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Applications for Food
& Beverage analysis

Phenols

Bisphenol A
Catechins
Flavonoids
Phenols
Antioxidants
Resveratrol
Epicatechin
Quercetin
Other polyphenols

Carbohydrates

Monosaccharides
Lactose
Other oligo- and
polysaccharides

Vitamins, minerals etc.

A, C, D, E, and K
Iodide
Q10, ubiquinol

Carbohydrate Analysis in Instant Coffee According to Adapted ISO Method 11292:1995

- **Mono- and disaccharides in food sciences**
- **HPAEC-PAD method on ALEXYS Analyzer**
- **Comparison of the standard method with adapted method**
- **Adapted method is 'fast and green'**

Summary

In this application note the analysis of carbohydrates in instant coffee, following the ISO 11292:1995 method, was evaluated using the ALEXYS carbohydrates analyzer. The method is based on separation of carbohydrates by High Performance Anion Exchange Chromatography followed by Pulsed Amperometric Detection (HPAEC-PAD) under standard bore conditions. An adapted method was evaluated, which uses narrow-bore conditions and a column with smaller particle size. In comparison with the standard method, the adapted method resulted in 50% shorter analysis times and 90% lower solvent use ('greener' solution). Both standard bore and narrow-bore methods provide excellent sensitivity, linearity and reproducibility, and are suitable for the routine analysis of carbohydrates in coffee applications using the ALEXYS carbohydrates analyzer.



Carbohydrate Analysis in Instant Coffee According to Adapted ISO Method 11292:1995

Introduction

Carbohydrates (also called saccharides) are the most abundant biomolecules in nature and a major constituent in many food and beverage products. These molecules provide the most easily accessible energy source for our body and are highly valued for their structural properties and (sweet) taste. Measurement of their presence in food and drinks is required for product labeling purpose, but carbohydrate composition is also a very good tracer for authenticity assessment^{1,2}.

Coffee is one of the most popular drinks in the world. Carbohydrates are the major constituents of coffee beans and they act as aroma binders, foam stabilizers and they increase the viscosity of the drink. Their analysis in coffee and coffee products is covered by method ISO 11292:1995, issued by the International Organization for Standardization (ISO). The principle of this method is the same as Method 995.13 which was issued in 1995 by the Association of Analytical Chemists (AOAC) and originally implemented as the UK national standard BS 5752-15:1995. This UK standard was withdrawn and replaced in 1997 by the unaltered text of the ISO method as BS 5752-15:1997. The text of the ISO method has further been approved by the governments as suitable for publication without deviations as the national standard method for use in India⁹ and Mexico¹⁰, which are major coffee producing countries. Germany, which is a coffee importing country, has adopted a translated version of the ISO method¹¹.

The principle of the ISO method for analysis of free and total carbohydrate content in soluble/instant coffee is:

- extraction of the free carbohydrates from the coffee with water, and hydrolysis of the coffee to obtain an extract of the monosaccharides for quantitation of the total carbohydrate content
- separation of the individual mono- and disaccharides on an Anion Exchange (AEX) HPLC column using water as the mobile phase
- detection on a gold electrode using Pulsed Amperometric Detection (PAD) after post-column NaOH addition.

The ISO method was developed in a time when applying normal bore HPLC in combination with large particles size was the best available commercial option. Nowadays, commercially available AEX columns with a smaller internal diameter are available, which reduces the need of solvents ('going green'). Another development has been the reduction of column particle size. The higher separation efficiencies of smaller particles can be used to reduce the analysis time. To apply the new column types, the only adaptation to the parameters given in the ISO method is to lower the given flow rate and injection volume to match with the narrower column.

This note shows the applicability of Antec's ALEXYS Analyzer with DECADE Elite electrochemical detector and SenCell flow cell (Figure 1) to analyze carbohydrates using the standard ISO method. The second part of this note shows the excellent results ('fast and green') when using the dedicated ALEXYS Carbohydrates Analyzer and a modern narrow-bore small particle sized AEX HPLC column.



Figure 1: ALEXYS Analyzer with 2nd pump for post-column addition

Method

The general ALEXYS LC-EC Analyzer with He sparging, low pressure solvent selector and post-column addition hardware (pump, backpressure generator, mixer) was used for method evaluation of the traditional normal bore conditions. The dedicated ALEXYS Carbohydrates Analyzer was used for testing the adapted 'fast and green' method.

For comparison purpose, a summary of the ISO method 11292: 1995 is given in Table 1, which is by no means intended to make the use of the official text superfluous.



