Carbohydrate in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method

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Application Note 280

Introduction

Brewed coffee has emerged as one of the most consumed beverages in the world.[1] In addition, green coffee (unroasted beans) is one of the most traded agricultural commodities in the world.[2] Coffee is grown in over 70 countries, primarily in Latin America, Southeast Asia, and Africa. As of 2010, coffee production was approximately 134 million bags (each bag containing 60 kg), with Brazil and Columbia contributing to nearly 40% of the total.[3] The top coffee importing countries are the United States, Germany, Japan, France, Italy, Spain, Canada, and the United Kingdom.

In recent years, there has been growing interest in the physiology and biochemistry of green coffee beans and their role in the final roasted coffee quality. Drinks made from green coffee beans have been introduced in the market.[4] Green and roasted coffee are tested at several stages of its production and processing. Tests conducted on green coffee beans include tests for bean density, brightness, acidity, pH, moisture content, and total soluble solids. Tests performed on roasted coffee include tests for caffeine, chlorogenic acids, lipids, carbohydrates, total polyphenols, total proteins, and aflatoxins.

Coffee carbohydrates constitute the major part (at least 50% of the dry weight) of raw coffee beans. The carbohydrates in coffee contribute to the flavor of the beverage as they undergo complex changes (react with amino acids, i.e., the Maillard reaction) during the roasting process. They act as aroma binders, foam stabilizers, and also impart viscosity to the coffee beverage. Carbohydrates are also good tracers for assessing the authenticity of soluble (instant) coffee.[5]

Currently, the Association of Analytical Chemists (AOAC) Official Method 995.13[6]—which is based on high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD)—is used for determining the free and total carbohydrates in instant coffee. This method is also used by the British Standards Institution for testing coffee and coffee products.[7]

This study first tested the AOAC official method 995.13 on a Thermo Scientific Dionex ICS-3000 system. Carbohydrates in extracts from instant coffee and green coffee beans were separated on a Thermo Scientific Dionex CarboPac PA1 column, and measured by electrochemical detection with disposable Au on polytetrafluoroethylene (PTFE) working electrodes. A few proposed modifications of the official method achieved separation of two pairs of sugars, which are otherwise difficult to resolve.

A fast method using the Dionex CarboPac™ SA10 column (with electrolytically generated eluent) was then tested for determining the common coffee carbohydrates. This column has been shown to achieve fast, high resolution separation of mono- and disaccharides commonly found in food samples.[8] The Dionex CarboPac SA10 column is composed of a wide-pore macroporous substrate coated with a strong anion-exchange latex of nano-beads. The combination of the high capacity provided by the substrate and the new internal chemistry of the nano-bead functionality delivers high resolution and short analysis time for the common sugars of interest in food and beverages.

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The testing here demonstrates the linearity, precision, and recovery of common coffee carbohydrates in samples ranging from instant coffee to green coffee beans. It compares the two methods and discusses their respective advantages and disadvantages. Note that the disposable electrodes used in these methods provide short equilibration times and greater electrode-to-electrode reproducibility compared to conventional electrodes. Additionally, compared to other disposable Au electrodes, the Au on PTFE electrodes have longer lifetimes and can operate at higher hydroxide concentrations.

Both the described methods provide good sensitivity, consistent response, and can be routinely used for sugar analysis in coffee applications. The fast method is recommended when rapid separation is desired, keeping in mind that two pairs of sugars (namely, rhamnose–galactose and fructose–ribose) are not resolved. In applications where all 11 common coffee carbohydrates need to be resolved, the AOAC official method 995.13 (with minor modifications) is recommended.

Equipment

Thermo Scientific Dionex ICS-5000 or ICS-3000 Ion Chromatography system including:

- Gradient or Isocratic Pump, with the vacuum degas option installed
- DC Detector/Chromatography Module
- Injection loop, 10 µL (for method based on AOAC official method 995.13)/injection valve with an internal 0.4 µL injection loop (P/N 074699) (for fast method)
- Electrochemical Detector (P/N 079830)
- Carbohydrate PTFE Disposable Au Working Electrodes (P/N 066480, package of 6)
- Ag/AgCl Reference Electrode (P/N 061879) PTFE gaskets, 2 mil (P/N 060141) or 15 mil (P/N 057364)
- Postcolumn Delivery Set with 125 µL reaction coil (P/N 53640)
- Thermo Scientific Dionex On Guard II Ag/H (P/N 057410), Dionex On Guard™ II RP (P/N 057083)

AS Autosampler

Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software

Eluent Organizer, including 2 L plastic bottles and pressure regulator

Polypropylene injection vials with caps (0.3 mL vial kit, P/N 055428)

Thermo Scientific Nalgene 125 mL HDPE narrow-mouth bottles (P/N 16057-062)

Nalgene™ 250 mL HDPE narrow-mouth bottles (P/N 16057-109)

Nalgene 250 mL 0.2 µm nylon filter units (P/N 28199-371)

Nalgene 1000 mL 0.2 µm nylon filter units (P/N 28198-514)

Reagents and Standards

Reagents

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better, filtered through a 0.2 µm filter immediately before use.

