HPAE-PAD analysis of Carbohydrates

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Laboratory

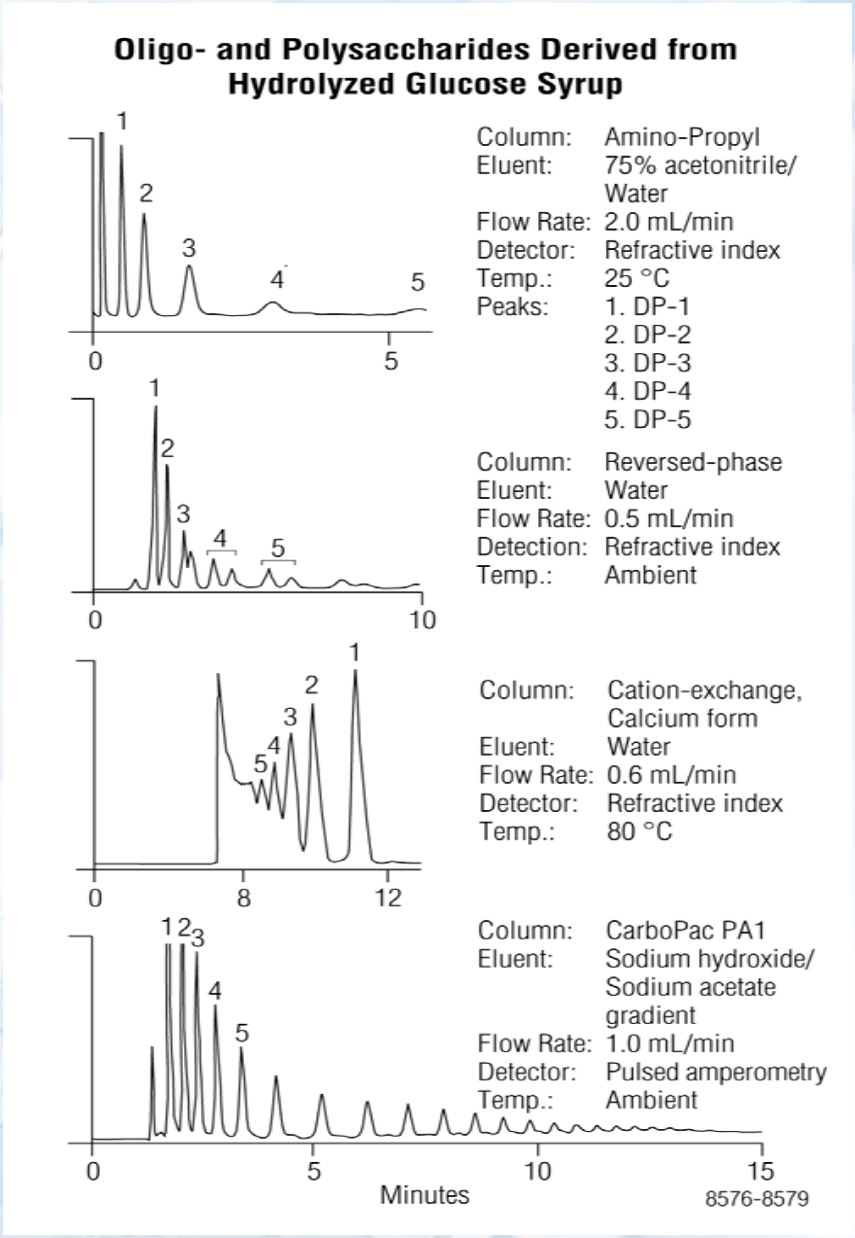
Presentation Outline

- Principles of High Performance Anion Exchange
- Column Technologies
- Principles of Pulsed Amperometric Detection
- Applications using HPAE-PAD

