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Analyzing Coffee Carbohydrates

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Outline

- Why do we need to determine coffee carbohydrates?
- Experiences with AOAC Official Method 995.13
- Fast Method

Key Messages

- Why do we need to determine coffee carbohydrates?
  - Flavor, authenticity, global coffee market
- AOAC Official Method 995.13
  - Long, 80-min run time
  - Two sugar pairs difficult to resolve
  - Recommendations proposed
- Fast Method
  - Run time 8 min
  - Two sugar pairs not resolved

Coffee Preparation: Bean to Brew

History and Botany

"It is difficult to imagine a world without coffee."

- Legend of Its Origins:
  Yemen goat herder story
- Botanical Studies: Ethiopia → Yemen
  (6th century)
- Arab World → Europe → America

- Coffee berries produced by several species of small evergreen bush of the genus *Coffea*
  - *Coffea arabica*
  - *Coffea canephora* (robusta)
- Five years for a coffee plant to start bearing fruit (cherries)

Five Steps to a Cup of Coffee

Drying

Green coffee beans are dried from a moisture level of 53% to 12% via sun-drying or machines. Hulls are removed by mechanical means and the beans are graded.

Blending

Manufacturers blend beans to achieve the unique flavor of their brand.

Roasting

Time, Temperature, and Humidity—manufacturers usually roast beans for five minutes at gas temperatures of 260 ºC.

Grinding

Average particle size affects the properties of the coffee when brewed, brewing time, and storage stability.

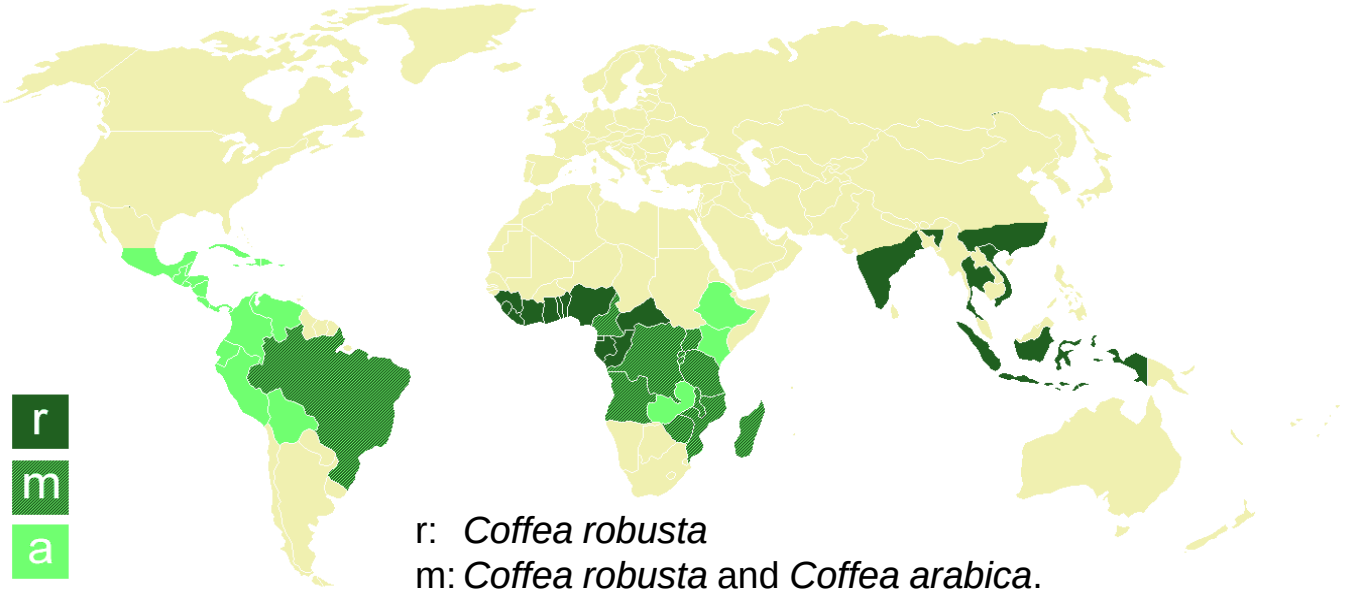
Brewing

Many variables influence the process of brewing—particle size, proportions of coffee and water, water temperature, the mixing mechanism of the brewing equipment, and brewing time.

Each step affects the carbohydrate profile.

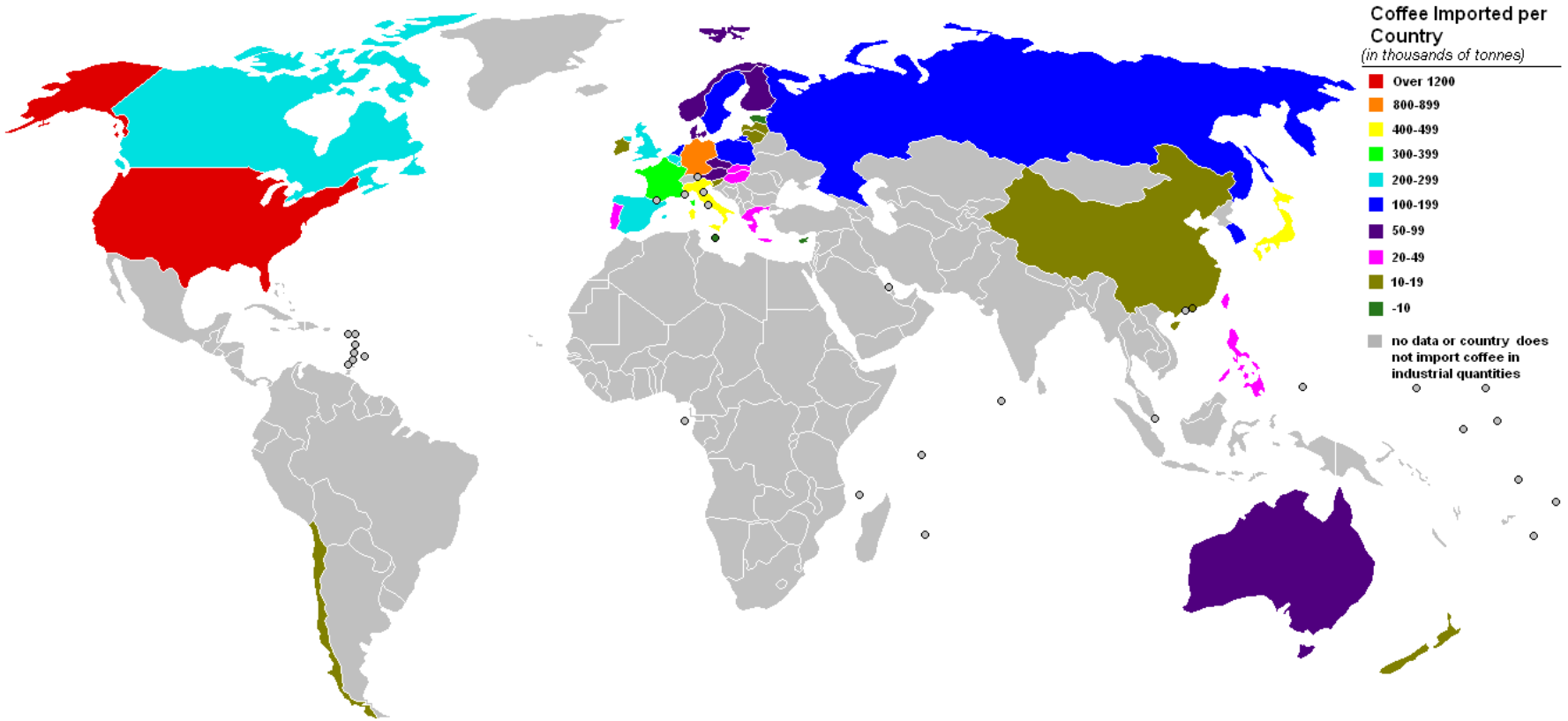
Economics of Coffee Consumption

- Ranks second only to petroleum in terms of dollars traded
- One of the most traded agricultural commodity
- 6.3 million tons produced worldwide
- Over 2.25 billion cups of coffee/day consumed
- Brazil, Vietnam, Columbia (over 70 countries grow coffee)