Standards

Fucose (Sigma-Aldrich®, St. Louis, USA, Cat. No. F2252)

Galactose (Sigma-Aldrich Cat. No. G-0625)

Mannose (Sigma-Aldrich Cat. No. M-6020)

Fructose (J.T. Baker Cat. No. M556-05)

Xylose (Sigma-Aldrich Cat. No. X107-5)

Sucrose (Sigma-Aldrich Cat. No. S-9378)

Glucose, monohydrate (J.T.Baker®, Mansfield, USA, Cat. No. 1910-01)

Arabinose (Sigma-Aldrich Cat. No. A-3131)

Ribose (Sigma-Aldrich Cat. No. R7500)

Rhamnose (Sigma-Aldrich Cat. No. 3875)

Mannitol (Sigma-Aldrich Cat. No. M-9546)

Conditions

Modified AOAC Official Method 995.13

Columns:	Dionex CarboPac PA1 Analytical, 4 × 250 mm (P/N 035391) Dionex CarboPac PA1 Guard, 4 × 50 mm (P/N 43096)
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (full loop)
Column Temp.:	25 °C
Detector Temp.:	30 °C
Back Pressure:	2400 psi
Eluent:	DI water from 0–50 min, 300 mM NaOH from 50–65 min DI water from 65–80 min (re-equilibration)
Postcolumn Base:	300 mM NaOH
Flow Rate for Postcolumn Base:	0.6 mL/min

Fast Method	
Columns:	Dionex CarboPac SA10 Analytical, 4 × 250 mm (P/N 074641)
	Dionex CarboPac SA10 Guard, 4 × 50 mm (P/N 074902)
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL (full loop)
Column Temp.:	45 °C
Detector Temp.:	30 °C
Back Pressure:	2500 psi
Eluent:	1 mM KOH
Eluent Source:	EGC II KOH with CR-ATC

Both	
Detection:	PAD
Background:	30–70 nC
Noise:	30–60 pC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrodes
Reference Electrode:	Ag/AgCl mode

Carbohydrate Waveform		
Time (s)	Potential (V)	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	–2.0	
0.42	–2.0	
0.43	+0.6	
0.44	–0.1	
0.50	–0.1	

Preparation of Solutions and Reagents

Eluent Solutions

Modified AOAC Official Method 995.13

Sodium Hydroxide, 1 M

It is essential to use high-quality water of high resistivity (18 MΩ-cm) containing as little dissolved carbon dioxide as possible. Biological contamination must be absent. Obtain source water using a water purification system consisting of filters manufactured without electrochemically active substances (e.g., glycerol). Filter prior to use through 0.2 µm porosity nylon under vacuum to remove particulates and reduce dissolved air. It is important to minimize contamination by carbonate, a divalent anion at high pH that binds strongly to the column, causing a loss of chromatographic resolution and efficiency. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and must not be used. A 50% (w/w) sodium hydroxide is much lower in carbonate and is the recommended source for sodium hydroxide.

Dilute 51.5 mL of a 50% (w/w) sodium hydroxide into 948.5 mL of thoroughly degassed water to yield a 1 M sodium hydroxide solution. Keep the eluents blanketed under 34–55 kPa (5–8 psi) of nitrogen at all times to reduce diffusion of atmospheric carbon dioxide and minimize microbial contamination.[9]

Fast Method

Potassium Hydroxide, 1 mM

Generate the potassium hydroxide (KOH) eluent online by pumping high-quality degassed deionized (DI) water through the EGC II KOH cartridge. Dionex Chromeleon™ software tracks the amount of KOH used and calculates the remaining lifetime. Although eluents can be prepared manually, if needed, we strongly recommend running this application with eluents prepared by an eluent generator and do not recommend using manually prepared eluents. Consistent preparation of a 1 mM hydroxide eluent or a 10 mM hydroxide eluent (if proportioning is used) is difficult due to variable carbonate contamination. The impact of carbonate contamination is significant when using low-concentration hydroxide eluents. If eluents must be prepared manually, use NaOH rather than KOH and prepare according to the general instructions for hydroxide eluents in Technical Note 71.[9] For this application, electrolytic eluent generation delivers superior performance and is used for all the fast method data in this study. Performance for this application with manually prepared eluents is not guaranteed.