Table 1

Summary of method ISO 11292: 1995	
HPLC	Metal free liquid chromatograph
Detector	Pulsed amperometric detector (PAD) with gold electrode
Column	High-performance anion-exchange column filled with pellicular polystyrene-divinylbenzene resin and guard column
Mobile phase (Eluent S1)	demineralized water (18 MΩ·cm)
Post-column addition and post-run flush solution (Eluent S2)	0.3 M NaOH in demineralized water
Flow rate mobile phase	1.0 mL/min
Flow rate post column addition	0.6 mL/min
Temperature	Ambient
Detector settings	Use the optimum conditions given by the manufacturer
Carbohydrate standards	Arabinose, fructose, galactose, glucose, mannose, sucrose, mannitol
V _{injection}	20 µL
Analysis	Inject a standard solution every 4 injections to account for any changes in retention times or peak integration

Table 2

LC-ECD conditions, based on ISO method 11292:1995	
HPLC	ALEXYS™ LC-EC Analyzer (with He-sparging hardware, in-line degassers, in-line 0.2 µm porosity filter, 2 low pulsation HPLC pumps, pulse dampers, detector and autosampler), passivated (metal free)
HPLC add-ons	low-pressure 2-way solvent selector, HDPE bottles
Detector	DECADE Elite™ electrochemical detector and SenCell™ with gold working electrode, HyREF™, AST 2
Mobile phase	Demineralized water (18 MΩ·cm, low TOC), continuously sparged with Helium 5.0
Post-column addition and post-run flush solution	0.3 M NaOH in demineralized water, continuously sparged with Helium 5.0
Temperature	32°C*
Detector settings	PAD wave-form (4-step) E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V t1, t2, t3, t4: 0.40, 0.02, 0.01, 0.07 s; ts: 200 ms
I-cell	0.2-1.0 µA
ADF	0.02 Hz
Range	20 µA/V (1 µA/V for measuring near-LOD levels)

* The ISO method states the application of 'ambient' temperature

Separation

Due to the numerous hydroxyl groups on carbohydrate molecules, they can be well separated in isocratic mode on an Anion Exchange HPLC separation column using water as the mobile phase. A 15-minute post-run column clean up with 0.3 M NaOH is part of the ISO method to keep column performance in check. A re-equilibration time of 15 minutes with mobile phase makes the column ready for the next injection. For these purposes, a post-column solvent addition pump and a step-gradient solvent selector are part of the system.

Temperature is a crucial parameter for separation, and stable reproducible retention times are only possible using a thermostatic compartment. The ISO method, however, states the use of ambient temperature, and the analysis of a standard every 4 injections to compensate for any changes in the chromatograms. The moment-to-moment temperature changes in a laboratory without air-conditioning have a significant effect on the retention times (data not shown), and therefore we chose to apply a fixed temperature that was only a few degrees above ambient.

The CarboPac PA1 (10 µm, 4 x 250 mm; Thermo Scientific) is mentioned in the ISO method as an example of a suitable column without being intended as an endorsement of this product by ISO. The CarboPac PA210 (4 µm, 2 x 150 mm; Thermo Scientific) with narrower internal diameter and smaller particles is chosen to make a 'fast and green' method. Note that both columns fit the materials description of the ISO method (see Table 1).

Traditional conditions:

The CarboPac PA1 (and matching precolumn) was chosen for method evaluation, using conditions given in Table 2 and Table 3. A schematic representation of the system is given in Figure 2. These conditions are advised in case there is no freedom to apply the reduced flow rate as needed for the adapted method.



Table 3

Conditions matching the ISO method settings	
Column + guard	CarboPac™ PA1 (10 µm, Thermo Scientific), 4 x 50 mm + 4 x 250 mm
Mixer	150 µL
Flow rate mobile phase	1.0 mL/min
Flow rate post column addition	0.6 mL/min
V _{injection}	20 µL

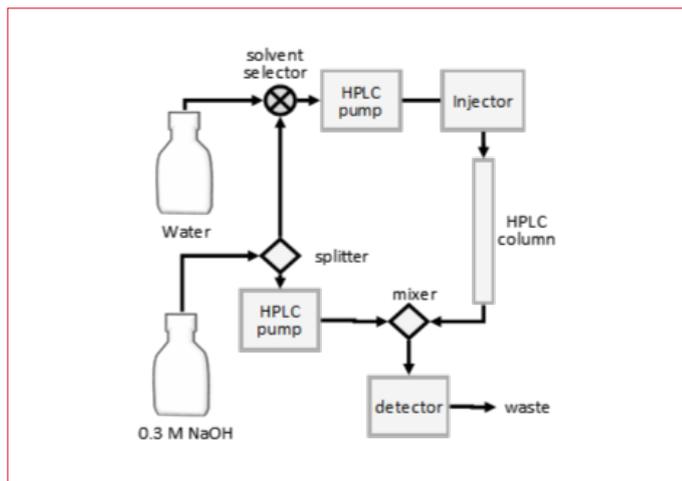


Figure 2: Schematic hardware representation of the set-up as used for testing the tradition conditions.

Adapted conditions for the 'fast and green' method:

The narrow-bore CarboPac PA210 and matching precolumn (Thermo Scientific) are chosen as suitable for the 'fast and green' method using conditions given in Table 2 and Table 4. Due to the narrow-bore dimensions of the column, a lower flow rate has to be applied to compensate for the narrower internal diameter, and the flow rate of the post-run NaOH addition is decreased accordingly.