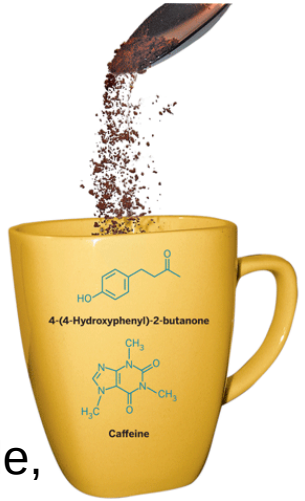
r: *Coffea robusta*
m: *Coffea robusta* and *Coffea arabica*.
a: *Coffea arabica*

Coffee Importers



The International Coffee Agreement (ICA) is an international commodity agreement to achieve a reasonable balance between the supply and demand of coffee.

History of Instant Coffee



1771: **Patent application for instant coffee (England)**

1901: **S. Kato (Japan)**

1906: **Commercial production (U.S.)**

- Concentrated coffee → evaporate the water to leave a soluble, dry coffee mix
- Convenient, quick to make, easy to carry, and increased shelf life
- The problem, of course, was taste……..

1930: **Nestle's Nescafe**

Nescafe began mixing soluble carbohydrates with concentrated coffee before drying.

1960: **Maxwell House introduced freeze-dried instant coffee.**

Today: **The global instant coffee market is estimated to be at a hefty $21 billion per year.**

Green Coffee—The New Antiaging Brew?

Green coffee beans: twice stronger antioxidant effect than green tea and grape seed extract.

Starbucks Eyes Expansion of Cold Beverage Line Green Coffee "Refreshers" Test Starts Aug. 4

"Ingredients include a 'flavor neutral' powdered extract made from unroasted green coffee and formulated to have less of a caffeine kick than regular coffee."

Reuters U.S. Edition. http://www.reuters.com/article/idUSN0319853720100804 Wed., Aug 4, 2010, 10:10am EDT (accessed Mar. 13, 2013).

Coffee Carbohydrates

- Provide the flavor of coffee
- Constitute the major part (at least 50% of the dry weight) of raw coffee beans
- Contain aroma binders
- Are foam stabilizers
- Impart viscosity
- Are very good tracers for the authenticity of instant coffee

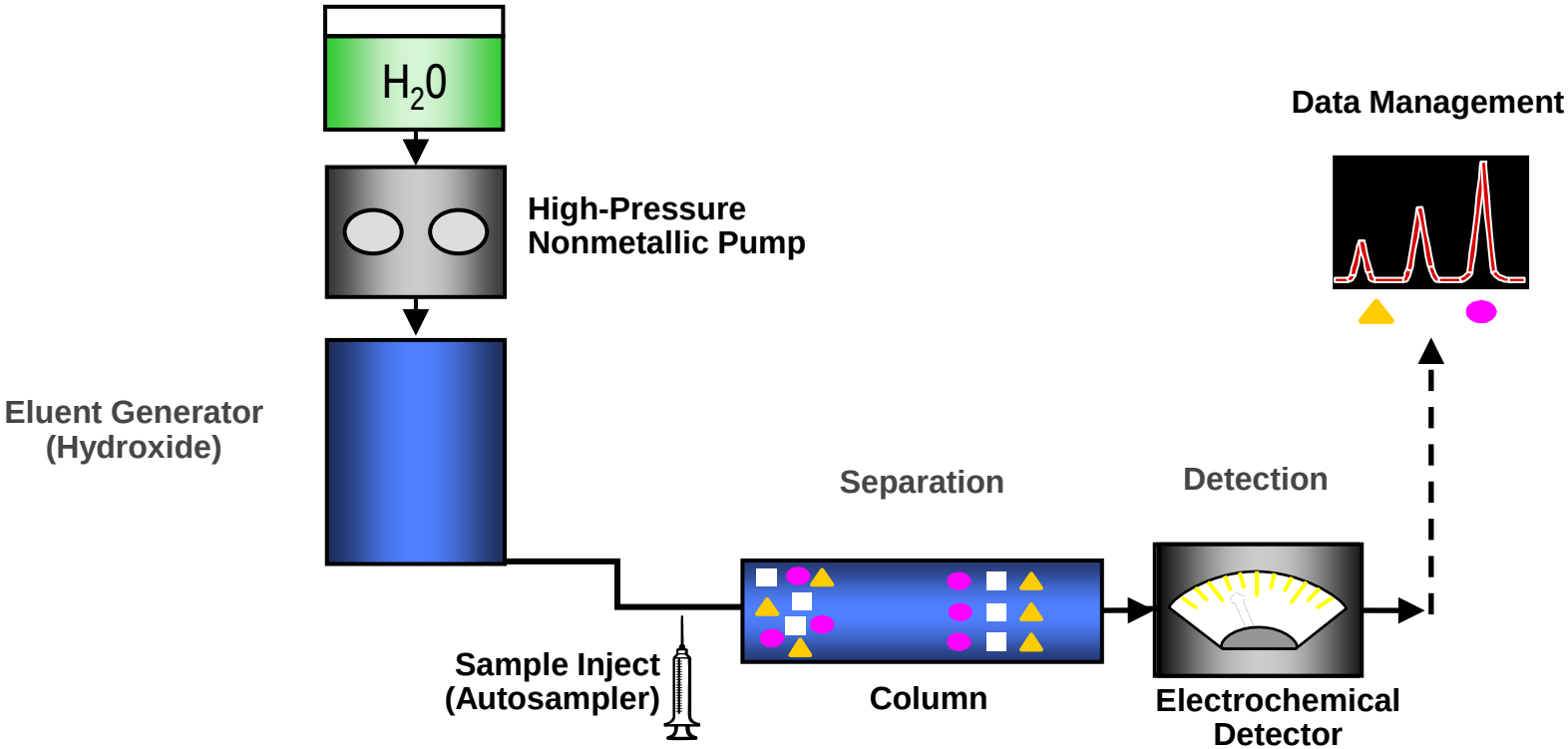
Arya, M.; Rao, L.J. An Impression of Coffee Carbohydrates. *Crit. Rev. Food Sci. Nutr.* **2007**, *47* (1), 51–67.