Stock Standard Solution

Dissolve solid standards in DI water to prepare a 200 mg/mL stock solution for each of the 11 carbohydrates. Maintain the stock solution at –20 °C until needed.

Mixed Carbohydrate Working Standard Solutions

Prepare the mixed carbohydrate working standards by diluting the stock solutions as required. Store working standards at 4 °C. Make all dilutions gravimetrically to ensure high accuracy.

Sample Preparation

Instant Coffee

Use soluble coffee without grinding or homogenization.

Free Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 70 mL of DI water and shake the flask until dissolution is complete. Dilute the solution to volume with DI water. Filter 5–10 mL of solution through a C18 cartridge. Discard the first 1 mL. Pass the filtrate through a 0.2 µm membrane filter prior to injection.

Total Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 50 mL of 1.0 M HCl and swirl the flask. Place the flask in a boiling water bath for 2.5 h (note: always keep the level of solution in the flask below that of water in the bath). Swirl the flask by hand every 30 min, then cool the flask to room temperature under tap water. Dilute the solution to 100 mL with DI water and filter through folded filter paper. Pass the filtrate (3–5 mL) through a disposable cation-exchange cartridge in the Ag form and a disposable cation-exchange cartridge in the hydronium form to eliminate the Cl- anion, neutralize the solution, and trap any Ag that might break through from the first cartridge (thus protecting the column and the working electrode). Discard the first 1 mL. Filter the remaining solution through a 0.2 µm membrane filter prior to LC injection.

Green Coffee

Weigh 1g of green coffee beans and mix with 10 mL of DI water. Sonicate this solution for 15 min. Pass the supernatant through a 0.2 µm filter, and dilute further with water if needed. Use the sample within 24 h. (Extractions from ground green coffee beans, obtained using this procedure, gave similar results.)

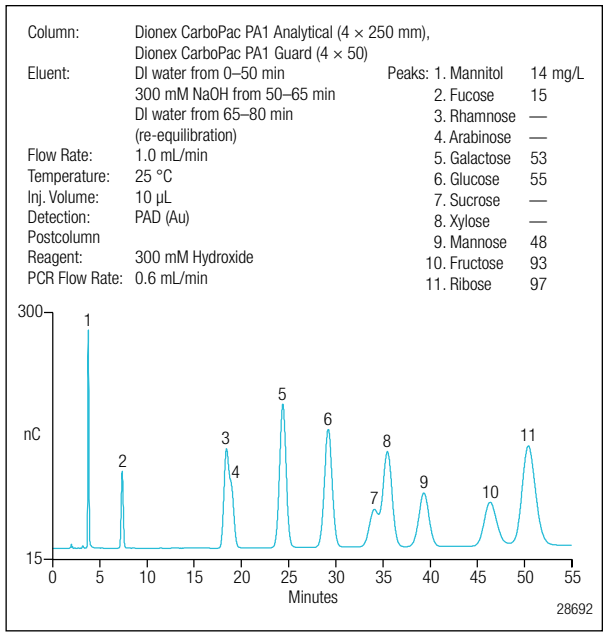

Figure 1. Chromatogram of mixed coffee carbohydrate standards, using the AOAC Official Method 995.13.

Results and Discussion

Modified AOAC Method 995.13

Separation

Figure 1 shows the separation of the carbohydrates present in a mix of standards. All the carbohydrates elute in 55 min with a total run time of 80 min (including column wash and equilibration steps). Note that the later eluting peaks are broader relative to the early eluting peaks, as expected from an isocratic method. Carbohydrate concentrations are calculated from the ratio of the peak response in the sample solution to that in the standard solution, and the concentration of the carbohydrate in the standard solution.[6]

Note that rhamnose–arabinose (Figure 1, peaks 3 and 4) and sucrose–xylose (Figure 1, peaks 6 and 7) are not completely resolved. The resolution issue for these sugars has been addressed in the official method. If the rhamnose–arabinose peaks are not resolved, the method suggests excluding rhamnose from the mixed standard solution. For the other pair, the AOAC method recommends 2–3 injections of the specified carbohydrates standard solution or an increase of the re-equilibrium time in order to achieve a good separation of glucose, sucrose, and xylose.