Carbohydrates -
Features of HPAE-PAD

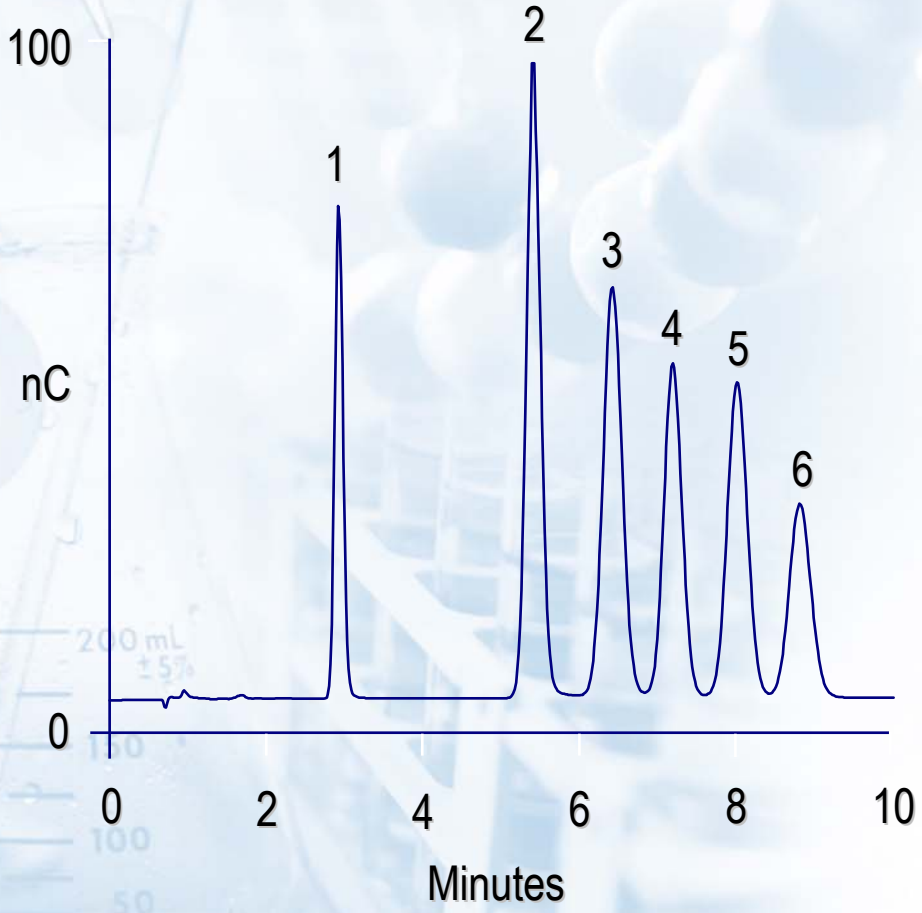
- **Simple, Direct Method**
  - No derivatization is necessary
- **High Sensitivity**
  - comparable to derivatization with fluorescence detection (fmol to low pmol).
  - Allows samples to be diluted 100 to 1000-fold, so simplifies sample prep
- **Minimal Sample Preparation is necessary**
- **Unique column technology**
- **Official Methods (using CarboPac PA1)**

Resolving Power of HPAE-PAD



Comparison of chromatographic techniques used for the analysis of a hydrolyzed glucose syrup illustrates the remarkable resolving power of the HPAE-PAD technique. Elution order is reversed with the CarboPac® PA1 column as compared with conventional metal loaded cation-exchange columns.

Rapid Monosaccharide Analysis with the CarboPac™ PA10



Column:	CarboPac PA10 and guard	
Eluent:	18 mM NaOH	
Flow Rate:	1.5 mL/min	
Detection:	Pulsed amperometry, gold electrode	
Peaks:	1. Fucose	1 nmol
	2. Galactosamine	1
	3. Glucosamine	1
	4. Galactose	1
	5. Glucose	1
	6. Mannose	1