Tests Done on Green and Roasted Coffee

Bean Density
Brightness
Titratable Acidity
pH
Moisture
Total Soluble Solids

Caffeine
Chlorogenic Acids
Lipids
Carbohydrates
Green Coffee (sucrose contributes to formation of aroma)
Total Polyphenols
Total Proteins
Ochratoxin A, Aflatoxins (AOAC Method)

Experimental Details



Chromatographic Conditions

AOAC Method 995.13

Columns:	Thermo Scientific™ Dionex™ CarboPac™ PA1, Guard & Analytical set
Eluent:	DI water, and 300 mM base for column wash and postcolumn delivery
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (Full Loop)
Temp:	25 ºC
Detection:	PAD (Au)

AOAC Method 995.13: Carbohydrates in Soluble Coffee

- Carbohydrates are separated on a pellicular ion-exchange column and measured by pulsed amperometric detection (PAD).
- Sample Preparation

  Free Carbohydrates

  - Coffee is dissolved in H_2O
  - Solution is filtered through C18 disposable cartridge, and then through 0.2 µm membrane filter

  Total Carbohydrates

  - Coffee is hydrolyzed with 1M HCl
  - Solution is filtered and then passed through a cation-exchange disposable cartridge to neutralize the solution and to eliminate the Cl anion prior to injection

AOAC Method 995.13

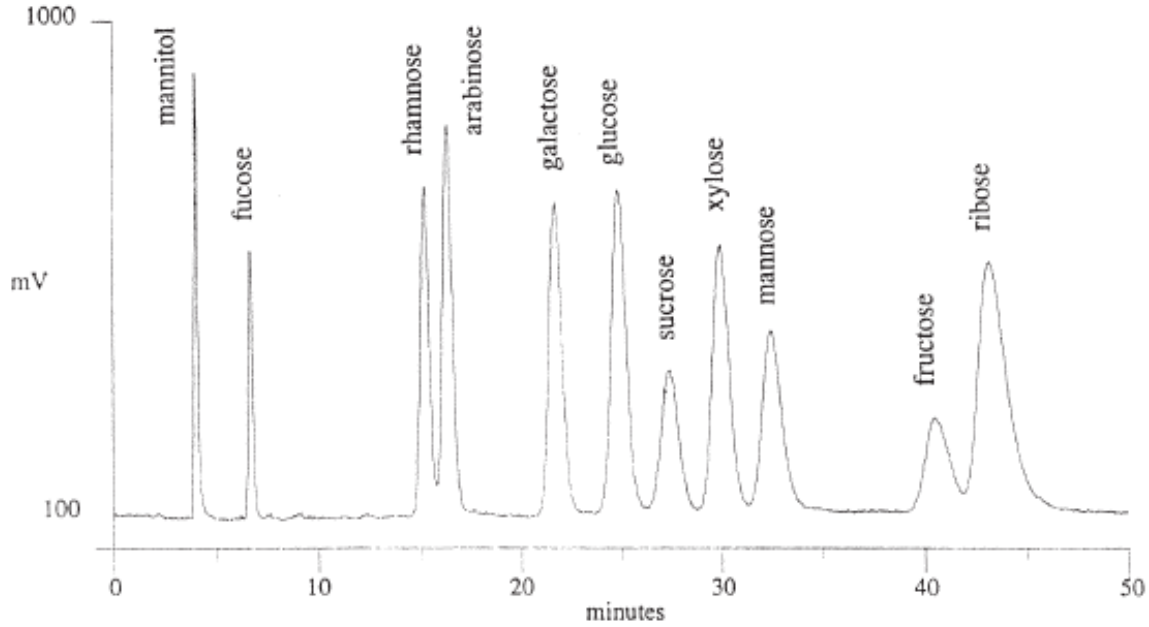
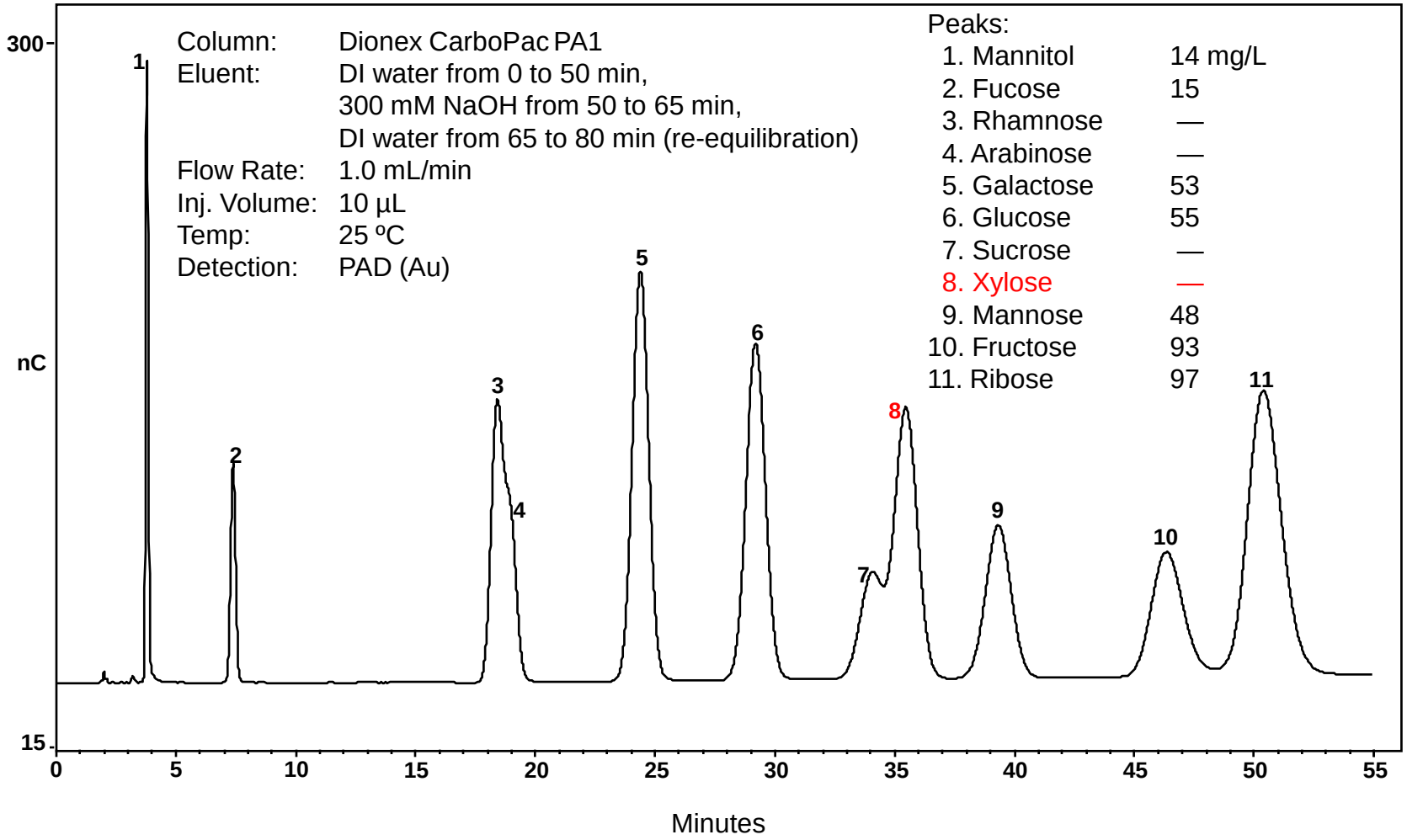