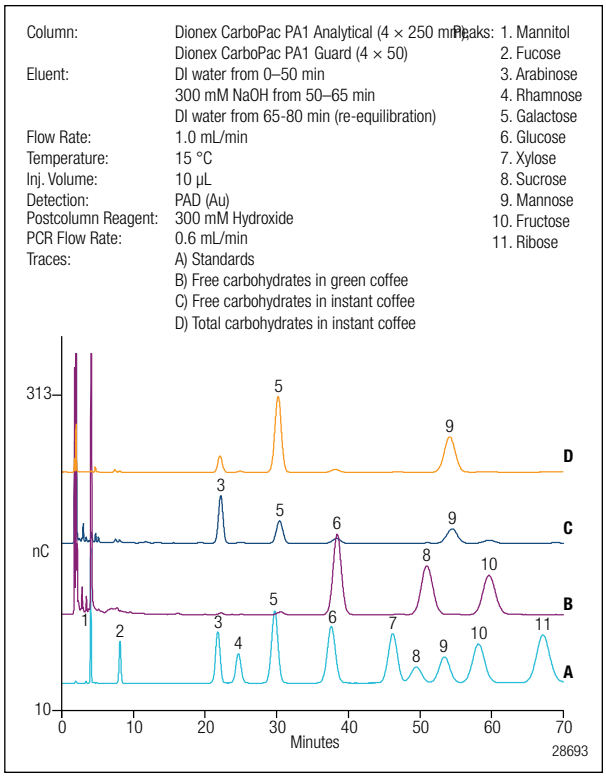

Figure 2. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrate in extract of green coffee beans (B), free carbohydrates in instant coffee (C), and total carbohydrates in instant coffee (D); using the modified AOAC official method 995.13 (T = 15 °C).

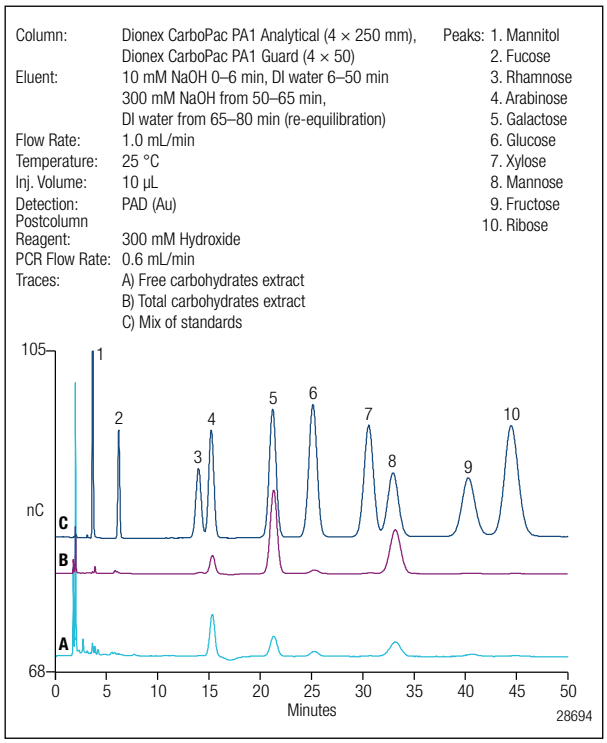

Figure 3. Chromatograms of free carbohydrates extract from instant coffee (A), total carbohydrates extract from instant coffee (B), and mixed carbohydrate standards (C); using the modified AOAC Official Method 995.13 (10 mM hydroxide for 6 min, and sucrose not included in mix of standards).

As an alternative, the column temperature may be lowered to 15 °C (referred to here as modification 1) to achieve separation of all 11 carbohydrates in the mixed standard solution. Note that the run time is increased (Figure 2), and all the sugars now elute in 70 min. In addition, arabinose elutes before rhamnose, and xylose before sucrose, compared to the elution order at 25 °C, suggesting that the interaction of these sugars with the stationary phase at low temperature (15 °C) is different than at 25 °C.

Figure 2 also shows representative chromatograms of extracts from green coffee beans (B), and extracts of free carbohydrates (C), and total carbohydrates (D) from instant coffee. The primary carbohydrates present in extracts from green coffee beans (B) were mannitol, glucose, sucrose, and fructose. In comparison, in the instant coffee sample tested, the major free carbohydrates were arabinose, galactose, and mannose, and the minor sugars were glucose and fructose. In the extract for total carbohydrates from instant coffee, the sugars were mainly arabinose, galactose, and mannose in the sample tested. This gives an indication of how the sugars present in green coffee have changed during roasting and other heat treatment processes (e.g., extraction, spray drying).