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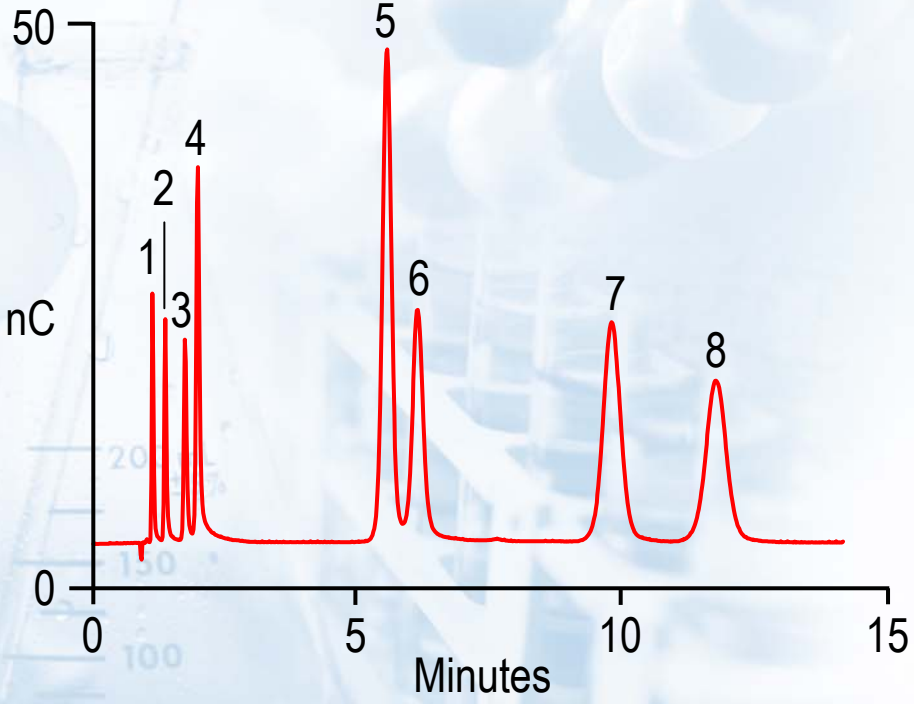
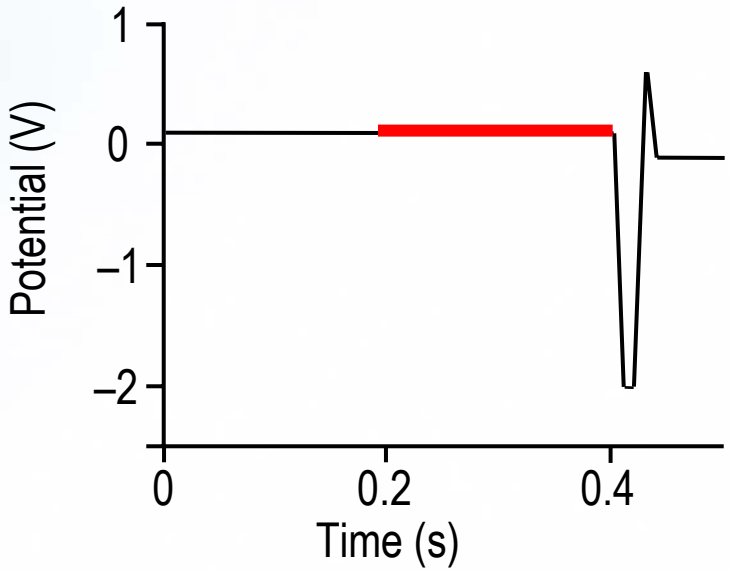
Detection of Food Sugars and Sugar Alcohols

Column:	CarboPac™ PA10, PA10 guard, Borate Trap®
Eluent:	52 mM sodium hydroxide
Flow Rate:	1.5 mL/min
Inj. Volume:	5 µL
Detection:	Pulsed amperometry, gold electrode

Peaks:		
1.	Glycerol	4.0 mg/mL
2.	Xylitol	2.0
3.	Sorbitol	1.5
4.	Mannitol	3.0
5.	Glucose	5.0
6.	Fructose	8.0
7.	Sucrose	2.0
8.	Lactose	8.0