Table 995.13K. Conditions of mobile phase for determination of free and total carbohydrates in soluble coffee by anion-exchange chromatographic method with pulsed amperometric detection

Time, min	Eluent A, %	Eluent B, %
0	100	0 (start acquisition)
50.0	100	0 (stop acquisition)
50.1	0	100 (start cleanup)
65.0	0	100 (stop cleanup)
65.1	100	0 (start re-equilibrium)
80.0	100	0 (stop re-equilibrium)

Results

Mixed Carbohydrate Standard on a Dionex CarboPac PA1 AOAC Method 995.13

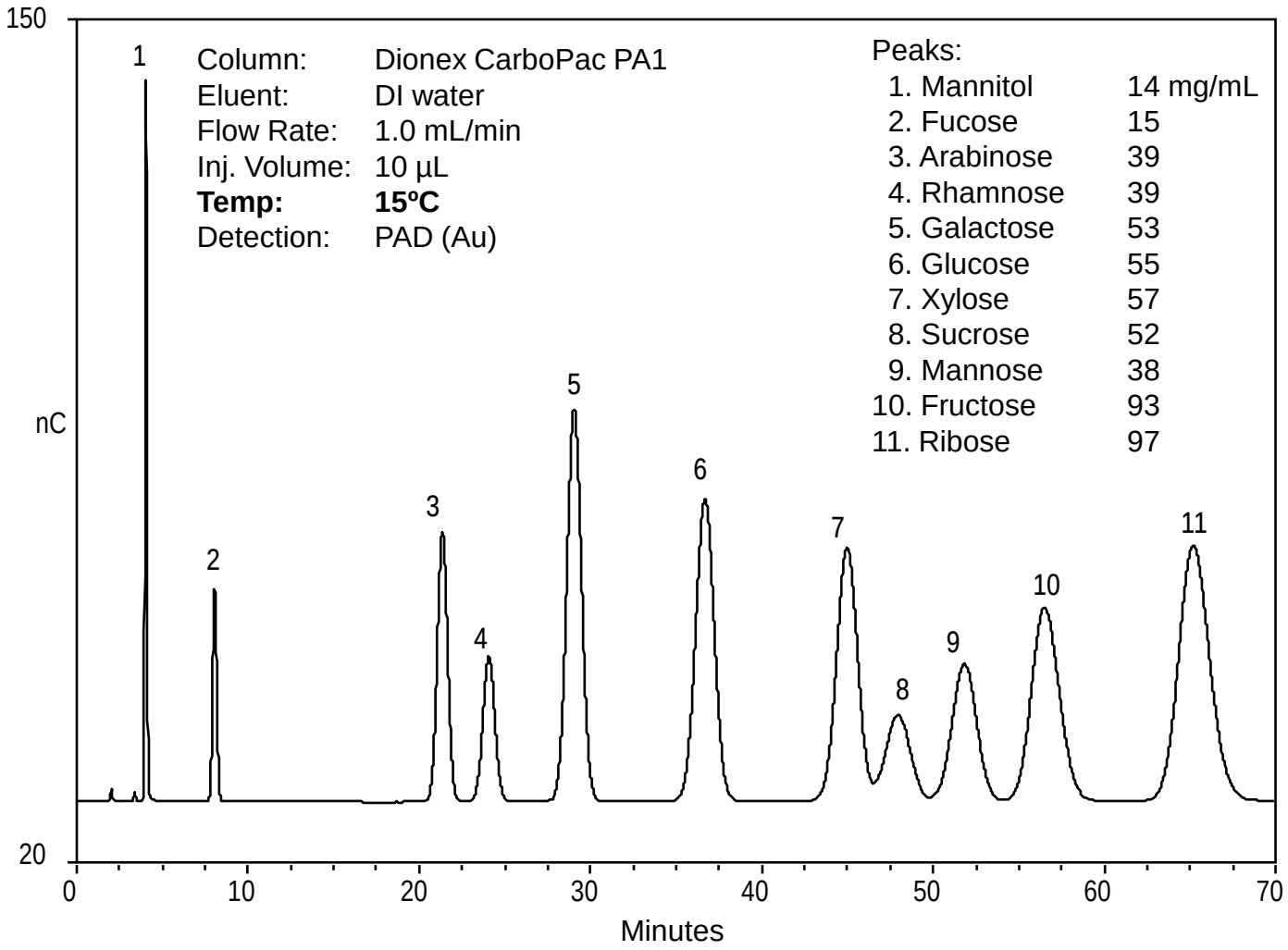


Separation of Mixed Carbohydrate Standard: AOAC Method

Known Issues and AOAC's Recommendations

- "If resolution of rhamnose from arabinose is difficult to achieve, do not add rhamnose to mixed standard solution."
- "It may be necessary to perform 2–3 injections of carbohydrates standard solution or to increase the re-equilibrium time in order to achieve a good separation of glucose, sucrose, and xylose."
- From our work we have developed two recommendations.

Recommendation #1: Lower Temperature



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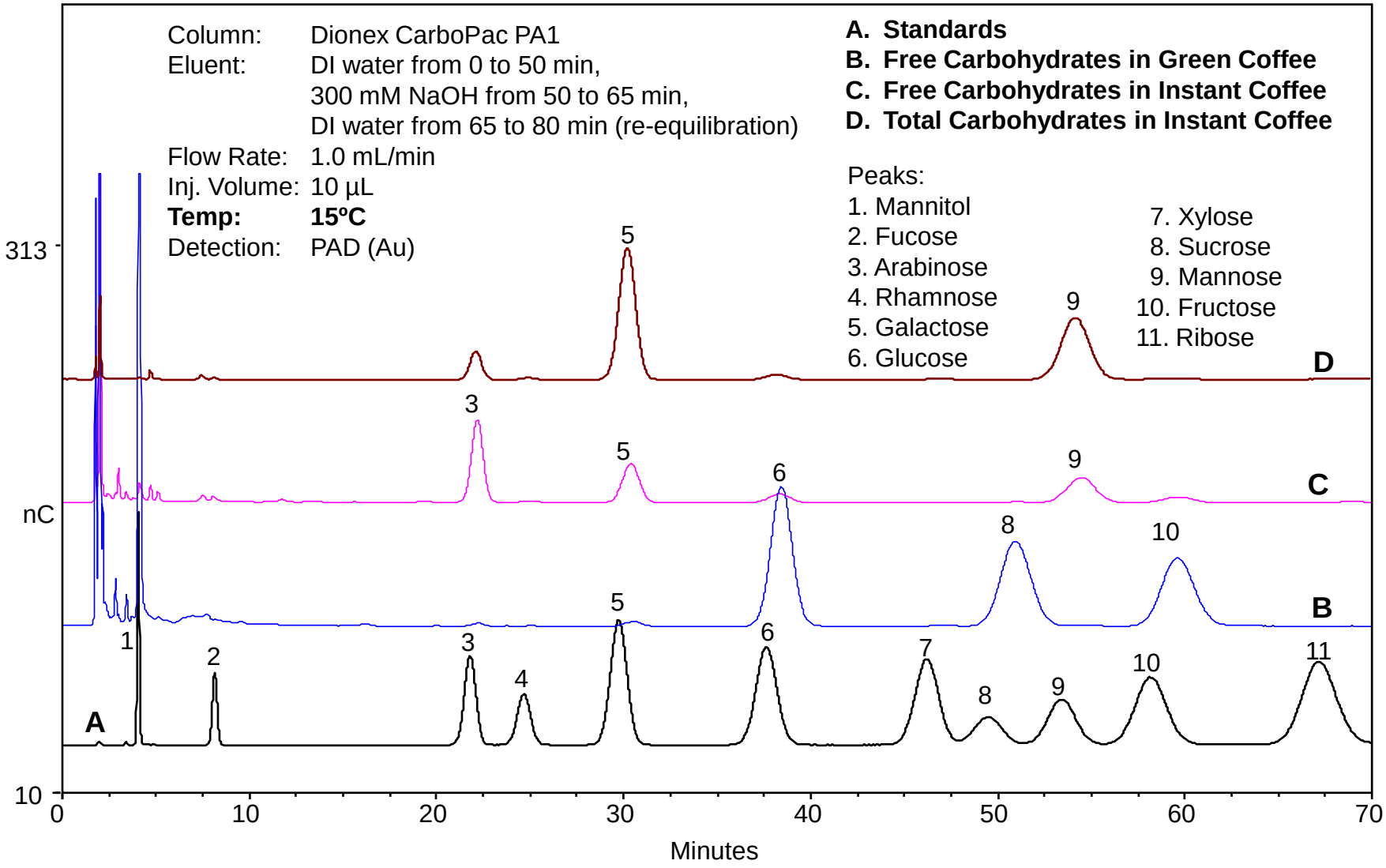
Recommendation #1: Applied to Coffee Analyses