The first set of co-eluting peaks, rhamnose and arabinose, was also resolved by modifying the conditions of the mobile phase: by eluting with 10 mM hydroxide for the first 6 min, then switching to DI water (i.e., using a step change; Figures 3 and 4). Note that only the mobile phase was modified; all other chromatography conditions were the same as in AOAC Method 995.13. Because extracts from instant coffee typically do not contain sucrose (Figure 2, C and D), sucrose can be eliminated from the mix of standards that will be used when analyzing samples from instant coffee (Figure 3 C).

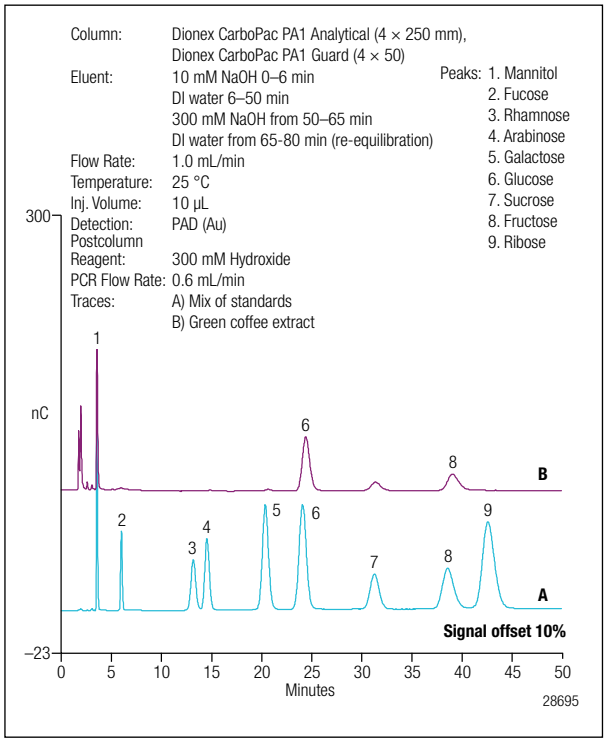

Figure 4. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrates extract from green coffee beans (B); using the modified AOAC official method 995.13 (10 mM hydroxide for 6 min, and xylose and mannose not included in mix of standards).

For determining sugars in instant coffee, the suggested changes (referred to as modification 2) to the official method include: (a) elution with 10 mM hydroxide for the first 6 min, then switch to DI water, and (b) exclude sucrose from the mix of standards. Note that flavored instant coffees are more likely to contain sucrose, so modification 1 will be more appropriate.

Typically, green coffee samples do not contain xylose and mannose (Figure 2 B). When analyzing green coffee samples, exclude xylose and mannose from the mix of standards (Figure 4 A). Similar to the original AOAC official method 995.13, all the sugars elute in 50 min. The suggested method changes (referred to as modification 3) for analyzing extracts from green coffee include: (a) elution with 10 mM base for the first 6 min, followed by DI water, and (b) exclusion of xylose and mannose from the mix of standards, while maintaining all other chromatography conditions in AOAC Method 995.13.

In summary, it may be difficult to achieve baseline resolution of some of the peaks using the official method. Three modifications have been proposed: (1) a lower column temperature to resolve the 11 common coffee carbohydrates in all samples (the caveat being increased run time); (2) for instant coffee samples, exclusion of sucrose in the mix of standard, and eluting with a step gradient with 10 mM base for the first 6 min, and water thereafter; and (3) for green coffee samples, avoiding xylose and mannose in the mix of standards and eluting with a step gradient with 10 mM base for the initial 6 min, followed with water.

Precision

The peak area and retention time (RT) precisions (RSDs) for six replicate injections of a mixture of sugar standards for the AOAC official method 995.13 with modification 1 (i.e., with column temperature 15 °C) are listed in Table 1. The retention time precisions ranged from 0.2–0.68% and the average peak area precision was 4.7%. The precisions for the official method with proposed modifications 2 and 3 are presented in Table 2. In these configurations, the RT precisions were in the range 0.19–1.84%, and the peak area precisions were 1.05–4.96%.

Table 1. Precisions for Coffee Carbohydrates Using Modified[a] AOAC Official Method 995.13

Analyte	Concentration Used for Precision Injections (mg/L)	RT Precision RSD	Peak Area Precision RSD
Mannitol	15	0.20	4.49
Fucose	15	0.24	4.69
Rhamnose	35	0.30	4.66
Arabinose	40	0.40	4.83
Galactose	50	0.42	4.72
Glucose	55	0.46	4.82
Sucrose	45	0.68	5.15
Xylose	55	0.42	4.88
Mannose	45	0.44	4.87
Fructose	90	0.47	4.45
Ribose	90	0.48	4.66

[a]Column temperature = 15 °C.