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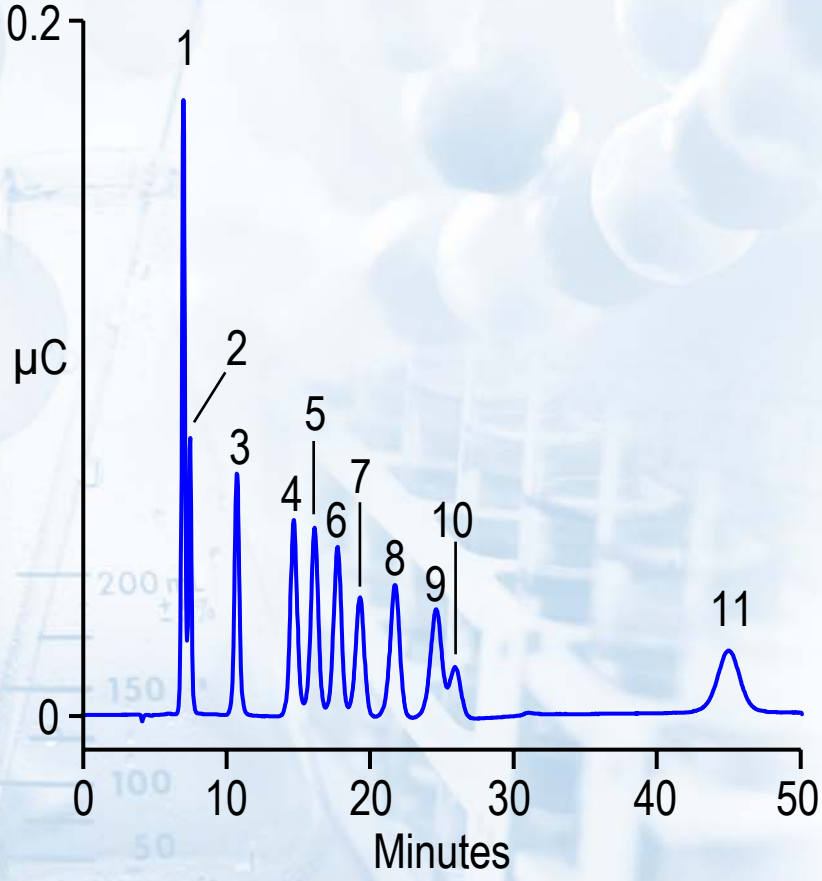
Separation of Reduced and Reducing Carbohydrates Commonly Found in Foods and Beverages

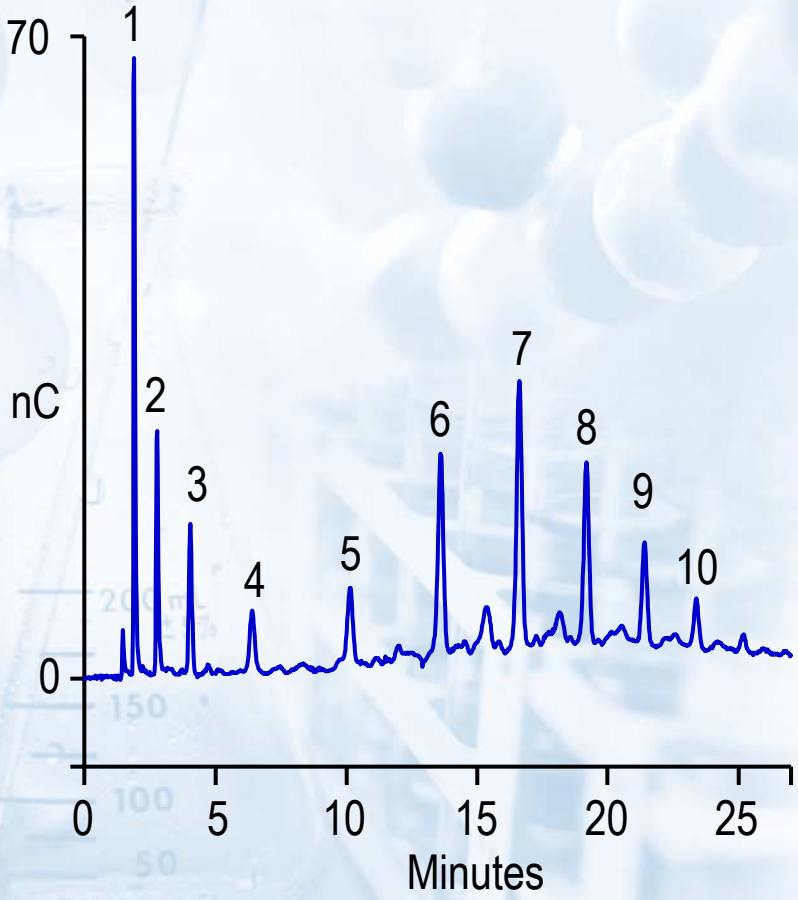


Column:	CarboPac™ MA1
Eluent:	480 mM sodium hydroxide
Flow Rate:	0.4 mL/min
Detector:	PED (gold)