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Precision: AOAC Method for Mixed Carbohydrate Standard

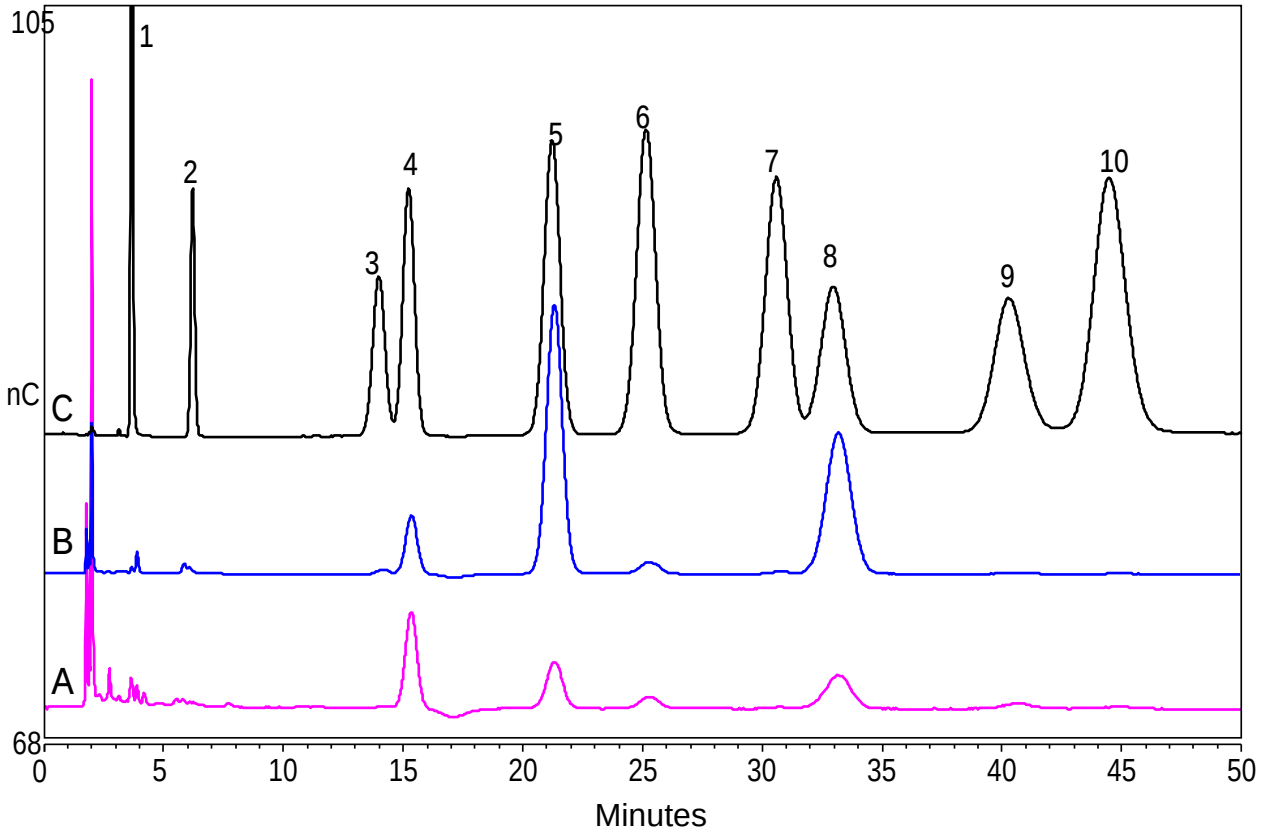
Recommendation #1: 15 ºC

Carbohydrate	Concn for Precision Injection (mg/mL)	Retention Time Precision (RSD)	Peak Area Precision (RSD)
Mannitol	15	0.20	4.49
Fucose	15	0.24	4.69
Rhamnose	35	0.40	4.83
Arabinose	40	0.30	4.66
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

n = Six Injections

Recommendation #2a (for Instant Coffee)

- 10 mM Base for 6 min, other parameters same as AOAC Method.
- Do not include sucrose in mix of standards.



Column:	Dionex CarboPac PA1
Eluent:	10 mM NaOH 0–6 min, DI water 6–50 min, 300 mM NaOH from 50 to 65 min, DI water from 65 to 80 min (re-equilibration)
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Temp:	25ºC
Detection:	PAD (Au)

A. Free Carbohydrates Extract
B. Total Carbohydrates Extract
C. Mix of Standards

Peaks:	
1. Mannitol	15 mg/L
2. Fucose	15
3. Rhamnose	35
4. Arabinose	40
5. Galactose	50
6. Glucose	55
7. Xylose	55
8. Mannose	45
9. Fructose	90
10. Ribose	90