The dedicated ALEXYS Carbohydrate Analyzer has a minimal dead volume, as it contains tubing with a narrow ID. This prevents plate loss for the narrow-bore column. The system also contains a high-pressure solvent selector just before the injector, which minimizes the gradient lag time. A splitter provides NaOH solution to the mixer at a flow rate of 90 µL/min and to the pressurized solvent reservoir (2 mL) at a flow rate of 210 µL/min. The solution in the pressurized solvent reservoir is used for automated post-run flush. A schematic representation of the system is given in Figure 3.

Table 4

Adapted 'fast and green' method	
Column + guard	CarboPac™ PA210 (4 µm, Thermo Scientific), 2 x 30 mm + 2 x 150 mm
Mixer	8 µL
Flow rate mobile phase	0.15 mL/min
Flow rate post column addition	0.090 mL/min
V _{injection}	5 µL

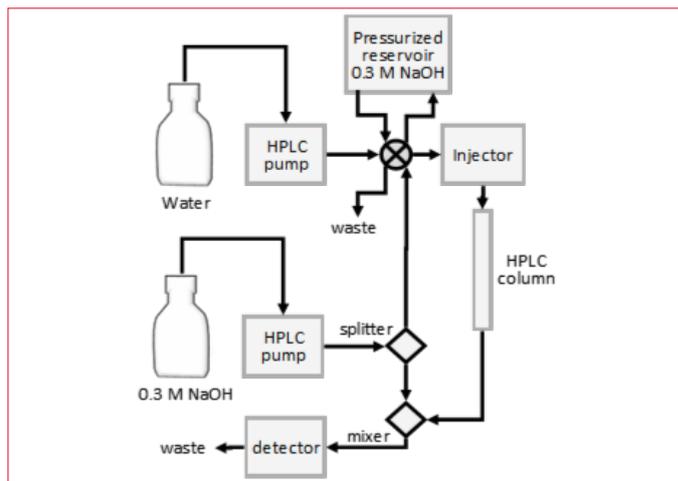


Figure 3: Schematic hardware representation of the set-up as used for testing the adapted 'fast and green' conditions.

Detection

Carbohydrates need a high pH to be detectable on a gold electrode with PAD. This makes post-column NaOH addition necessary when running pure water as the mobile phase. Under the recommended mixing ratio, the acidity inside the electrochemical flow cell is above pH 13. The optimum conditions for the PAD settings are a 4-step potential waveform as shown in Figure 4. This has been claimed to have as benefit (1) a consistent long-term peak area response and (2) minimal electrode wear¹¹. The DECADE Elite applies this 4-step pulse to the gold electrode of SenCell, which has a three-electrode configuration. For analysis of carbohydrates, a combination of gold working electrode (WE), HyREF (Pd/H₂) reference electrode (REF) and stainless steel auxiliary electrode (AUX) is advised. The chromatograms are recorded using Clarity software (Data Apex), which is also used to control the system.

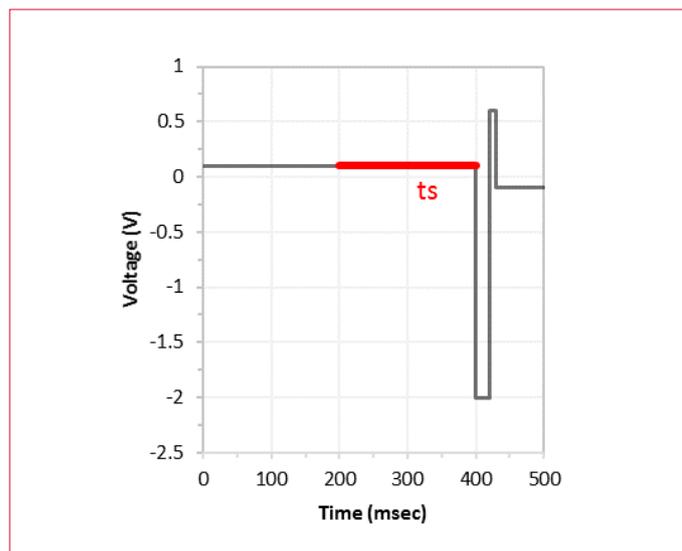


Figure 4: 4-step PAD potential waveform for the detection of monosaccharides and other carbohydrates. The duration of the sampling time (t_s) is highlighted in red.

Performance evaluation

Performance of both traditional and ‘fast and green’ methods were evaluated using standard measurements for repeatability ($n=6$), linearity and Limit of Detection (LOD). The LOD levels were calculated for each carbohydrate as the concentration that would show a signal-to-noise ratio of 3, using the response of a 10 μM standards mix chromatogram recorded under a range setting of 1 $\mu\text{A/V}$ and an ADF setting of 0.02 Hz. The ASTM noise level was calculated as the average from 30 subsequent 0.5 min segment peak-to-peak values.

