



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 coffee  
Carbohydrates in Dutch candy  
Carbohydrates in honey  
Oligo- and Polysaccharides in honey

**Prebiotics Food Additives**

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

**Lactose Free Products**

Lactose in dairy & meat  
Lactose in lactose-free products

**Artificial Sweeteners**

Sugar alcohols  
Sucralose

## Carbohydrates in Coffee

- **ALEXYS™ Carbohydrate Analyzer**
- **SweetSep™ AEX20 anion-exchange column**
- **AOAC 995.13/ISO 11292:1995**
- **Simple, fast and 'green' method**
- **Instant coffee & green coffee beans**

### Summary

Coffee is one of the most widely consumed beverages worldwide and a frequent target of food fraud through adulteration with carbohydrate-based fillers such as glucose syrups. To ensure authenticity, the official method for carbohydrate analysis in coffee (AOAC 995.13/ISO 11292:1995) employs high-performance anion exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD). However, this method has several limitations, including a long elution time (45 minutes) and the need for post-column sodium hydroxide addition. This study presents an improved analysis method using a novel polymeric anion-exchange column SweetSep™ AEX20, offering fast separation of 11 carbohydrates in under 13 minutes with baseline resolution. The method does not require post-column addition of sodium hydroxide, simplifying the analytical workflow. In addition, the use of narrow-bore (2.1 mm ID) columns reduces mobile phase consumption and waste, aligning with green chemistry principles. A variety of coffee products (four instant coffees and four green coffees) were analyzed using the presented method, demonstrating its versatility for carbohydrate analysis and its potential as a more efficient and user-friendly alternative for coffee authenticity verification.

## Introduction

Coffee is one of the most popular drinks in the world. World-wide coffee consumption in the 2023/2024 period is estimated at 10.62 million tons of coffee. The high global demand for coffee is driven by its stimulating properties, primarily due to caffeine, as well as its potential health benefits. Carbohydrates constitute a major part (more than 50% dry weight) of raw coffee beans. In the raw coffee beans, it contributes to the aroma of the bean. During the roasting process, carbohydrates react with other chemical compounds present in the coffee beans, such as amino acids in the Maillard reaction. Therefore, carbohydrates also contribute to the color of coffee beans. Furthermore, carbohydrates influence the flavor and the viscosity of brewed coffee. Due to the high consumption and price of coffee worldwide, coffee is frequently subjected to food fraud, where coffee beans are often adulterated with more inexpensive additives, such as glucose syrups, soybean, maltodextrin, wheat, etc.

Therefore, carbohydrates serve as suitable markers to assess the authenticity of coffee products, including coffee beans and instant coffee. The official chromatographic method used to quantify the carbohydrate content of instant coffee is described in AOAC 995.13, published in 1995 [4]. This method is based on HPAEC-PAD, which enables selective separation and sensitive detection of carbohydrates without derivatization. The International Organization for Standardization (ISO) adopted the AOAC method and released it as official standard ISO 11292:1995 [3]. In 2011, ISO published a related complementary standard, ISO 24114:2011, describing the

criteria for the authenticity of instant coffee [2,5]. ISO 24114 is based on the analysis of the carbohydrate contents using the method specified in ISO 11292. According to the ISO 24114, the maximum carbohydrate content in pure soluble coffee is 2.46% (%w/w) of glucose and 0.45% (%w/w) of xylose. The original AOAC 995.13 method is based on anion-exchange separation of carbohydrates using type I ultrapure water as mobile phase and therefore requires post-column sodium hydroxide addition to increase pH to be able to detect the carbohydrates using PAD.

In this note, we present an improved HPAEC-PAD method derived from the AOAC 995.13 for the analysis of carbohydrates in coffee using a novel polymeric anion-exchange column SweetSep™ AEX20. This stationary phase consists of highly monodisperse 5 µm ethylvinylbenzene-divinylbenzene copolymer (80% crosslinked) substrate particles coated with functionalized nanobeads containing dual ion exchange sites (quaternary amine and tertiary amine). The high uniformity and small particle size of the resin allow for a fast and high-resolution separation of carbohydrates without the need for post-column sodium hydroxide addition, addressing the limitations of the AOAC 995.13 and other existing methods for more accurate and user-friendly analysis. Additionally, following the green chemistry principles, 2.1 mm ID columns were used to reduce the flow rate and thus mobile phase consumption. A total of eight coffee samples were analyzed to demonstrate the applicability of the method for quantification of the sugar content in coffee and identifying potential adulteration in coffee.

## Method

The carbohydrate analyses of coffee samples were performed using the Antec Scientific ALEXYS™ Carbohydrate Analyzer (figure 1) and the conditions specified in table 1. This high-pressure ion chromatography system with metal-free flow path consists of the ET210 eluent tray, P6.1L quaternary LPG pump, CT2.1 column thermostat, and DECADE Elite electrochemical detector. The ET210 eluent tray has an integrated gas distribution system to blanket the headspace of the eluent bottles with inert gas (Nitrogen or Helium), to avoid diffusion of CO<sub>2</sub> into the eluents and the build up of carbonate ions (CO<sub>3</sub><sup>2-</sup>) which act like a strong 'pushing agent'.

## Separation

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. Therefore, under alkaline conditions (pH > 12) carbohydrates can be separated by means



**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.



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 pKa value and increases significantly with molecular weight.

In HPAEC-PAD carbohydrate analysis, small concentrations (low ppb level) of borate contaminants present in hydroxide mobile phases can negatively affect chromatographic performance due to peak tailing and loss of peak symmetry of specific sugars. Borate ions ( $\text{BO}_3^{-3}$ ) can easily complex with vicinal hydroxyl groups present in some carbohydrates, for example, mannose, sugar alcohols, and fructose. Therefore, as a precaution, a borate ion trap column (50 × 2.1 mm ID) was installed between

**Table 1**

HPAEC-ECD Conditions	
HPLC system	ALEXYS™ Carbohydrate Analyzer
Columns	SweetSep™ AEX20, 2.1 × 200 mm analytical column, 5 μm SweetSep™ AEX20, 2.1 × 50 mm precolumn, 5 μm Borate ion trap, 2.1 × 50 mm column, 10 μm (Antec Scientific)
Mobile phase (MP)	A: 10 mM NaOH B: DI Water (resistivity > 18 MOhm.cm and TOC < 5ppb) C: 100 mM NaOH + 100 mM NaOAc Eluents blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min
Back pressure	about 220 bar
Injection	3 μL
Temperature	25 °C for separation, 45 °C for 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 0.1 - 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 - 10	5 mM NaOH	50	50	0	Elution & detection
10 - 15	100 mM NaOH, 100 mM NaOAc	0	0	100	Elution, detection & column clean-up/ regeneration
15 - 49	5 mM NaOH	50	50	0	Equilibration to the starting condition

the pump and the injector. Another possible source of borate ions is glassware, which can leach borate especially at high pH. Therefore, all solutions were prepared in polypropylene bottles, and the samples were stored in a polypropylene vial.