Precision: Mixed Carbohydrate Standard by AOAC Method 995.13

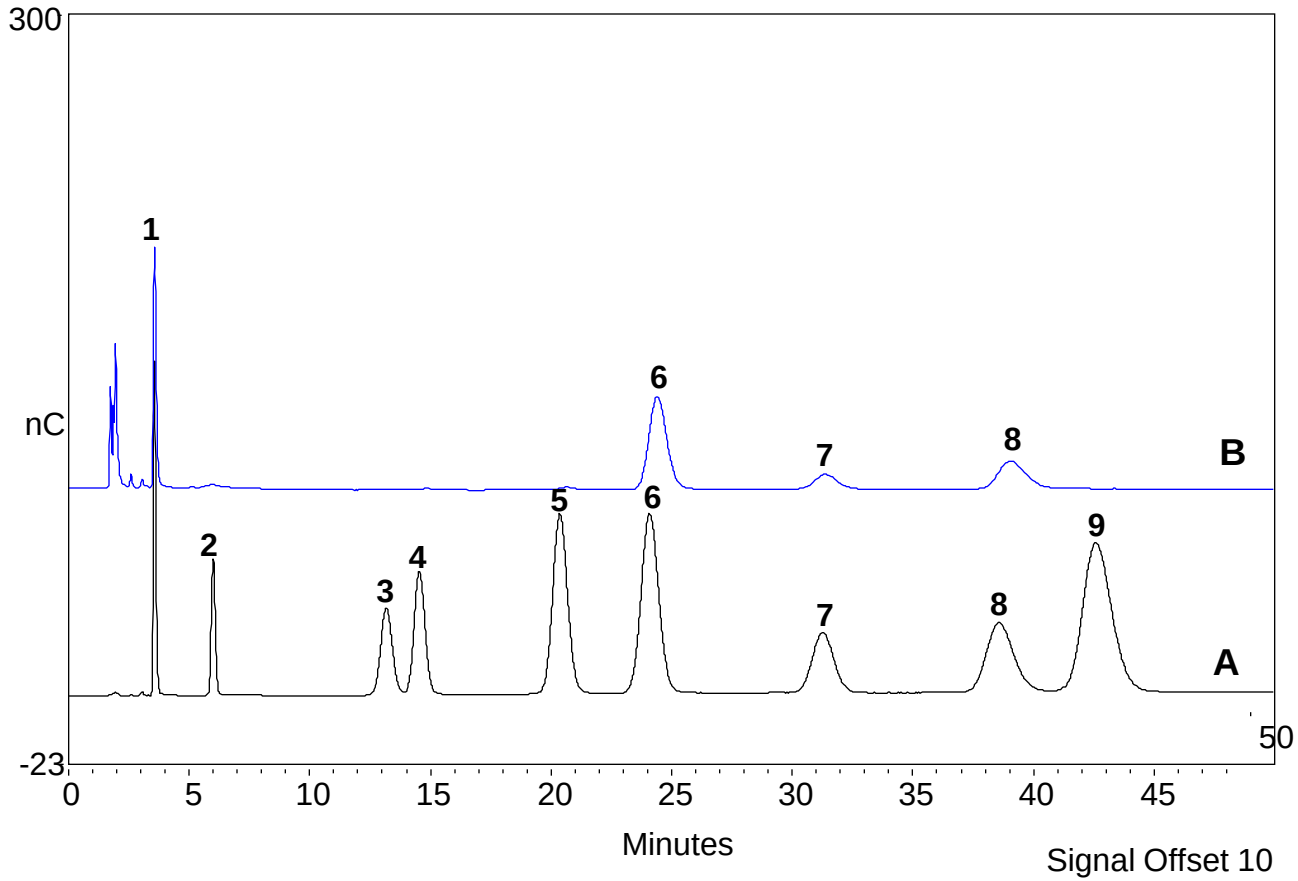
- Recommendation #2a as applied to instant coffee: 10 mM Base for 6 min
- Do not include sucrose in mix of standards

Carbohydrate	Concn for Precision Injection (µg/mL)	Retention Time Precision (RSD)	Peak Area Precision (RSD)
Mannitol	15	0.09	2.8
Fucose	15	0.46	2.6
Rhamnose	35	0.81	4.1
Arabinose	40	0.41	3.2
Galactose	50	0.27	3.2
Glucose	55	0.35	3.0
Xylose	55	0.33	5.0
Mannose	45	0.50	4.2
Fructose	90	0.42	3.0
Ribose	90	0.37	3.4

n = Six Injections

Recommendation #2b: for Green Coffee

- 10 mM Base for 6 min
- Do not include xylose and mannose in mix of standard for green coffee samples



Column: Dionex CarboPac PA1
Eluent: 10 mM NaOH 0–6 min, DI water 6–50 min, 300 mM NaOH from 50 to 65 min, DI water from 65 to 80 min
Flow Rate: 1.0 mL/min
Temp: 25ºC
Inj. Volume: 10 µL
Detection: PAD (Au)

A. Standards
B. Green Coffee Extract

Peaks:
1. Mannitol
2. Fucose
3. Rhamnose
4. Arabinose
5. Galactose
6. Glucose
7. Sucrose
8. Fructose
9. Ribose

Analyte Recovery: Total Carbohydrates—Instant Coffee

Carbohydrate Recoveries in Extract of Total Carbohydrates from Instant Coffee (n = Three Days) Using Modified[a,b] AOAC Method 995.13

Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	97.3	105.0	107.5	9.3
Fucose	99.5	82.0	82.1	9.1
Rhamnose	106.0	101.0	71.1	14.0
Arabinose	91.5	186.0	88.8	15.0
Galactose	102.0	817.0	114.4	15.4
Glucose	92.8	113.0	84.6	9.9
Xylose	129.0	106.0	76.2	14.2
Mannose	200.0	819.0	59.8	18.0
Fructose	103.0	89.7	87.1	12.4
Ribose	98.4	79.1	80.3	5.8

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

b. Exclusion of sucrose from mix of standards

Analyte Recovery: Free Carbohydrates—Instant Coffee

Carbohydrate Recoveries in Extract of Free Carbohydrates from Instant Coffee (n = Three Days) Using Modified[a,b] AOAC 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 base 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 mix of standards

Analyte Recovery: Free Carbohydrates—Green Coffee

Carbohydrate Recoveries in Extract of Free Carbohydrates from Green Coffee (n = Three Days) Using Modified[a,b] AOAC Method 995.13