Table 2. Precisions for Coffee Carbohydrates Using Modified[a, b] AOAC Official Method 995.13

Analyte	Concentration Used for Precision Injections (mg/L)	RT Precision RSD	Peak Area Precision RSD
Mannitol	15	0.19	1.79
Fucose	15	0.75	2.09
Rhamnose	35	1.84	3.44
Arabinose	40	1.05	2.57
Galactose	50	0.71	1.05
Glucose	55	0.80	2.05
Sucrose[b]	45	0.31	1.79
Xylose[b]	55	0.33	4.96
Mannose[b]	45	0.50	4.20
Fructose	90	0.72	1.17
Ribose	90	0.66	1.27

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13.

[b]Exclusion of sucrose from the mix of standards when analyzing instant coffee samples, and xylose and mannose when analyzing green coffee samples.

Accuracy

The accuracy of the method was evaluated by measuring recoveries in spiked coffee samples (Tables 3–5). Samples were spiked with analytes at a level that was 50–100% of the amount determined in the original sample. Recoveries were calculated from the difference in response between the spiked and unspiked samples. The average recovery for the sugars (using modification 2 with the official method) in the instant coffee samples ranged from 70–116%. For green coffee samples (using the official method with modification 3), the average recovery ranged from 73–95%. The between-day recovery precision for the coffee sugars in the spiked samples averaged 12% over three days. These recovery values indicate that the modified methods are accurate for analyzing coffee carbohydrates.

Table 3. Carbohydrate Recoveries in an Extract of Total Carbohydrates from Instant Coffee (n = 3 days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	97.3	105	107.5	9.3
Fucose	99.5	82.0	82.1	9.1
Rhamnose	106	101	71.1	14.0
Arabinose	91.5	186	88.8	15.0
Galactose	102	817	114.4	15.4
Glucose	92.8	113	84.6	9.9
Xylose	129	106	76.2	14.2
Mannose	200	819	59.8	18.0
Fructose	103	89.7	87.1	12.4
Ribose	98.4	79.1	80.3	5.8

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13.

[b]Exclusion of sucrose from the mix of standards.

Table 4. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Instant Coffee (n = 3 Days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	39.5	47.7	116.1	18.1
Fucose	41.4	29.3	71.3	11.1
Rhamnose	45.1	40.5	89.5	6.8
Arabinose	36.6	61.0	77.9	20.6
Galactose	45.2	56.3	83.5	15.5
Glucose	42.2	43.5	92.4	9.6
Xylose	41.2	43.0	104.4	7.9
Mannose	41.2	58.7	83.3	19.2
Fructose	39.2	44.2	94.8	11.0
Ribose	49.9	43.2	85.1	17.3

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13.

[b]Exclusion of sucrose from the mix of standards.

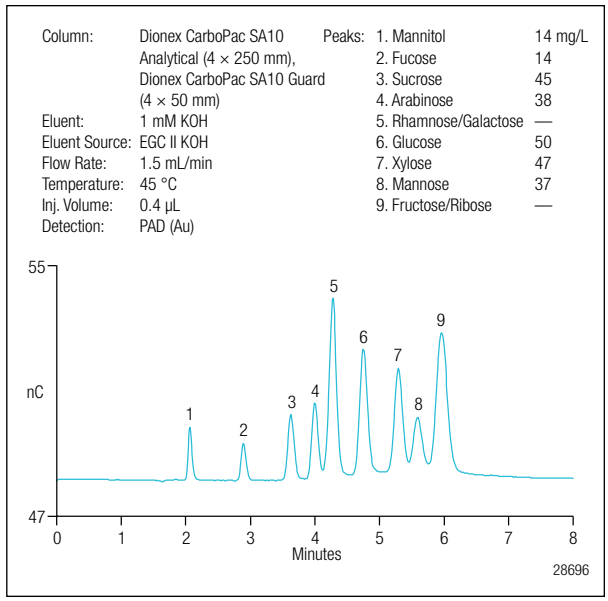

Figure 5. Chromatogram of mixed coffee carbohydrate standards using the fast method.

Table 5. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Green Coffee (n = 3 Days) Using Modified[a,b] AOAC Official Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	42.9	41.3	76.6	6.2
Fucose	95.2	90.7	95.4	12.8
Rhamnose	111	83.5	75.6	8.6
Arabinose	97.7	81.1	83.0	2.5
Galactose	104	97.8	92.2	7.6
Glucose	101	129	88.7	23.1
Sucrose	88.4	233	69.5	32.1
Fructose	106	140	73.3	14.9
Ribose	109	90.7	83.3	5.6

[a]10 mM NaOH in the eluent in the first 6 min, followed by water; all other chromatography conditions same as AOAC Method 995.13.

