer</b> |   |
| 180.0057W                          | ALEXYS Carbohydrates Analyzer - gradient (quaternary LPG) |
| 116.4321                           | SenCell 2 mm Au HyREF                                     |
| 186.ATC00                          | CT2.1 Column Thermostat                                   |
| <b>Column</b>                      |   |
| 260.0010                           | SweetSep™ AEX200, 4 x 200 mm column, 5 µm                 |
| 260.0015                           | SweetSep™ AEX200, 4 x 50 mm precolumn, 5 µm               |
| 260.0030                           | Borate ion trap, 4 x 50 mm column, 10 µm                  |
| 260.0100*                          | Pre-column filter PEEK, 0.5 µm                            |
| <b>Software<sup>#</sup></b>        |   |
| 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 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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#### Antec Scientific (USA)

info@AntecScientific.com

www.AntecScientific.com

T 888 572 0012

#### Antec Scientific (worldwide)

info@AntecScientific.com

www.AntecScientific.com

T +31 (172) 268888