The separations were performed at 25°C on a SweetSep AEX20 analytical column (200 × 2.1 mm ID). A SweetSep AEX20 precolumn (50 × 2.1 mm ID) was installed in series upfront the analytical column for protection against contaminants and particulate matter. The sugars were separated using isocratic elution with 5 mM NaOH at a flow rate of 0.18 mL/min for 10 minutes (see gradient program in table 2). After the elution, a column clean-up step with 100 mM NaOH and 100 mM NaOAc was executed for 5 minutes, followed by an equilibration step to the starting condition for 34 minutes, resulting in a total run time of 49 minutes.

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 ultrapure type 1 water (resistivity > 18 MΩ.cm, TOC < 5 ppb), which was sparged with Nitrogen 5.0 (purity 99.999%). During analysis the mobile phase headspace is blanketed with Nitrogen gas (0.2–0.5 bar N<sub>2</sub> pressure) using the ET210 eluent tray to ensure reproducible analysis.

## Detection

The ALEXYS Carbohydrate Analyzer was equipped with a DECADE Elite electrochemical detector in combination with a SenCell™ electrochemical flow cell for the pulsed amperometric detection [6]. The SenCell has a confined wall-jet flow cell design and consists of a gold working electrode (WE), HyREF palladium reference electrode (RE), and stainless-steel auxiliary electrode (AE). The flow cell has an adjustable spacer and was set to position 2, which corresponds to a 50 μm spacing and a 160 nL working volume. A 4-step potential waveform was applied: E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, and E<sub>4</sub> were +0.10, -2.0, +0.6, and -0.1 V, respectively, with pulse duration of t<sub>1</sub> = 0.40 s, t<sub>2</sub> = 0.02 s, t<sub>3</sub> = 0.01 s, and t<sub>4</sub> = 0.07 s, resulting in a total pulse time of 0.5 s (corresponding to a data rate of 2 Hz). The signal (cell current) is acquired for 200 ms at t<sub>1</sub> between 0.20 - 0.40 s. This particular 4 -step waveform has several benefits: (1) long-term reproducible response factor for all analytes of interest and (2) minimal electrode wear [7]. The detection temperature was set to 45°C to improve detection sensitivity at low NaOH concentration in the eluent. Under the conditions specified, the typical cell current was 0.2 μA. The filter setting for the detection was set to 0.05 Hz.

## 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 were stored in the freezer at -30°C. The stock standards under these conditions were stable for more than one month. Working standards in the concentration range of 1-100 µM were prepared by serial dilution of the stock standards with type I ultrapure water. This concentration corresponds to the range of 0.2 -18.2 mg/L for mannitol, 0.2 - 16.4 mg/L for fucose and rhamnose, 0.2 - 15.0 mg/L for arabinose, xylose, and ribose, 0.2 - 18.0 mg/L for galactose, glucose, mannose, and fructose, 0.3 - 34.2 mg/L for sucrose.

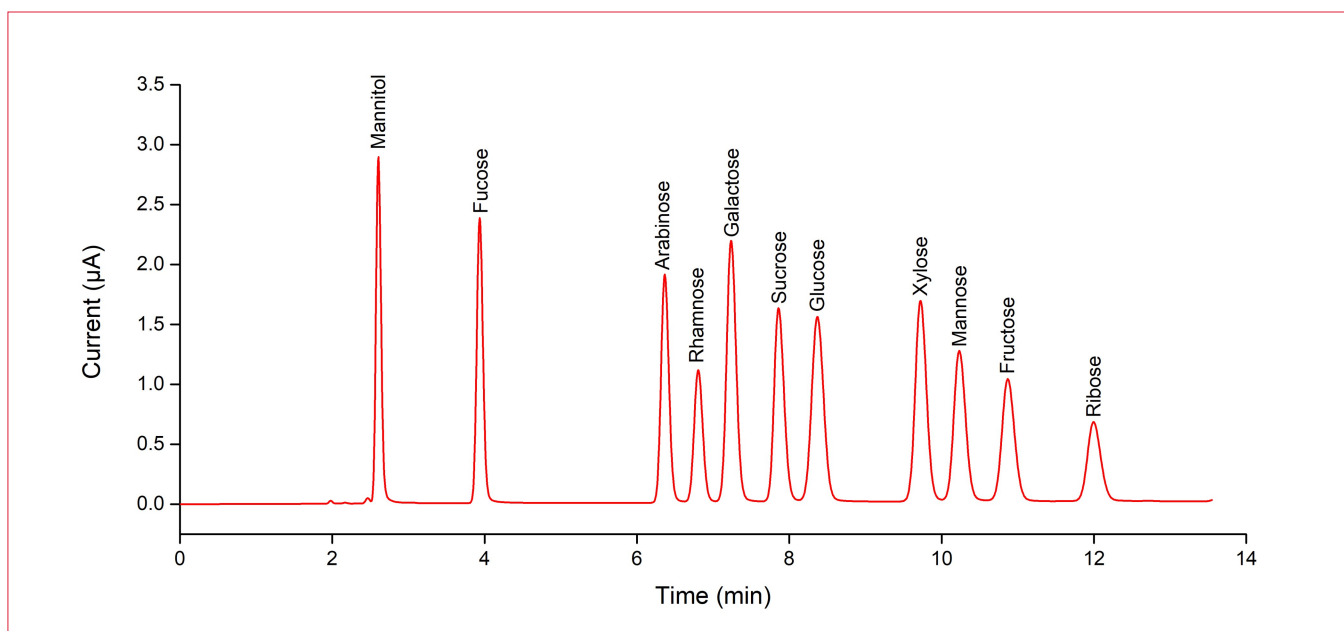
**Instant coffee:** A total of eight coffee samples (four instant coffee products and four green coffee beans) were analyzed. Two instant coffee products originated from The Netherlands, while the other two were from Turkey and Indonesia. The instant coffees were treated using two different sample preparation methods based on the AOAC 995.13 [4].