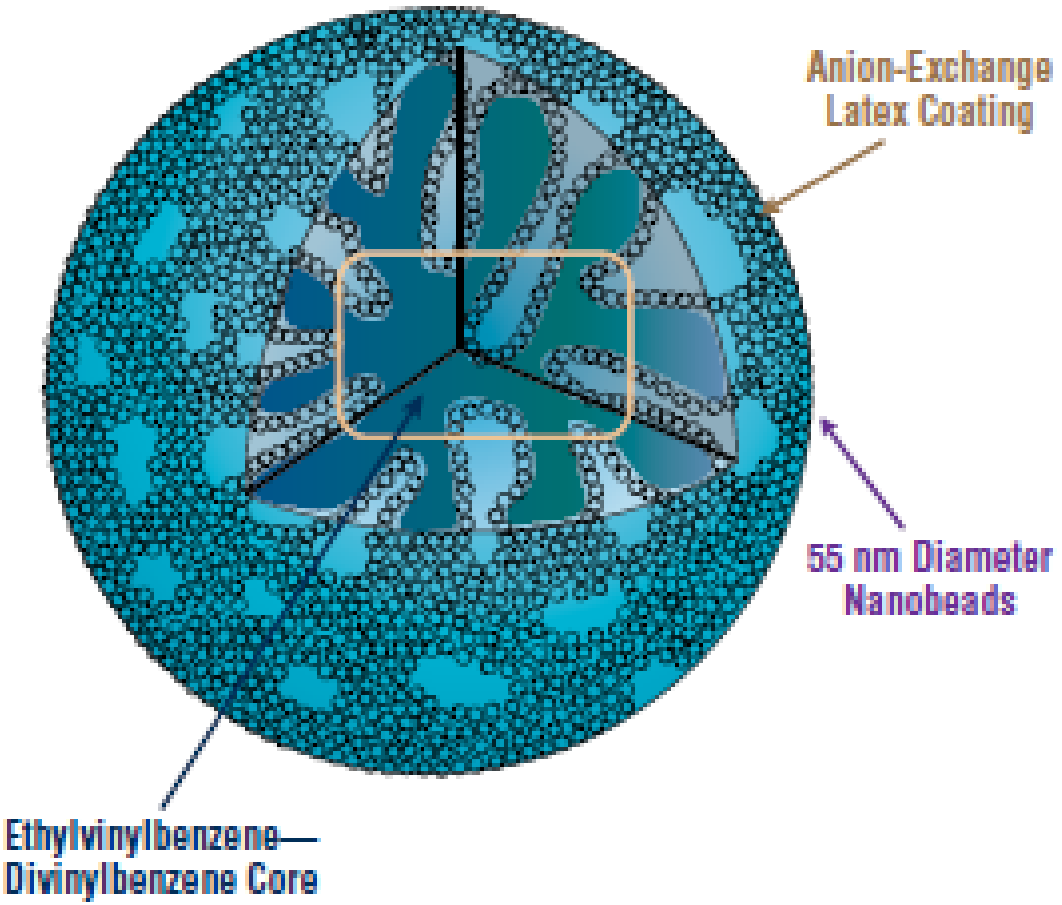
Analyte	Amount Added (mg/L)	Amount Detected (mg/L)	Recovery (%)	RSD
Mannitol	42.9	41.3	76.6	6.2
Fucose	95.2	90.7	95.4	12.8
Rhamnose	111.0	83.5	75.6	8.6
Arabinose	97.7	81.1	83.0	2.5
Galactose	104.0	97.8	92.2	7.6
Glucose	101.0	129.0	88.7	23.1
Sucrose	88.4	233.0	69.5	32.1
Fructose	106.0	140.0	73.3	14.9
Ribose	109.0	90.7	83.3	5.6

a. 10 mM base 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 mix of standards

Faster Method

Column for Rapid Separation of Plant Monosaccharides

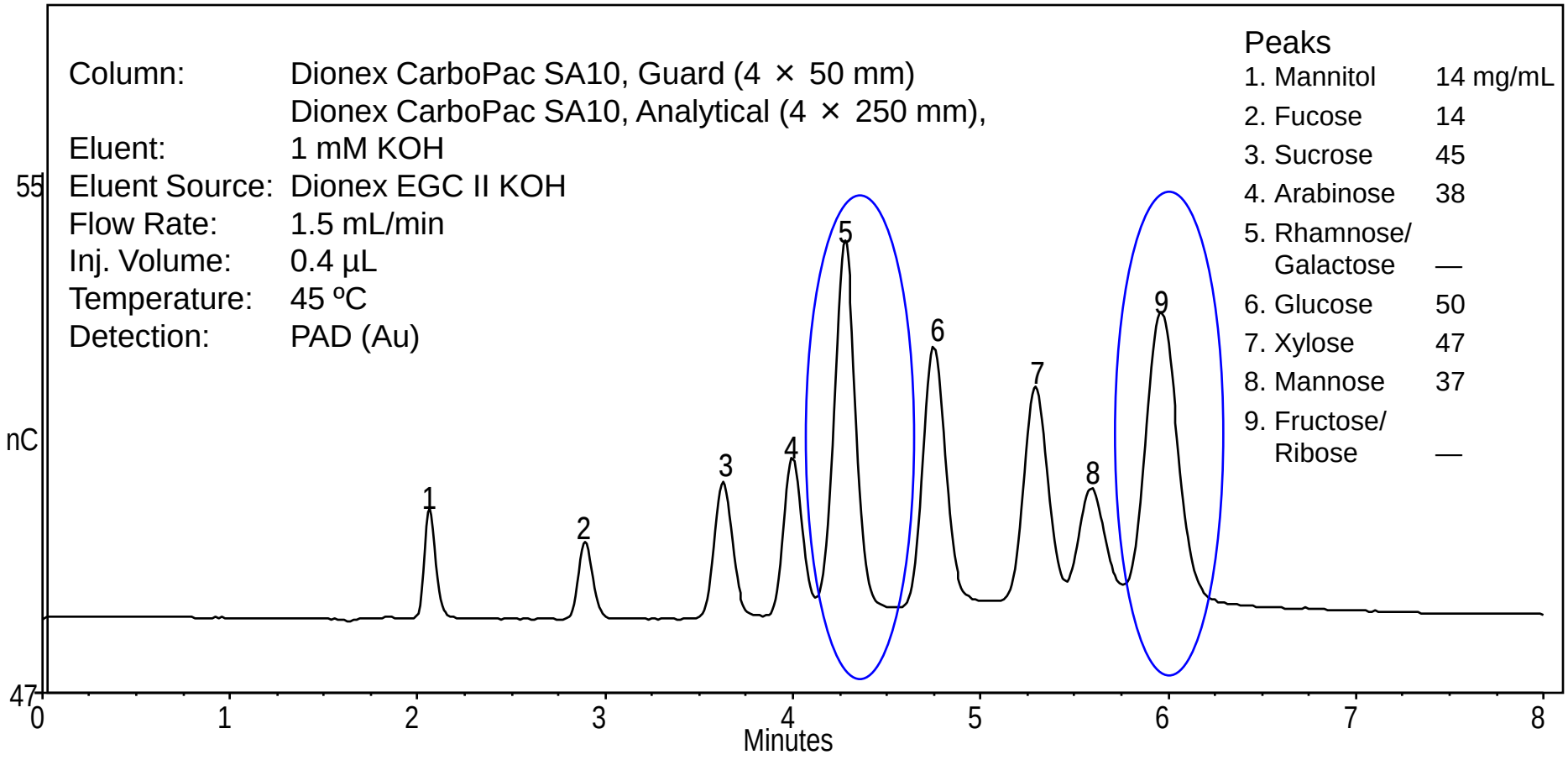
Dionex CarboPac SA10 Column



Chromatographic Conditions for Fast Method

Column:	Dionex CarboPac SA10, Guard (4 × 50 mm)
	Dionex CarboPac SA10, Analytical (4 × 250 mm)
Eluent:	1 mM KOH
Eluent Source:	Thermo Scientific™ Dionex™ EGC II KOH
Flow Rate:	1.5 mL/min
Inj. Volume:	0.4 µL (Internal Loop)
Temperature:	45 ºC
Detection:	PAD (Au)