[b]Exclusion of xylose and mannose from the mix of standards.

Fast Method

Separation

The mixture of coffee carbohydrate standards separated on a Dionex CarboPac SA10 column is shown in Figure 5. All the sugars elute within 8 min. This is significantly faster than the other methods used for analyzing common sugars in food and beverages. However, note that two pairs of sugars co-elute under the current configuration. These are rhamnose–galactose and fructose–ribose (Figure 5, peaks 5 and 9).

Figure 6 shows representative chromatograms for extracts from green coffee beans and extracts from instant coffee. The green coffee sample has mannitol, sucrose, glucose, and fructose (assignment is based on the knowledge that green coffee samples have minimal or no ribose), whereas the instant coffee samples have arabinose, galactose (assignment is based on the knowledge that instant coffee samples have minimal or no rhamnose), glucose, and mannose.

Figure 7 shows extracts from three kinds of green coffee beans, all of which contain mannitol, sucrose, glucose, and fructose.

Peaks (mg/mL)	Standard (A)	Instant Free (D)	Instant Total (C)	Green (B)
1. Mannitol	—	0.017	0.006	0.054
2. Fucose	0.040	0.001	—	—
3. Sucrose	0.046	—	—	0.315
4. Arabinose	0.042	0.041	0.126	—
5. Rhamnose and Galactose	—	—	—	—
6. Glucose	0.047	—	0.032	0.059
7. Xylose	0.045	—	—	—
8. Mannose	0.041	0.028	0.603	0.001
9. Fructose and Ribose	—	—	—	—

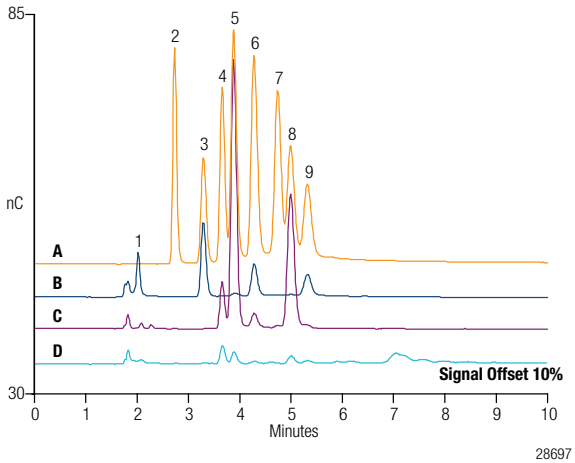

Figure 6. Chromatograms of a mixture of coffee carbohydrate standards (A), free carbohydrates from green coffee beans (B), free carbohydrates (C), and total carbohydrates (D) extract from instant coffee; using the fast method. Chromatographic conditions same as listed in Figure 5.

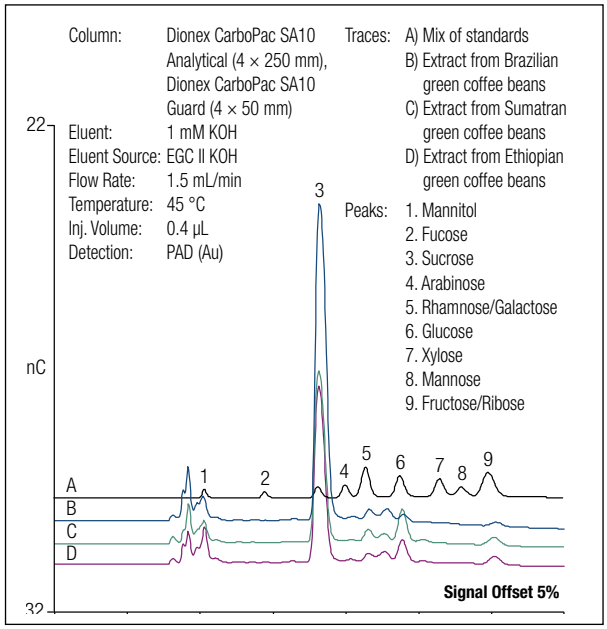

Figure 7. Chromatograms of a mix of coffee carbohydrate standards (A), free carbohydrates in green coffee beans (ground green coffee beans dissolved in water), Brazilian beans (B), Sumatran beans (C), and Ethiopian beans (D).