The first method was used to determine the free carbohydrate content in the sample. In this method, 150 mg of the coffee sample was added to a 50 mL volumetric flask. 35 mL of DI water was added, and the resulting mixture was swirled until dissolution, or a homogenous suspension was obtained. The mixture was then diluted to a total volume of 50 mL. Subsequently, 5 – 10 mL of the mixture was filtered through Waters Sep-Pak® C<sub>18</sub> cartridge, where the first few milliliters

were discarded. The filtrate was passed through a 0.22 µm PES (Polyethersulfone) syringe filter and diluted 100× prior to injection.

The second method was used to determine the total carbohydrate content in the instant coffee sample via acid hydrolysis of the oligo- and polysaccharides into mono- and disaccharides. The sample preparation was performed as follows: 150 mg of coffee sample was added into a 50 mL volumetric flask. Subsequently, 25 mL of hydrochloric acid (1 M) was added, and the resulting mixture was swirled. The flask was incubated in a water bath at 98 ± 2 °C for 2.5 hours, taking into account that the flask was sufficiently submerged to assure efficient heat transfer to the sample and optimal extraction of the sugars (the meniscus of the sample solution in the flask in this case was always below the water level in the water bath). Every 30 minutes the flask was manually swirled to homogenize the sample mixture. After incubation, the mixture was cooled down to room temperature by running tap water onto the flask as coolant. The mixture was further diluted to a total volume of 50 mL, and filtered through a Whatman™ filter (589/1, 125 mm). The resulting solution was diluted 100× prior to injection. 3 µL of the samples were injected into the LC system and analyzed.

**Green coffee:** The four green coffee bean samples originated from India, Guatemala, Ethiopia, and Brazil. The green coffee beans were processed with a sample preparation method to determine the free carbohydrate content only. In this study,



**Figure 2.** Chromatogram obtained from a 3 µL injection of 100 µM carbohydrate standard mix containing galactose, fructose, glucose, sucrose, mannitol, fucose, arabinose, rhamnose, xylose, mannose, and ribose in ultrapure water.



the coffee beans were analyzed as-is (as recommended by the AOAC method 995.13) and crushed to see whether crushing affected the carbohydrate content. The sample preparation was performed as follows: 1 g of the intact green coffee beans or crushed green coffee beans were weighed in a volumetric flask and 10 mL of DI water was added. This solution was sonicated for 15 minutes and passed through a 0.22  $\mu\text{m}$  PES (Polyethersulfone) syringe filter. For the green coffee bean samples, a dilution of 1000 $\times$  was performed prior to injection.

## Results

The official AOAC method 995.13 specifies 11 carbohydrates that can be detected in instant coffee. The 11 carbohydrates are galactose, fructose, glucose, sucrose, mannitol, fucose, arabinose, rhamnose, xylose, mannose, and ribose. These carbohydrates are commonly found in both instant coffee products and/or raw green coffee beans. A chromatogram of 3  $\mu\text{L}$  injection of a 10  $\mu\text{M}$  mix of 11 carbohydrates is shown in Figure 2. All carbohydrates in the standard mix were eluted within less than 13 minutes and all analytes were baseline separated ( $>1.5$ ), even the potentially problematic peak pair rhamnose and arabinose. The elution time is at least 3 $\times$  faster compared to the condition specified in the AOAC official method 995.13. The peak asymmetry values for all carbohydrates were either 1.0 or 1.1, showing no significant

peak tailing. The peak efficiencies found for all carbohydrates ranged from 33000 – 91500 theoretical plates/meter

## Validation

The method validation involved assessing repeatability, linearity, and determination of the limit of detection and quantification.

### Repeatability

The relative standard deviation (RSD) for the retention time and peak area were determined for 10 repetitive injections of a 1  $\mu\text{M}$ , representing the lowest concentration of the standard, as well as a 10  $\mu\text{M}$  standard mix in DI water. The results are listed in Table 3 and 4. Low RSD values were obtained for the retention time and peak area for all compounds. The retention time RSD values for all compounds in both the 1  $\mu\text{M}$  and 10  $\mu\text{M}$  standard were  $< 0.28\%$ . The peak area RSD values for the 10  $\mu\text{M}$  standard were slightly better compared to the 1  $\mu\text{M}$  standard due to the lower concentration injections having smaller peak areas, and therefore the errors are slightly higher. Nevertheless, the peak area RSD values remained  $< 1.90\%$ , which are significantly lower than the average RSD values specified in the ISO 11292:1995 (4.5%) [3]. These low RSD values for peak area demonstrate the good repeatability of the analysis of all 11 carbohydrates using this method.

**Table 3**

Repeatability of 3  $\mu\text{L}$  injections of 10  $\mu\text{M}$  sugar standard mix standard in DI water (n=10)

Compound	RSD (%)	
	10 $\mu\text{mol/L}$	
	$t_R$	Area
Mannitol	0.22	0.56
Fucose	0.15	0.50
Arabinose	0.19	0.74
Rhamnose	0.17	1.10
Galactose	0.22	0.44
Sucrose	0.28	0.73
Glucose	0.23	0.75
Xylose	0.20	0.89
Mannose	0.18	0.88
Fructose	0.19	0.36
Ribose	0.17	0.60