Mixed Carbohydrate Standard Separated by the Fast Method

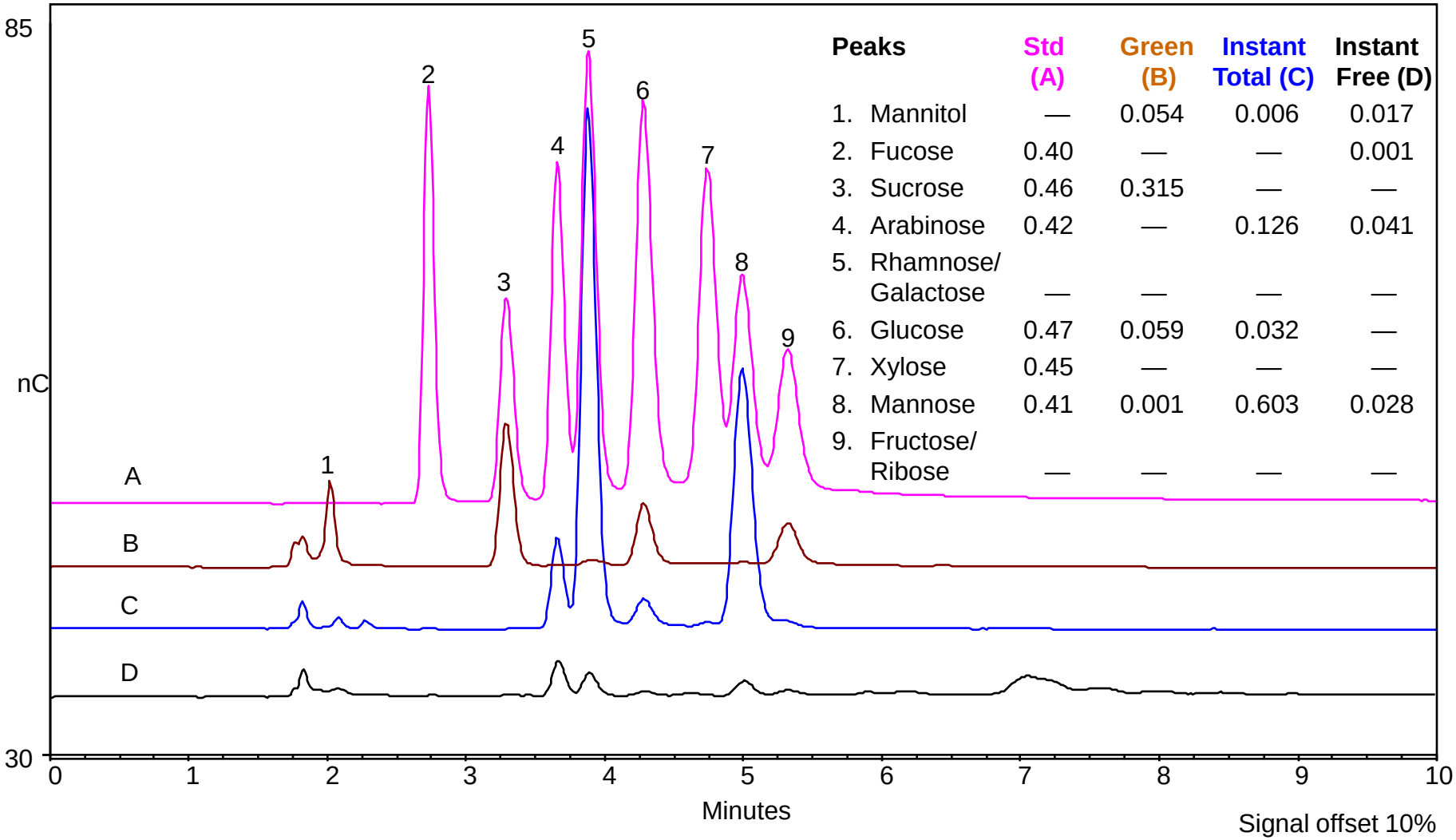


Calibration and Precisions Using the Fast Method

Linear Range and Precisions for Coffee Sugars

Analyte	Range (mg/mL)	Coeff of Deter.	Concn for Precision (mg/L)	Retention Time (min)	Retention Time Precision (RSD)	Peak Area (nC*min)	Peak Area Precision (RSD)
Mannitol	0.005–0.2	0.99917	15	2.06	0.21	0.16	1.35
Fucose	0.006–0.2	0.99980	15	2.89	0.15	0.13	3.25
Sucrose	0.01–0.8	0.99587	45	3.61	0.19	0.29	3.28
Arabinose	0.018–0.3	0.99973	40	3.99	0.13	0.33	4.24
Glucose	0.013–0.9	0.99631	55	4.74	0.20	0.75	3.64
Xylose	0.01–0.74	0.99668	55	5.28	0.18	0.71	4.64
Mannose	0.006–0.7	0.99417	45	5.58	0.15	0.86	3.85

Green and Instant Free and Total Carbohydrates Coffee Extracts Fast Method



Peaks	Std (A)	Green (B)	Instant Total (C)	Instant Free (D)
1. Mannitol	—	0.054	0.006	0.017
2. Fucose	0.40	—	—	0.001
3. Sucrose	0.46	0.315	—	—
4. Arabinose	0.42	—	0.126	0.041
5. Rhamnose/ Galactose	—	—	—	—
6. Glucose	0.47	0.059	0.032	—
7. Xylose	0.45	—	—	—
8. Mannose	0.41	0.001	0.603	0.028
9. Fructose/ Ribose	—	—	—	—

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Accuracy of the Fast Method for Green Coffee Extract

Sugar Recoveries in an Extract of Free Sugars from Green Coffee (n = Three Days)

Analyte	Amount Added (mg/mL)	Amount Detected (mg/mL)	Recovery (%)	RSD
Fucose	0.10	0.08	86.5	8.6
Sucrose	0.16	0.39	73.9	7.3
Arabinose	0.09	0.09	97.4	16.9
Glucose	0.11	0.14	83.5	9.5
Xylose	0.11	0.08	75.3	14.4
Mannose	0.10	0.08	78.0	17.8

Fast Method Accuracy: Instant Coffee Extract—Total and Free Carbohydrates

Sugar Recoveries in an Extract of Total Sugars from Instant Coffee (n = Three Days)

Analyte	Amount Added (mg/mL)	Amount Detected (mg/mL)	Recovery (%)	RSD
Fucose	0.10	0.11	**105.9**	**12.6**
Sucrose	0.09	0.10	**107.7**	**6.9**
Arabinose	0.09	0.11	**127.7**	**5.1**
Glucose	0.09	0.37	**101.3**	**7.1**
Xylose	0.10	0.16	**114.5**	**7.8**
Mannose	0.09	0.11	**120.5**	**11.5**

Sugar Recoveries in an Extract of Free Sugars from Instant Coffee (n = Three Days)

Analyte	Amount Added (mg/mL)	Amount Detected (mg/mL)	Recovery (%)	RSD
Fucose	0.04	0.04	**85.4**	**1.4**
Sucrose	0.05	0.04	**81.4**	**13.1**
Arabinose	0.03	0.03	**102.4**	**14.2**
Glucose	0.05	0.08	**98.0**	**9.9**
Xylose	0.04	0.03	**78.9**	**24.9**
Mannose	0.05	0.04	**80.0**	**16.0**

Summary

- Dionex CarboPac PA1 (AOAC Method) has two reported issues. Our recommendations to resolve these issues:
  - Use low temp to resolve all 11 carbohydrates.
  - Use initial base (step change) to resolve rhamnose-arabinose.
  - For instant coffee analysis, remove sucrose from the standard mix.
- Dionex CarboPac SA10 reduces analysis time eightfold.
  - However, two pairs (rhamnose-galactose and ribose-fructose) are not resolved.

Thank you!