Sample measurements

The soluble coffee sample preparation was done according to the standardized method¹⁰, which comprises (1) the extraction of ‘free’ carbohydrates with water, and (2) generating a hydrolyzed fraction that yields the total carbohydrate content as a mix of monosaccharides:

Sample preparation for analysis of free carbohydrates

Extract the free carbohydrates from approximately 300 mg of instant coffee with 70 mL water by shaking in a 100 mL volumetric flask until dissolution is complete. Dilute to the mark with water and pass 5-10 mL of the solution through a C18 cartridge. Discard the first few milliliters and pass the filtrate through a 0.2 μm membrane filter before injection.

Sample preparation for analysis of total carbohydrates

Hydrolyze the carbohydrates by heating approximately 300 mg of instant coffee dissolved in 70 mL 1.0 M HCl in a boiling water bath for about 2.5 h. Keep the level of the solution below that of the water and swirl every 30 min. Cool to room temperature with running tap water before diluting to the mark with water. Filter the solution through a folded filter paper and pass 3 mL of the solution through a disposable cartridge in the silver form to eliminate the chloride ions. Discard the first few milliliters and pass the filtrate through a 0.2 μm membrane filter before injection.

Standards

For quantification purpose, stock solutions of individual carbohydrates in water were mixed and diluted with water to prepare calibration standards in the range of 1-10 μM (10x higher galactose and arabinose concentrations). An additional calibrator standard mix (Table 5) was prepared with concentrations close to the levels as found in the hydrolyzed fraction (single point calibration).

Table 5

Calibrator concentrations for quantification of hydrolyzed fractions of coffee using traditional conditions as given in Table 3

Compound	μM	mg/L
Mannitol	2	0.4
Arabinose	500	75
Galactose	500	90
Glucose	500	90
Sucrose	50	17
Xylose	10	1.5
Mannose	100	18
Fructose	50	9



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Results, using traditional conditions

Figure 5 shows the chromatogram for a standard mix of the 8 carbohydrates in water that are mentioned in the ISO method for the analysis of instant coffee, plus xylose. All the peaks are well separated (resolution ≥ 1.5), including the potentially problematic peak pair of sucrose and glucose (Table 6). The total analysis time for each sample is 80 min, which includes the post-run step gradient for column clean-up and 15 min re-equilibration time.

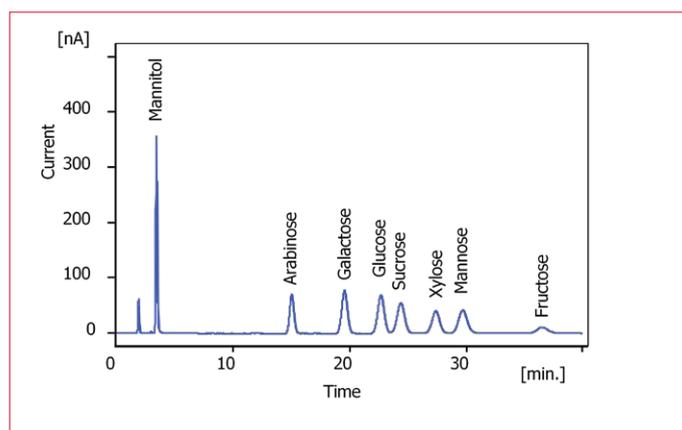


Figure 5: Chromatogram of a 20 μL injection of 10 μM standard mix of 8 carbohydrates in water, analyzed with ISO method 11292:1995 as detailed in Table 2 and Table 3. Peak parameters given in Table 6.

Table 6

Peak table of chromatogram given in Figure 5

Compound	Tr [min]	Area [nA.s]	Height [nA]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
Mannitol	3.5	3051	356	1.14	3301	11005	
Arabinose	15.1	1845	72	1.05	7526	25086	24.9
Galactose	19.6	2677	78	1.02	7018	23392	5.6
Glucose	22.7	2743	69	1.04	7303	24342	3.1
Sucrose	24.4	2503	55	1.01	6417	21391	1.5
Xylose	27.4	1847	40	0.98	7918	26392	2.5
Mannose	29.7	2136	42	1.05	7483	24944	1.8
Fructose	36.6	686	11	1.10	7299	24330	4.5

Repeatability

The ISO method states the advice to analyze a standard after every 4 injections to account for any changes in retention time or response. The repeatability of retention time was $>2\%$ RSD ($n=4$) when applying ambient temperature (data not shown). With the use of the ALEXYS system and application of thermostatted instead of ambient temperature, excellent repeatability

of chromatograms ($n=6$) is evident from the obtained RSD values: $<0.25\%$ for retention time and $<2\%$ for peak area for most of the carbohydrates (Table 7).