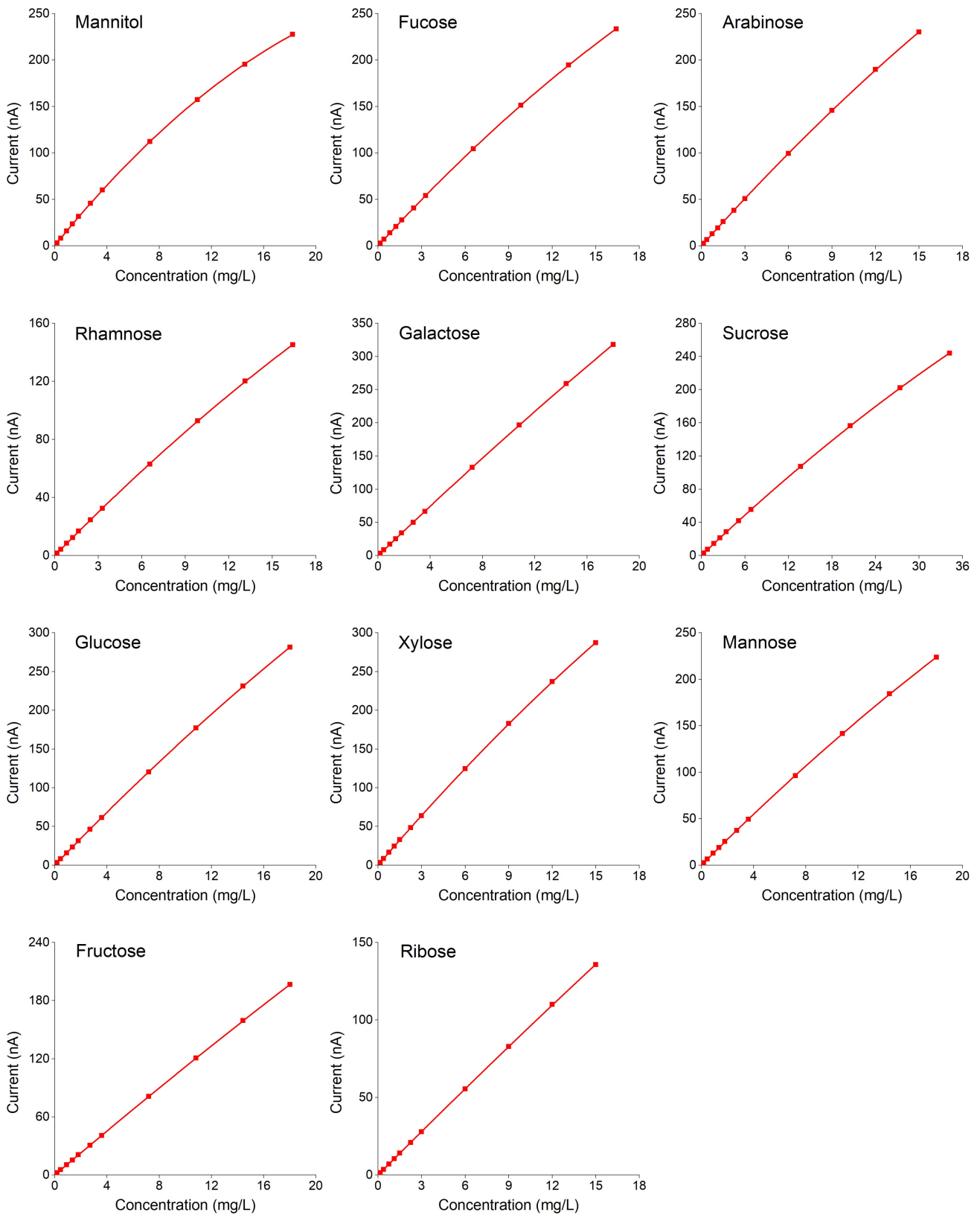
**Table 4**

Repeatability of 3  $\mu\text{L}$  injections of 1  $\mu\text{M}$  sugar standard mix standard in DI water (n=10)

Compound	RSD (%)	
	1 $\mu\text{mol/L}$	
	$t_R$	Area
Mannitol	0.25	1.10
Fucose	0.22	0.91
Arabinose	0.21	1.19
Rhamnose	0.15	1.90
Galactose	0.17	1.17
Sucrose	0.23	1.13
Glucose	0.18	1.53
Xylose	0.17	1.13
Mannose	0.18	1.23
Fructose	0.16	1.14
Ribose	0.16	1.07



# Carbohydrates in Coffee



**Figure 3.** Calibration curves of the 11 sugars in the range of 0.2 - 18.2 mg/L for mannitol, 0.2 - 16.4 mg/L for fucose and rhamnose, 0.2 - 15.0 mg/L for arabinose, xylose, and ribose, 0.2 - 18.0 mg/mL for galactose, glucose, mannose, and fructose, 0.3 - 34.2 mg/L for sucrose. A quadratic fit was applied for all sugars, were the origin was ignored and a weight factor of  $1/\text{concentration}^2$  was used.



## Calibration

The linearity of the method was investigated over a concentration range of 1 – 100  $\mu$ M (11 calibration points) for all carbohydrates, corresponding to:

- Mannitol: 0.2 -18.2 mg/L
- Fucose and rhamnose: 0.2 - 16.4 mg/L
- Arabinose, xylose, and ribose: 0.2 - 15.0 mg/L
- Galactose, glucose, mannose, and fructose: 0.2 - 18.0 mg/L
- Sucrose: 0.3 - 34.2 mg/L

The calibration curves of all 11 sugars are shown in Figure 3. Electrochemical detection of carbohydrates has been known to have a broad linear dynamic range. However, the calibration curve may deviate from linearity at a high analyte concentration, and therefore a quadratic fit was used for fitting all the calibration points for all 11 compounds. The quadratic fitting was applied ignoring the origin and using a weighted factor of  $1/\text{concentration}^2$ .

Table 5

### Relative standard error (RSE)

Compound	RSE (%)
Mannitol	1.220
Fucose	0.986
Arabinose	1.307
Rhamnose	1.346
Galactose	0.979
Sucrose	1.411
Glucose	0.937
Xylose	1.334
Mannose	1.405
Fructose	1.417
Ribose	0.944

The relative standard error was calculated to assess the goodness-of-fit of the calibration curves, see table 5. The predicted concentration closely matched the actual concentration of the standards. A relative standard error of  $\leq 15 - 20\%$  is typically advised, and in this case the relative standard error (RSE) for all compounds are less than 1.5%, demonstrating high accuracy of the calibration curves [7,8]. The calibration curves in Figure 3 were used for the actual quantification of the carbohydrates in the samples.

## Detection limit

The limit of detections (LOD) and limit of quantifications (LOQ) for all carbohydrates are summarized in Table 6. The LOD values were calculated as the analyte response corresponding to  $3\times$  average peak-to-peak baseline noise (ASTM noise).

Table 6

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

Compound	LOD		LOQ
	nmol/L	ng/mL	ng/mL
Mannitol	14	3	8
Fucose	20	3	11
Arabinose	27	4	14
Rhamnose	46	8	25
Galactose	25	5	15
Sucrose	31	11	35
Glucose	33	6	20
Xylose	30	5	15
Mannose	41	7	25
Fructose	56	10	34
Ribose	89	13	45

The ASTM noise was calculated based on 0.5-minute segments between  $t = 2.5$  min and  $t = 12.5$  min in a blank injection (total of 20 segments). The average analyte responses of 10 replicate injections of the lowest concentration standard (1  $\mu$ M) were used to calculate the LOD. The detection limits range from 3 – 13 ng/mL for all compounds. The LOQ was calculated in a similar way to the LOD, using a  $10\times$  signal-to-noise (S/N) ratio. The LOQ for all carbohydrates ranges from 8 – 45 ng/mL, indicating the high sensitivity of this method for quantification of carbohydrates.