Table 6. Linear Range and Precisions for Coffee Carbohydrates Using the Fast Method

Analyte	Range (mg/mL)	Coeff. Of Determ. (r^2)	Concentration Used for Precision (mg/L)	RT (min)	Retention Time Precision (RSD)	Peak Area (nC*min)	Peak Area Precision (RSD)
Mannitol	0.005–0.2	0.9992	15	2.06	0.21	0.16	1.35
Fucose	0.006–0.2	0.9998	15	2.89	0.15	0.13	3.25
Sucrose	0.01–0.8	0.9959	45	3.61	0.19	0.29	3.28
Arabinose	0.018–0.3	0.9997	40	3.99	0.13	0.33	4.24
Glucose	0.013–0.9	0.9963	55	4.74	0.20	0.75	3.64
Xylose	0.01–0.74	0.9967	55	5.28	0.18	0.71	4.64
Mannose	0.006–0.7	0.9942	45	5.58	0.15	0.86	3.85

Table 7. Carbohydrate Recoveries in an Extract of Total Carbohydrates from Instant Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	105	109.9	105.9	12.6
Fucose	93.7	100.7	107.7	6.9
Sucrose	85.9	109.5	127.7	5.1
Arabinose	94.4	368.3	101.3	7.1
Glucose	97.8	161.5	114.5	7.8
Xylose	91.5	109.8	120.5	11.5
Mannose	620	1658	74.2	7.9

Table 8. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Instant Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	41.1	39.0	85.4	1.4
Fucose	39.7	39.9	81.4	13.1
Sucrose	38.1	33.6	102.4	14.2
Arabinose	49.9	84.1	98.0	9.8
Glucose	42.4	33.0	78.9	24.9
Xylose	46.4	37.1	80.0	16.0
Mannose	38.7	69.0	131.9	9.1

Linearity and Precision

The linearity of the method was determined by injecting calibration standards in triplicate, covering the expected range of the sugars of interest in the samples (ranging from 5–900 mg/L) (Table 6). The coefficients of determination obtained from the calibration curves were between 0.9942–0.9998, using least squares regression fits.

Table 9. Carbohydrate Recoveries in an Extract of Free Carbohydrates from Green Coffee (n = 3 Days) Using the Fast Method

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	96.1	139	81.3	8.3
Fucose	96.8	83.2	86.5	8.6
Sucrose	163	385	73.9	7.3
Arabinose	92.2	87.9	97.4	16.9
Glucose	111	136	83.5	9.5
Xylose	109	80.7	75.3	14.4
Mannose	103	78.8	78.0	17.8

The peak area and RSDs were determined for seven replicate injections of a mixture of sugar standards. The concentrations of the carbohydrates in the mix of standards used for precision are listed in Table 6. The RSDs ranged from 0.13–0.21%. The peak area precisions were in the range 1.35–4.65%. These precisions suggest that the method based on separation with the Dionex CarboPac SA10 column can be used for the determination of coffee carbohydrates.

The two methods (the AOAC official method and the fast method) gave similar intra-day and between-day (over three days) RT and peak area precisions (data not shown).

Accuracy

The accuracy of the method was evaluated by measuring recoveries in spiked coffee samples (Tables 7–9). Samples were spiked with analytes at a level that was 50–100% of the amount determined in the original sample. Recoveries were calculated from the difference in response between the spiked and unspiked samples. Intra-day carbohydrate concentration RSDs for coffee extracts were in the range of 0.2–1.8%. The average recovery for the sugars in the three types of coffee samples (free and total carbohydrates extract from instant coffee and free carbohydrate extract from green coffee) ranged from 74–127%. The between-day recovery precision for the coffee sugars in the spiked samples ranged from 1.5–24% (average 10%) over three days. These recoveries fall within the accepted range for food matrices.

Conclusion

This study describes HPAE-PAD methods for the determination of carbohydrates in extracts from instant coffee and green coffee beans. Two methods (the AOAC official method 995.13 and a fast method using the Dionex CarboPac SA10 column) were compared. The former method has a longer run time (80 min) compared to the fast method (10 min). For certain sugars that might be difficult to resolve with the official method, minor modifications are suggested. The fast method, proposed for determining coffee carbohydrates, resolves 7 of the 11 coffee carbohydrates in 8 min (two additional peaks are coelutions of two pairs of carbohydrates) and needs only the addition of DI water for continuous operation.

Both methods have high precisions and acceptable recoveries for the carbohydrates in instant and green coffee extracts. In addition, disposable gold working electrodes provide consistently high detector response for both methods, assuring greater instrument-to-instrument and lab-to-lab reproducibility. In summary, both the AOAC Official Method 995.13 (with suggested modifications) and the fast method are sensitive, accurate, reliable, and differ primarily in their total analysis time and peak resolutions for coffee carbohydrate determinations.

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