Table 7

Repeatability ($n=6$) using 20 μL of 10 μM carbohydrate standards mix in water, analyzed with ISO method 11292:1995 as detailed in Table 2 and Table 3

Compound	%RSD retention time	%RSD peak area
Mannitol	0.16	0.5
Arabinose	0.15	0.8
Galactose	0.14	0.4
Glucose	0.19	0.7
Sucrose	0.24	1.0
Xylose	0.20	1.1
Mannose	0.21	1.2
Fructose	0.20	3.8

Linearity

The peak area responds linear in the tested range of 1-10 μM (and 10-100 μM for galactose and arabinose) with a correlation coefficients of at least 0.998 (except 0.995 for fructose). As a double check, the carbohydrate concentrations in the calibrator solution for the hydrolyzed samples (Table 5) were quantified using the standards generated to evaluate linearity, and the outcome deviated less than 2% from the concentrations that they were supposed to represent, except for fructose (showing 10% deviation). Fructose seems to behave slightly different compared to the other tested carbohydrates, as it was also showing the highest RSD value when checking repeatability.

Detection limit

The Limit of Detection (LOD) was calculated for each carbohydrate as the concentration that would show a signal-to-noise ratio of 3, using the response and noise level in a 10 μM standards mix chromatogram. The noise level was 0.1 nA and the calculated LOD's were in the range of 0.01 - 0.40 μM or 0.002 - 0.07 mg/L (Table 8). Considering the ISO method prescription where 300 mg of sample is extracted with 100 mL water, the detection limit for the various carbohydrates is in the range of 0.1 - 2 mg/100 g product using the ALEXYS system and the traditional conditions.



Table 8

Calculated LOD ($S/N = 3$) for 20 μL injections using ISO method 11292:1995 as detailed in Table 2 and Table 3 (Range 1 $\mu\text{A/V}$; ADF: 0.02 Hz)

Compound	μM	mg/L
Mannitol	0.01	0.002
Arabinose	0.07	0.010
Galactose	0.06	0.010
Glucose	0.07	0.012
Sucrose	0.10	0.036
Xylose	0.13	0.019
Mannose	0.14	0.024
Fructose	0.41	0.074

Sample measurement: instant coffee

A sample of instant coffee was analyzed using the traditional conditions, and the concentrations of free and total carbohydrates (Table 9) were calculated based on the peak responses from the chromatograms shown in Figure 6 and Figure 7. The high level of total glucose ($>>2.46\%^{1,2}$) suggests that the tested instant coffee is not pure.

The main peaks in the chromatogram of the instant coffee sample were well separated and identified.

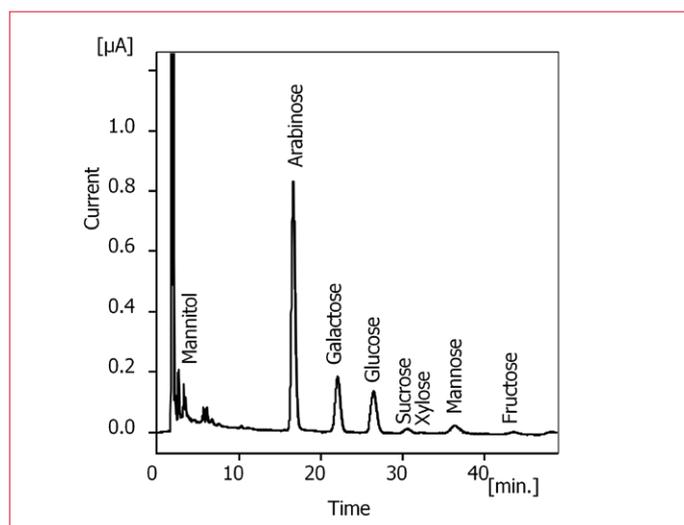


Figure 6: Chromatogram of the free carbohydrate fraction (non-hydrolyzed) of an instant coffee sample extracted and analyzed according to ISO method 11292:1995 with details as given in Table 2 and Table 3.

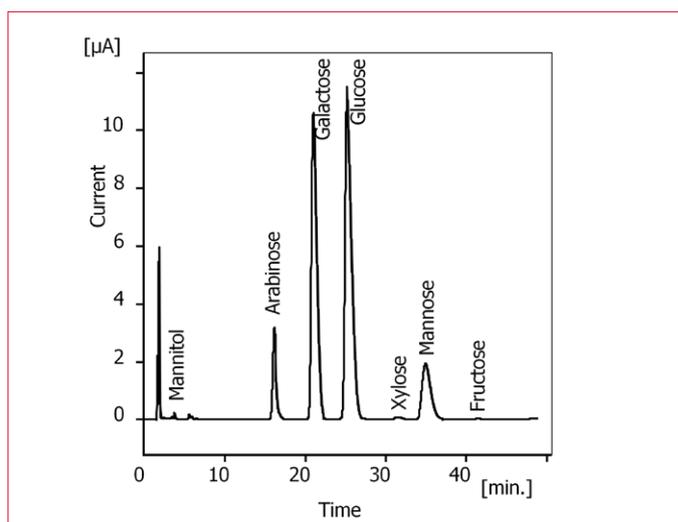


Figure 7: Chromatogram of the total carbohydrate fraction (hydrolyzed) of an instant coffee sample extracted and analyzed according to ISO method 11292:1995 with details as given in Table 2 and Table 3.