## Sample measurement: instant coffee

To evaluate the method's applicability, four commercially available instant coffee products were analyzed. The instant coffee samples were diluted  $100\times$  before injection. For peak identification, the samples were spiked with a known amount of carbohydrate standards. The chromatograms obtained from the free- and total carbohydrate analysis of all instant coffees are shown in Figure 4 - 7.



It is evident from the chromatograms of the instant coffee samples that most of the carbohydrates targeted in AOAC 1995.13 were present in the samples after acid hydrolysis. Among the detected carbohydrates, galactose and mannose are the most dominant carbohydrates present in the sample, which most likely originated from the hydrolysis of galactomannans as the most abundant polysaccharide in coffee [10,11]. Interestingly, fucose and sucrose are not detected in any of the samples after acid hydrolysis. Both carbohydrates are highly susceptible to acidic degradation (e.g. hydrolysis), explaining their absence after the hydrolysis step.

The free- and total carbohydrate content were determined based on the calibration curves (figure 3) and are reported in g/100g (%w/w), see table 8. The instant coffee samples #1 & #2 (The Netherlands) contained 11 carbohydrates in their free form. In contrast, the instant coffee samples #3 & #4 (Turkey & Indonesia, respectively) contained only minimal amount of free carbohydrates.

ISO 24114:2011 specifies the maximum allowable total carbohydrate content in pure instant coffee, setting limits at 2.46% (%w/w) for glucose and 0.45% (%w/w) for xylose. Based on these authenticity criteria, sample #4 from Indonesia, although labelled as 100% pure, is considered as an adulterated instant coffee. It contained an exceptionally high glucose content (more than 9x higher than the threshold value), and in addition, also the xylose contents was a factor 1.4x higher than allowed. This example demonstrates that this analytical method can be used for authenticity verification. [2].

While ISO 24114:2011 does not specify the maximum carbohydrate content of the other sugars, the ISO 11292:1995 standard describes the average carbohydrate content values in instant coffee, as listed in Table 7. The free and total carbohydrate content in the analyzed coffee samples closely aligns with these reference values. However, the total arabinose content found in all samples were smaller than the reported average values. This can be attributed to the roasting process, as arabinose is highly susceptible to thermal degradation, especially if arabinose is present as a side chain in the arabinogalactans [11-13].

### Sample measurement: green coffee beans

Four types of green coffee beans from four different geographical regions were analyzed. The green coffee bean samples were diluted 1000x before injection. These samples were also spiked with a known concentration of the standards for peak identification. The chromatograms obtained from the green coffee bean samples (see figure 8 - 11) exhibited notable

differences from those of instant coffee samples. The major carbohydrate detected in the green coffee bean samples was sucrose, which is a major constituent in unroasted coffee [14]. As expected, sucrose is lost during the roasting process of coffee beans, explaining its significantly lower concentration in instant coffee samples above, even as a free carbohydrate.

The carbohydrate content of all green coffee samples is summarized in Table 8. In all cases, fucose, rhamnose, and xylose were not detected. All other sugars were found at very low levels, except for sucrose. It was previously reported that sucrose in green coffee beans makes up 7.3% of the dry-weight or even as high as 10.1% for some coffee beans, and the values listed for the whole green coffee bean samples were consistent with previously reported data [14-15]. Notably, it was found that the free carbohydrate contents in the crushed coffee bean samples were significantly smaller compared to the whole bean sample. Previous studies have reported that grounded coffee beans gave similar results to whole beans [16]. This study suggests that sample preparation may influence the amount of carbohydrates found in the green coffee sample and therefore highlights the need for careful sampling when analysing green coffee beans.

Table 7

Average carbohydrate content in instant coffee based on ISO 11292:1995

Compound	Average carbohydrate content in instant coffee (%w/w)	
	Free carbohydrates	Total carbohydrates
Mannitol	0.02 – 1.5	0.15 – 1.8
Arabinose	0.5 – 1.3	3.5 – 4.8
Galactose	0.2 – 0.6	8.0 – 18.0
Glucose	0.04 – 2.0	0.7 – 17
Sucrose	0 – 1.3	–
Xylose	0 – 0.03	0.03 – 1.8
Mannose	0.3 – 1.0	2.0 – 19.0
Fructose	0.05 – 3.6	0 – 2.0

### Recovery

In order to assess the accuracy of the sample analysis, the recoveries were calculated based on the response of the analytes in the sample, the spiked sample and 5 µM standard (the final spike concentration in the sample was 5 µM).



**Table 8**

**Concentration of free and total carbohydrates (g/100 g) in a sample of instant coffees**

Component	Coffee sample 1		Coffee sample 2		Coffee sample 3		Coffee sample 4	
	Free	Total	Free	Total	Free	Total	Free	Total
Mannitol	0.04	0.03	0.04	0.02	0.03	0.02	-	0.03
Fucose	0.02	-*	0.02	-	-	-	-	-
Arabinose	0.80	2.45	0.73	2.22	0.02	2.48	-	2.39
Rhamnose	0.03	0.33	0.04	0.37	-	0.21	-	0.22
Galactose	0.54	14.99	0.58	12.98	-	9.37	-	7.72
Sucrose	0.07	-	0.01	-	0.17	-	0.41	-
Glucose	0.11	0.85	0.19	0.85	-	0.57	0.05	22.75
Xylose	0.02	0.12	0.02	0.30	-	0.15	-	0.65
Mannose	0.98	16.35	0.81	11.02	-	13.15	-	8.45
Fructose	0.31	0.27	0.31	0.24	-	0.09	0.06	0.15
Ribose	0.09	0.10	0.05	0.07	-	0.04	-	0.04
<b>Total</b>	<b>3.01</b>	<b>35.49</b>	<b>2.80</b>	<b>28.06</b>	<b>0.23</b>	<b>26.07</b>	<b>5.01</b>	<b>42.40</b>

\* - = not detected

**Table 9**

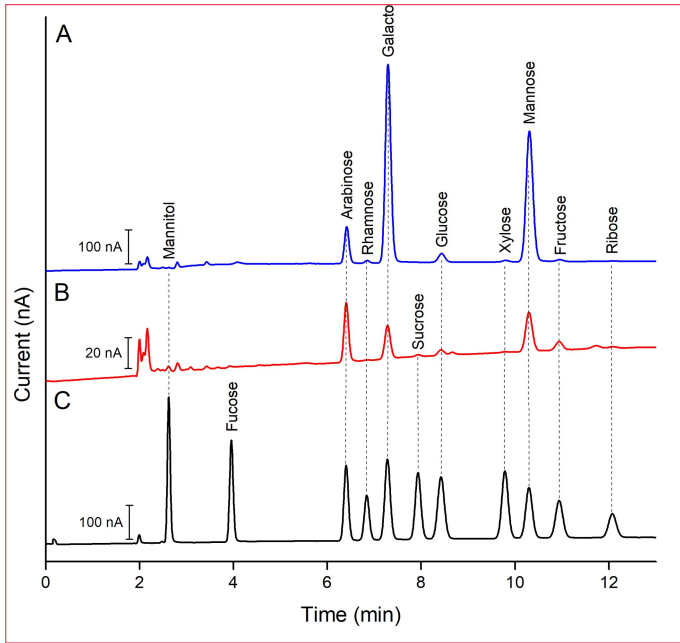
**Concentration carbohydrates (g/100 g) in green coffee beans**