Peaks:

1. Inositol	18 mg/L
2. Glycerol	9
3. Arabitol	15
4. Sorbitol	18
5. Dulcitol	18
6. Mannitol	18
7. Mannose	18
8. Glucose	18
9. Galactose	18
10. Fructose	18
11. Sucrose	34

Separation of Maltose Oligomers

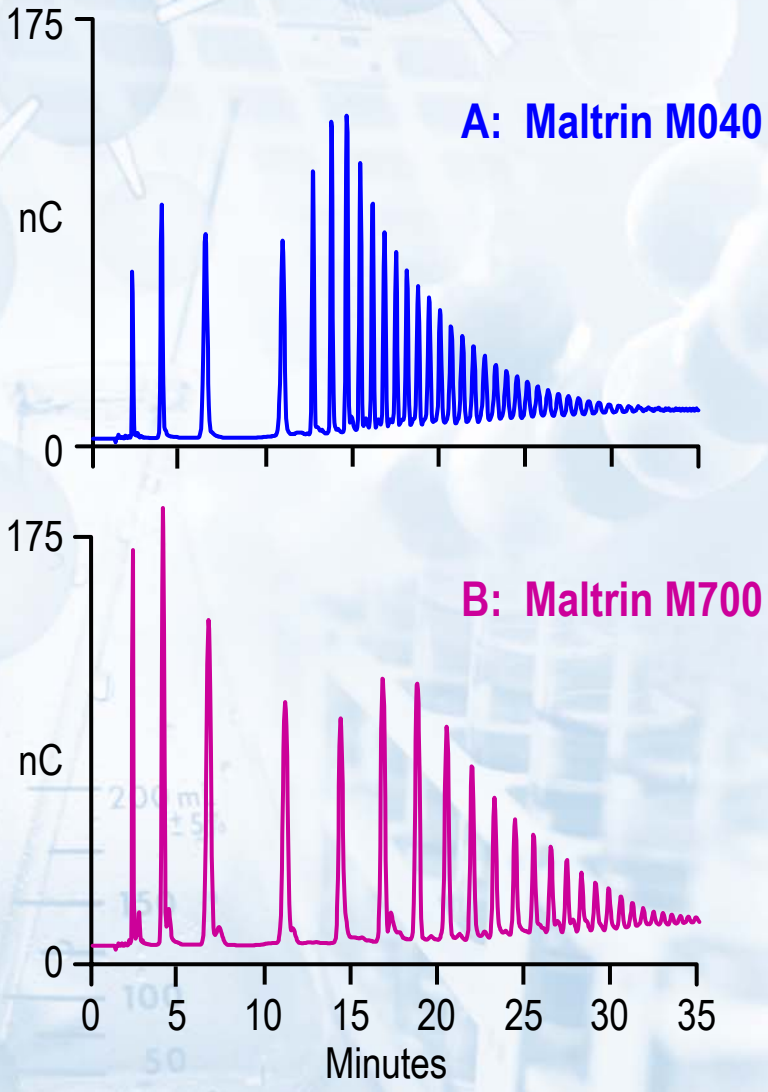


Column:	CarboPac™ PA-100 and guard
Eluent:	NaOH/sodium acetate gradient
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	Pulsed amperometry, Au electrode

Peaks:

1. Glucose
2. Maltose
3. Maltotriose
4. Maltotetraose
5. Maltopentaose
6. Maltohexaose
7. Maltoheptaose
8. Maltooctaose
9. Maltononaose
10. Maltodecaose