Table 8

Concentration of carbohydrates (g/100 g) in a sample of instant coffee, analyzed using traditional version of ISO method 11292:1995 as detailed in Table 2 and Table 3

Component	A- free	B- total
Mannitol	0.013	0.015
Arabinose	1.0	4
Galactose	0.22	13
Glucose	0.20	19
Sucrose	0.061	-
Xylose	0.006	0.15
Mannose	0.07	5
Fructose	0.09	0.19
Total	1.7	41

Results, using adapted 'fast and green' method

Using the dedicated ALEXYS Carbohydrates Analyzer and the narrow-bore column CarboPac PA210, the analysis of the standards mix resulted in the chromatogram shown in Figure 8. All the peaks are well separated (resolution ≥ 1.5) and the total analysis time for each sample is reduced to 35 min, which includes a 10 min post-run step gradient for column clean-up and 10 min re-equilibration time. The reduction in solvent use is almost 90% per analysis, compared to the traditional settings.



Carbohydrate Analysis in Instant Coffee According to Adapted ISO Method 11292:1995

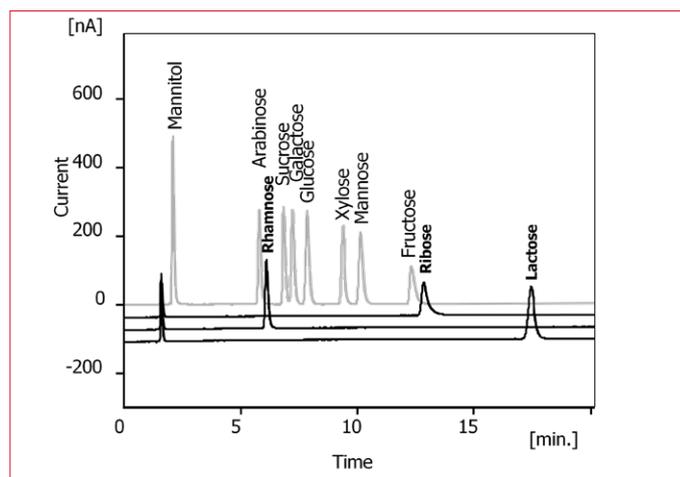
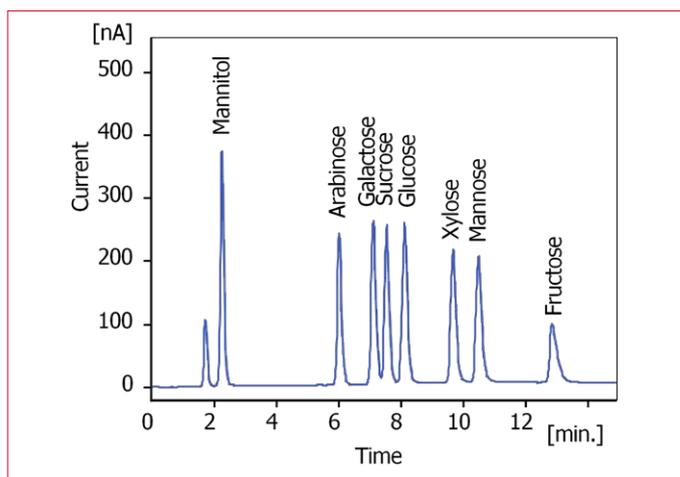


Figure 8: Chromatogram of a 5 µL injection of 10 µM standard mix of 8 carbohydrates in water, analyzed with adapted ‘fast and green’ version of ISO method 11292:1995 as detailed in Table 2 and Table 4. Peak parameters given in Table 10.

Figure 9: Chromatogram overlay of 5 µL injections of 10 µM standard mix of different carbohydrates in water, analyzed with adapted ‘fast and green’ version of ISO method 11292:1995 as detailed in Table 2 and Table 4. The light grey trace is the same standard mix of 8 carbohydrates as presented in Figure 8 for comparison purpose.

Table 10

Peak table of chromatogram given in Figure 8

Compound	Tr [min]	Area [nA.s]	Height [nA]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
Mannitol	2.3	3030	371	1.31	1578	8764	
Arabinose	6.0	2337	241	1.50	8889	49381	15.7
Galactose	7.1	2760	260	1.29	9140	50780	4.0
Sucrose	7.5	2671	253	1.38	11318	62880	1.5
Glucose	8.1	2970	255	1.33	10837	60203	1.9
Xylose	9.7	2531	211	1.39	14092	78288	4.9
Mannose	10.5	2604	200	1.61	15197	84428	2.4
Fructose	12.8	1573	92	2.65	12847	71374	6.0

Repeatability

The excellent repeatability of chromatograms using the ALEXYS system is evident from the obtained RSD values, which are <0.4% for retention time and <0.3% for peak area, except for fructose (Table 11).