Component	India		Guatemala		Ethiopia		Brazil	
	Whole bean	Crushed bean	Whole bean	Crushed bean	Whole bean	Crushed bean	Whole bean	Crushed bean
Mannitol	0.05	0.01	0.05	0.02	0.18	0.04	0.04	0.03
Fucose	-*	-	-	-	-	-	-	-
Arabinose	-	0.01	0.01	-	0.01	-	-	-
Rhamnose	-	-	-	-	-	-	-	-
Galactose	0.02	0.01	0.15	0.03	0.06	0.02	0.07	0.02
Sucrose	4.98	3.83	9.27	4.18	4.88	2.69	5.79	2.98
Glucose	0.20	0.09	0.16	0.01	0.32	0.02	0.08	0.05
Xylose	-	-	-	-	-	-	-	-
Mannose	0.01	0.01	0.01	-	0.01	-	-	0.01
Fructose	0.34	0.25	0.20	0.03	0.50	0.10	0.16	0.12
Ribose	-	-	0.01	-	0.01	-	0.01	-
<b>Total</b>	<b>5.59</b>	<b>4.21</b>	<b>9.86</b>	<b>4.28</b>	<b>5.96</b>	<b>2.87</b>	<b>6.14</b>	<b>3.19</b>

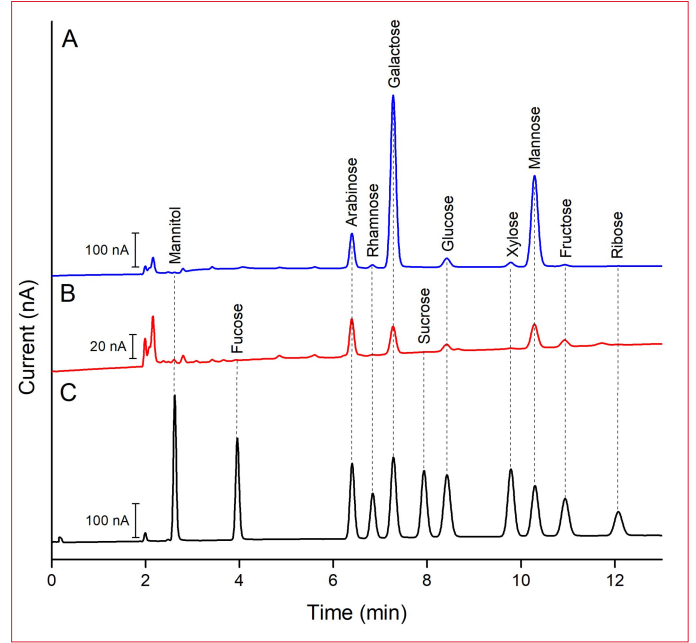
\* - = not detected



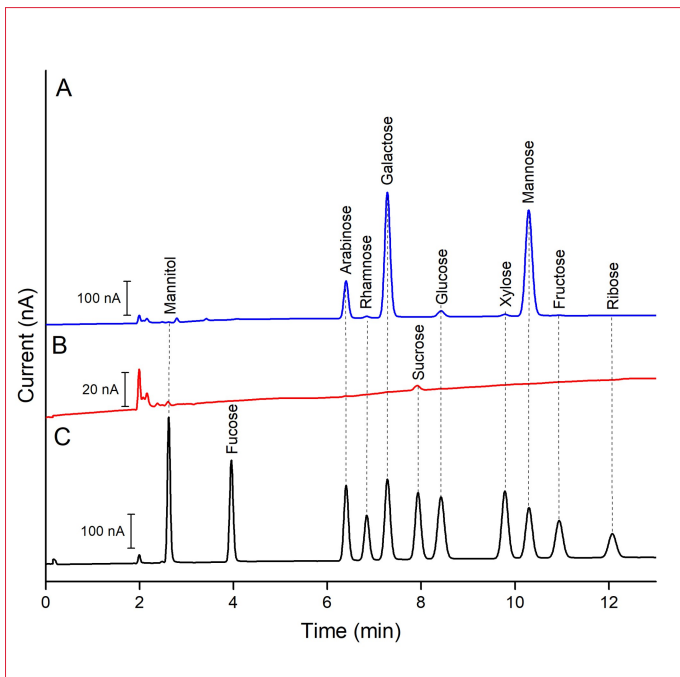
# Carbohydrates in Coffee



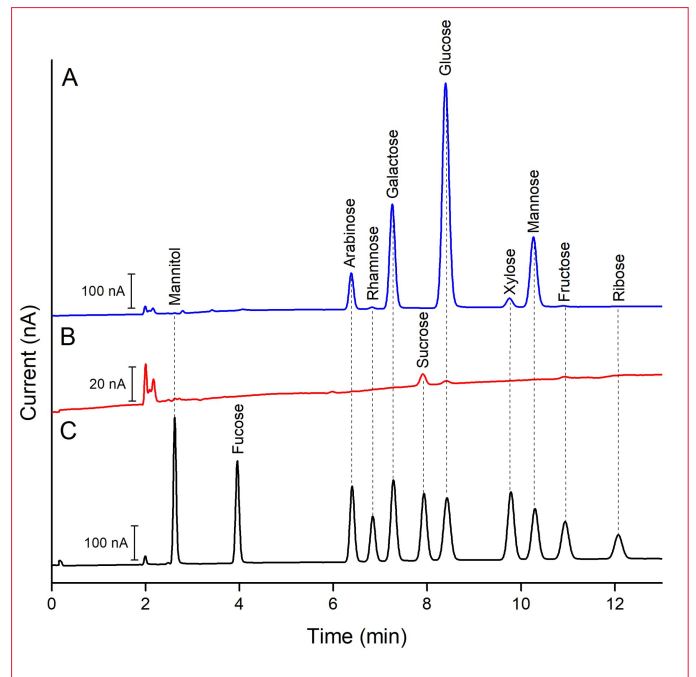
**Figure 4.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] instant coffee sample #1 - total carbohydrate determination (blue curve), [B] instant coffee sample #1 - free carbohydrate determination (red curve), and [C] 10  $\mu\text{M}$  standard



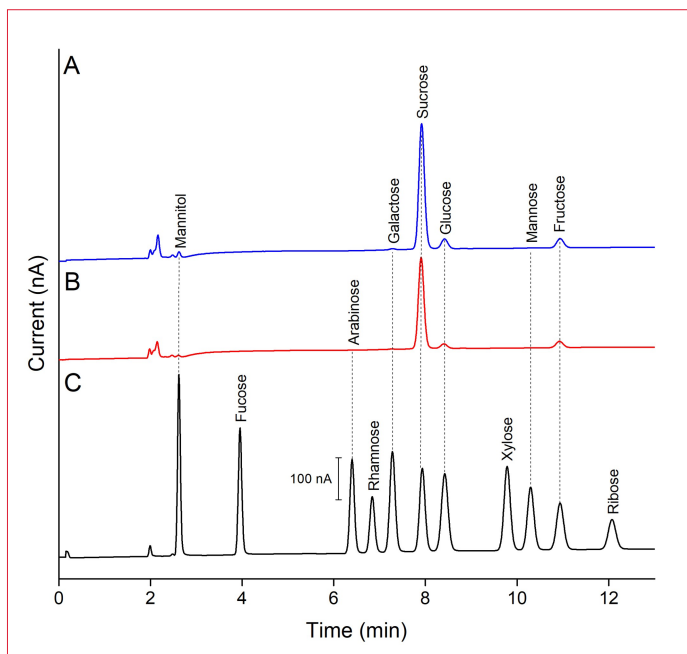
**Figure 5.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] instant coffee sample #2 - total carbohydrate determination (blue curve), [B] instant coffee sample #2 - free carbohydrate determination (red curve), and [C] 10  $\mu\text{M}$  standard



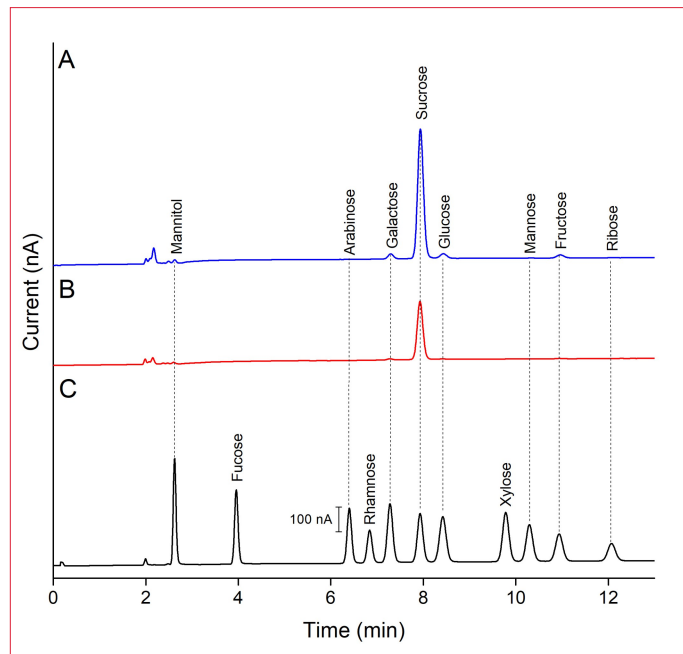
**Figure 6.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] instant coffee sample #3 - total carbohydrate determination (blue curve), [B] instant coffee sample #3 - free carbohydrate determination (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



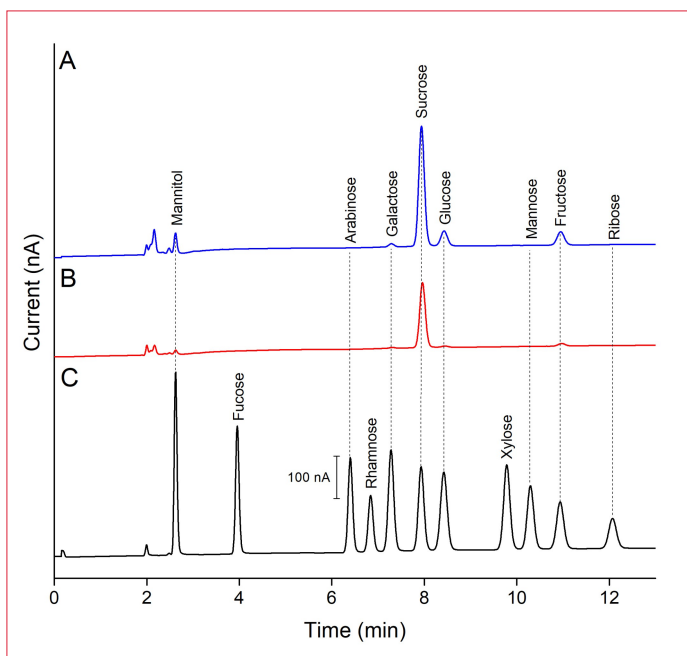
**Figure 7.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] instant coffee sample #4 - total carbohydrate determination (blue curve), [B] instant coffee sample #4 - free carbohydrate determination (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



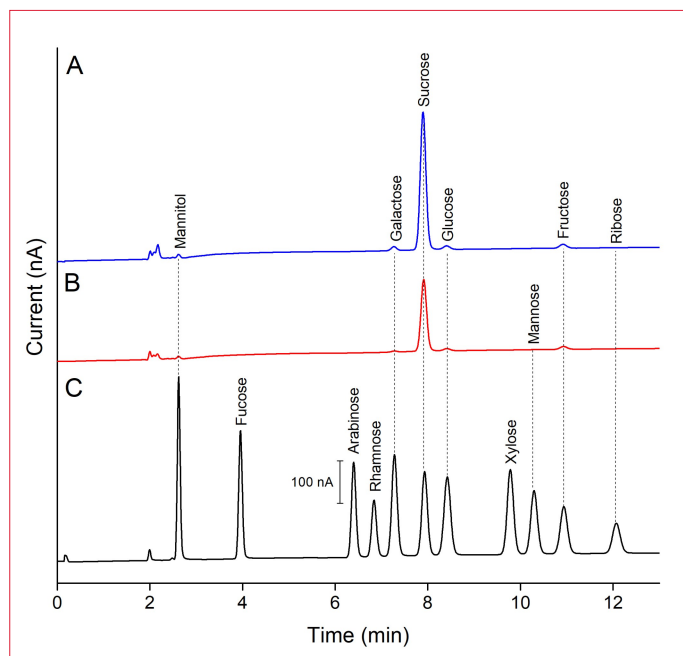
**Figure 8.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] intact green coffee beans from India (blue curve), [B] crushed green coffee beans from India (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



**Figure 9.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] intact green coffee beans from Guatemala (blue curve), [B] crushed green coffee beans from Guatemala (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



**Figure 10.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] intact green coffee beans from Ethiopia (blue curve), [B] crushed green coffee beans from Ethiopia (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



**Figure 11.** Overlay of chromatograms obtained from 3  $\mu\text{L}$  injections of [A] intact green coffee beans from Brazil (blue curve), [B] crushed green coffee beans from Brazil (red curve), and [C] 10  $\mu\text{M}$  standard mix containing 11 carbohydrates in ultrapure water (black curve).