Table 11

Repeatability (n=6) using 5 µL of 10 µM carbohydrate standards mix in water, analyzed with adapted ‘fast and green’ version of ISO method 11292:1995 as detailed in Table 2 and Table 4.

Compound	%RSD retention time	%RSD peak area
Mannitol	0.29	0.1
Arabinose	0.18	0.1
Galactose	0.18	0.2
Glucose	0.15	0.2
Sucrose	0.17	0.2
Xylose	0.15	0.2
Mannose	0.12	0.3
Fructose	0.37	1.8

According to the ISO method, rhamnose and arabinose are a potentially problematic pair to separate, and the advice is not to add rhamnose in case they co-elute. Figure 9 shows a chromatogram overlay that indicates that rhamnose, ribose and lactose (relevant for other applications) are not co-eluting with the other peaks.



Linearity

The peak area responds linear in the tested range of 0.2 - 10 μM and 10 - 250 μM with a correlation coefficients of 0.999 for all carbohydrates, except sucrose. Concentrations higher than 250 μM result in increased peaks asymmetry and relatively lower area response. For sucrose, the response stops being linear above 50 μM (=17 mg/L).

Detection limit

With a noise level of 0.2 nA, the calculated LOD's were better than 0.03 μM for most tested carbohydrates, except for fructose (Table 12). Comparing the LOD values with those reported using the already highly sensitive traditional conditions, about 4x lower LOD levels are found for the adapted 'fast and green' method. Note that the injection volume was also a factor 4 lower (20 vs 5 μL), giving a total on-column detection sensitivity improvement of a factor around 16 (with some deviations among components).

Table 12

Calculated LOD (S/N = 3) for 5 μL injections using adapted 'fast and green' version of ISO method 11292:1995 as detailed in Table 2 and Table 4 (Range 1 $\mu\text{A/V}$; ADF: 0.02 Hz)

Compound	nM	$\mu\text{g/L}$
Mannitol	17	3
Arabinose	25	4
Galactose	24	4
Glucose	24	4
Sucrose	24	8
Xylose	29	4
Mannose	31	6
Fructose	61	11

Sample measurement: instant coffee

Both hydrolyzed and non-hydrolyzed samples of the same instant coffee were re-analyzed using the adapted 'fast and green' method, resulting in the chromatograms shown in Figure 10, Figure 11 and the carbohydrate composition given in Table 13.

The hydrolyzed sample had to be diluted 10x with water before analysis, as undiluted sample showed tailing peaks with an asymmetry factor of 3, indicating column saturation (chromatogram not shown). The levels of the most abundant carbohydrates in the hydrolyzed fraction were quantified to reach up to 3 mM, which far exceeded the linear response range for the adapted method. An additional sample dilution step is therefore advised for the hydrolyzed fraction when using the CarboPac PA210 narrow-bore column and adapted conditions.

When comparing the sample composition tables and chromatograms as obtained with the traditional and adapted 'fast and green' conditions, it can be observed that the data are comparable among methods. The small differences in quantification data may have developed during the storage of the samples before re-analysis.



Carbohydrate Analysis in Instant Coffee According to Adapted ISO Method 11292:1995

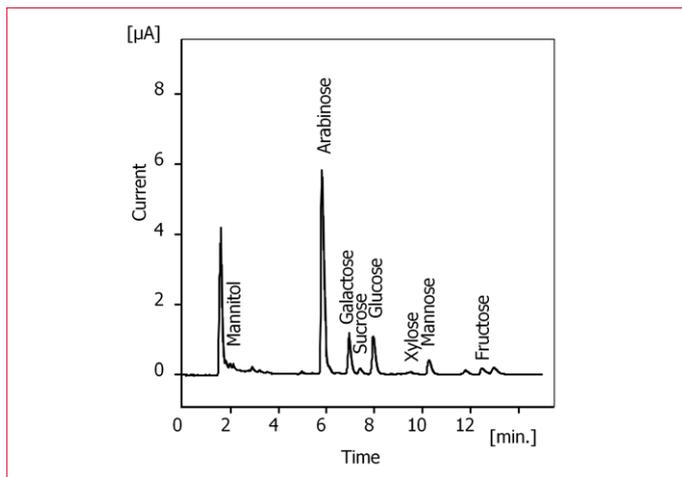


Figure 10: Chromatogram of the free carbohydrate fraction (non-hydrolyzed) of an instant coffee sample extracted according to ISO method 11292:1995 and analyzed with column type CarboPac PA210 (adapted 'fast and green' conditions) as detailed in Table 2 and Table 4.

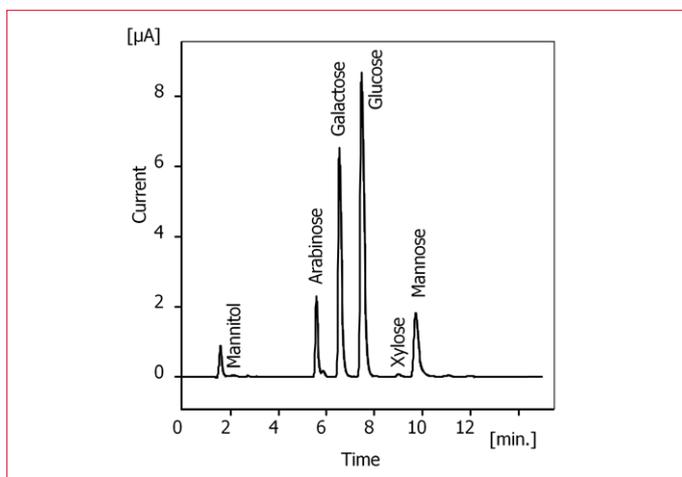


Figure 11: Chromatogram of the total carbohydrate fraction (hydrolyzed) of an instant coffee sample extracted according to ISO method 11292:1995 and analyzed with column type CarboPac PA210 (adapted 'fast and green' conditions) as detailed in Table 2 and Table 4.

Table 13

Concentration of carbohydrates (g/100 g) in a sample of instant coffee, analyzed using adapted 'fast and green' version of ISO method 11292:1995 as given in Table 2 and Table 4.

Component	A- free	B - total
Mannitol	0.012	0.04
Arabinose	1.1	4
Galactose	0.24	13
Glucose	0.23	19
Sucrose	0.07	-
Xylose	0.020	0.16
Mannose	0.12	5
Fructose	0.09	-
Total	1.9	42

Sample measurement: breakfast cereal

To show that this detection method is more generally applicable, a chromatogram was made from an extract of breakfast cereal (Figure 12). According to the labelling information the sugar content was 15 g/100 g product. The main 'free' carbohydrate was found to be sucrose, at the concentration stated on the label. An alternative sweetener to sucrose is high glucose-fructose corn syrup, but in the tested product the main sweetener is clearly sucrose.

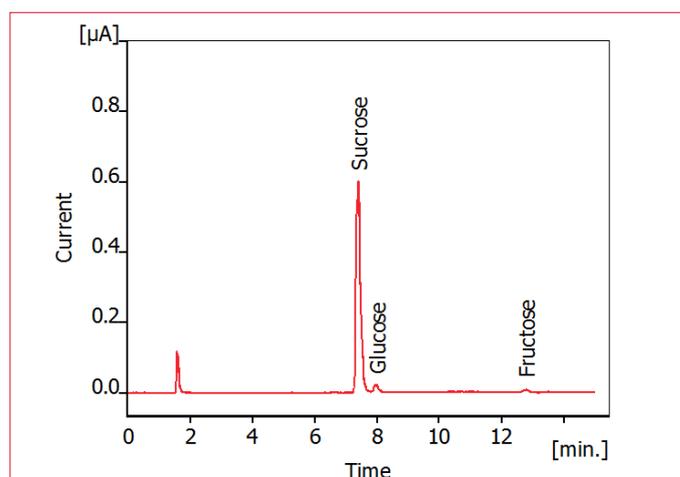


Figure 12: Chromatogram of an extract of 'Special flakes' breakfast cereal from Albert Heijn. About 10 g of product was extracted with 200 mL water for 10 min, centrifuged, filtered through 0.2 µm filter and 1000x diluted with water before injection (5 µL). Chromatogram recorded with conditions as detailed in Table 2 and Table 4. Sucrose, glucose and fructose contents were quantified as respectively 15.4, 0.3 and 0.2 g/100g product.



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Conclusion

The DECADE Elite detector and SenCell flow cell can be used to run the ISO method 11292:19956 for the analysis of carbohydrates in instant coffee. Using the dedicated ALEXYS Carbohydrates Analyzer, a narrow-bore column with smaller particle size and an adapted lower flow rate results in a significant increase in sensitivity, 50% decrease in analysis time and a 90% reduction of solvents-use compared to the traditional conditions.



Carbohydrate Analysis in Instant Coffee According to Adapted ISO Method 11292:1995

Ordering information

'ALEXYS Carbohydrate Analyzer' with in-line degassers, 2 pumps, high pressure solvent selector, pulse dampers, and autosampler. Included detector and flow cell are listed here additionally for convenience.

175.0035	DECADE Elite SCC
116.4321	SenCell 2 mm Au HyREF

The CarboPac columns used in this application are manufactured and sold by Thermo Scientific:

057179*	CarboPac PA1 guard column; 50 x 4.0 mm ID
057178*	CarboPac PA1 analytical column; 250 x 4.0 mm ID
088956*	CarboPac PA210-Fast-4 μ m guard column; 30 x 2.0 mm ID
088954*	CarboPac PA210-Fast-4 μ m analytical column; 150 x 2.0 mm ID

* The columns can be ordered at Thermo Scientific

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. 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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