## Carbohydrates in Coffee

**Table 10**

### Recovery values for the instant coffee samples

Compound	Recovery (% non-hydrolyzed sample)				Recovery (% hydrolyzed sample)			
	Sample #1 (The Netherlands)	Sample #2 (The Netherlands)	Sample #3 (Turkey)	Sample #4 (Indonesia)	Sample #1 (The Netherlands)	Sample #2 (The Netherlands)	Sample #3 (Turkey)	Sample #4 (Indonesia)
Mannitol	108.2	97.4	98.8	97.7	98.2	95.8	97.1	96.1
Fucose	109.3	99.0	99.8	96.8	97.9	95.7	97.6	99.2
Arabinose	80.0	97.4	99.4	97.3	96.1	94.0	99.1	95.6
Rhamnose	108.9	97.2	99.5	97.3	96.2	93.6	97.5	96.5
Galactose	92.5	100.0	99.4	95.7	93.5	80.4	105.4	96.1
Sucrose	108.8	98.1	98.7	96.3	94.9	91.8	95.7	95.5
Glucose	106.1	101.0	99.5	97.2	98.9	95.2	99.2	86.3
Xylose	109.9	97.8	99.6	96.7	97.6	95.1	97.4	96.7
Mannose	80.2	98.0	99.7	96.7	83.3	80.1	95.9	89.6
Fructose	100.2	100.0	99.3	96.8	99.1	96.7	98.7	97.0
Ribose	108.3	96.3	99.7	97.0	99.7	97.7	98.7	97.8

**Table 11**

### Recovery values for green coffee bean samples

Compound	Recovery (%) (Intact bean sample)				Recovery (%) (Crushed sample)			
	Brazil	Ethiopia	Guatemala	India	Brazil	Ethiopia	Guatemala	India
Mannitol	96.6	97.3	94.9	96.9	99.8	99.5	99.7	97.6
Fucose	96.3	96.4	93.5	96.1	99.2	99.2	98.8	96.0
Arabinose	96.6	96.4	94.1	96.0	99.2	99.3	99.0	96.5
Rhamnose	94.6	95.7	93.2	95.2	98.1	97.4	98.0	95.2
Galactose	96.7	97.8	96.5	98.0	100.6	100.6	100.7	98.5
Sucrose	84.9	88.9	82.2	90.6	100.1	98.0	95.4	93.6
Glucose	97.3	96.3	97.0	96.2	99.6	98.5	98.2	96.4
Xylose	96.4	97.0	94.2	96.6	99.0	99.0	99.3	97.0
Mannose	94.9	94.3	91.2	94.1	97.6	97.0	97.5	93.8
Fructose	97.1	97.1	94.4	97.3	100.1	100.2	100.7	98.0
Ribose	97.8	97.5	95.3	97.1	100.2	100.5	100.7	98.5



The recovery is calculated using the equation below:

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

The calculated recoveries are listed in table 10 for the instant coffee samples (hydrolyzed and non-hydrolyzed) and in table 11 for the green coffee bean samples. The recovery values for all samples ranged between 80 – 110%. These values are in accordance with the specifications drawn by the European Commission related to the use of analytical methods for food products, and therefore ensured the accuracy of the sample analysis [17].

## Conclusion

An improved HPAEC-PAD method for the analysis of carbohydrates in coffee has been developed based on direct detection, without post-column addition of sodium hydroxide. The method enables fast, high-resolution separation ( $r > 1.5$ ) of the 11 carbohydrates of interest within 13 minutes using the ALEXYS carbohydrate analyzer in combination with the new microbore SweetSep™ AEX20 column.

Compared to the official AOAC 995.13/ISO 11292:1995 method, the optimized method is significantly faster with a 1.6 x shorter runtime.

The use of a narrow-bore column minimizes solvent consumption and waste, thus reducing environmental impact. The applicability of the method was demonstrated through the successful analysis of a total of eight commercial instant coffee and green coffee beans. Notably, one commercial instant coffee sample labelled as 100% pure soluble coffee was found to be adulterated, containing nine times the maximum allowable glucose content specified in ISO 24114:2011.

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## Ordering information

<b>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>Columns</b>	
260.0021	SweetSep™ AEX20, 2.1 x 200 mm column, 5 µm
260.0026	SweetSep™ AEX20, 2.1 x 50 mm precolumn, 5 µm
260.0031	Borate ion trap, 2.1 x 50 mm column, 10 µm
260.0100*	Pre-column filter PEEK, 0.5 µm
<b>Software#</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.

## Reagents, standards and sample prep accessories

NaOH 50%, carbonate –free	Fisher Scientific, pn SS254-500
Sodium acetate (NaOAc), 100%	Sigma Aldrich, pn 79714
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
Hydrochloric acid 2M	Fisher, pn J/4315/15
Galactose	Sigma Aldrich, pn G0750
Fructose	Sigma Aldrich, pn F0127
Glucose	Sigma Aldrich, pn G8270
Sucrose	Sigma Aldrich, pn S9378
Mannitol	Sigma Aldrich, pn 3340-100G
Fucose	Sigma Aldrich, pn F2252-5G
Arabinose	Sigma Aldrich, pn A3131
Rhamnose	Sigma Aldrich, pn W373011
Xylose	Sigma Aldrich, pn X1500
Mannose	Sigma Aldrich, pn M4625
Ribose	Sigma Aldrich, pn R7500
C <sub>18</sub> - SPE cartridges	Waters Sep-Pak®
Syringe filter	0.22 µm PES (Polyethersulfone) 25 mm Ø FFL/MLS
Filter paper	Whatman™ filter (589/1, 125 mm)

*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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