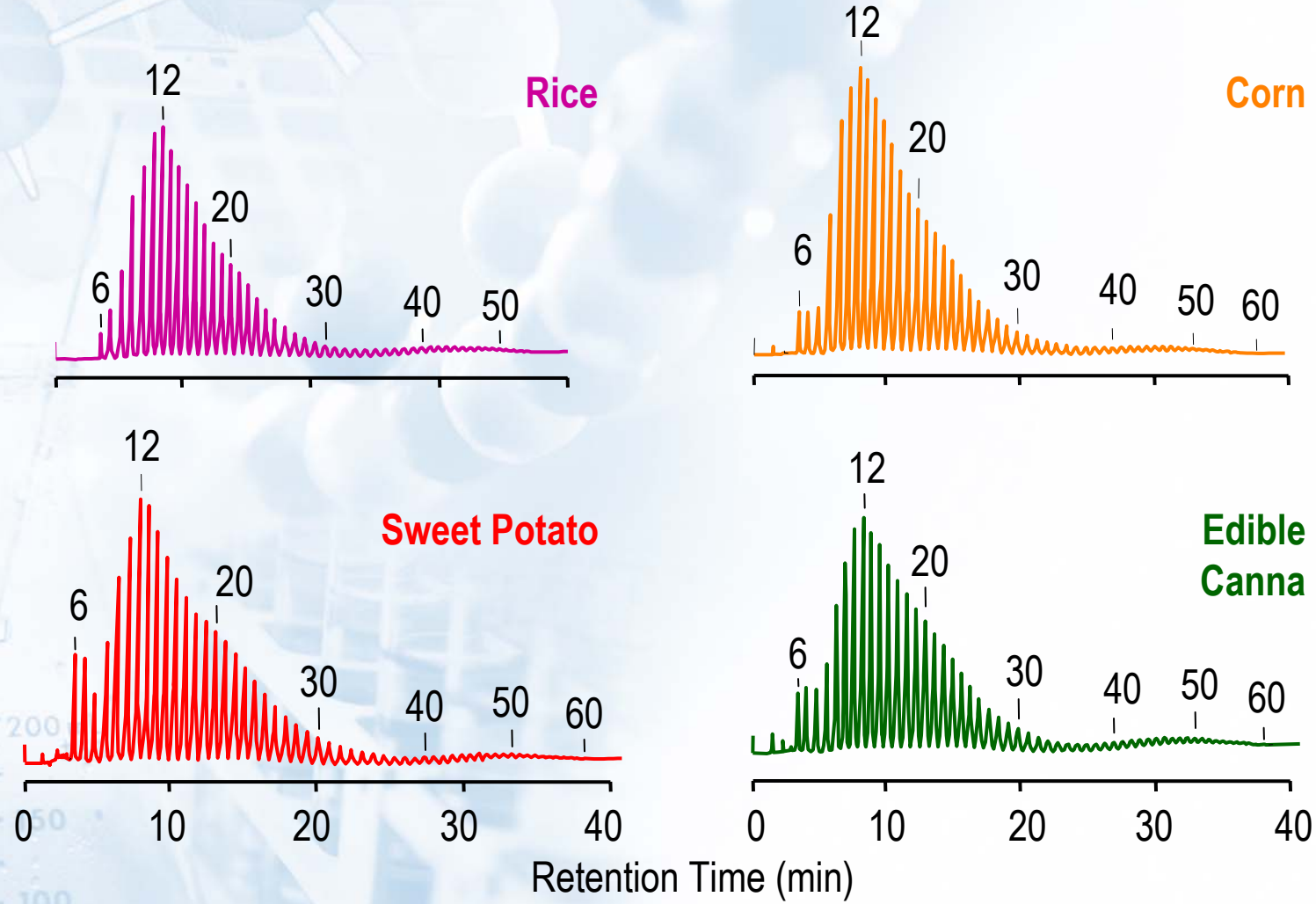
Maltodextrins—Maltrin® M040 and M700



Column:	CarboPac™ PA10
Eluent:	Sodium hydroxide/ sodium acetate gradient
Flow Rate:	1 mL/min
Inj. Volume:	25 µL
Detection:	Pulsed amperometry, Au electrode

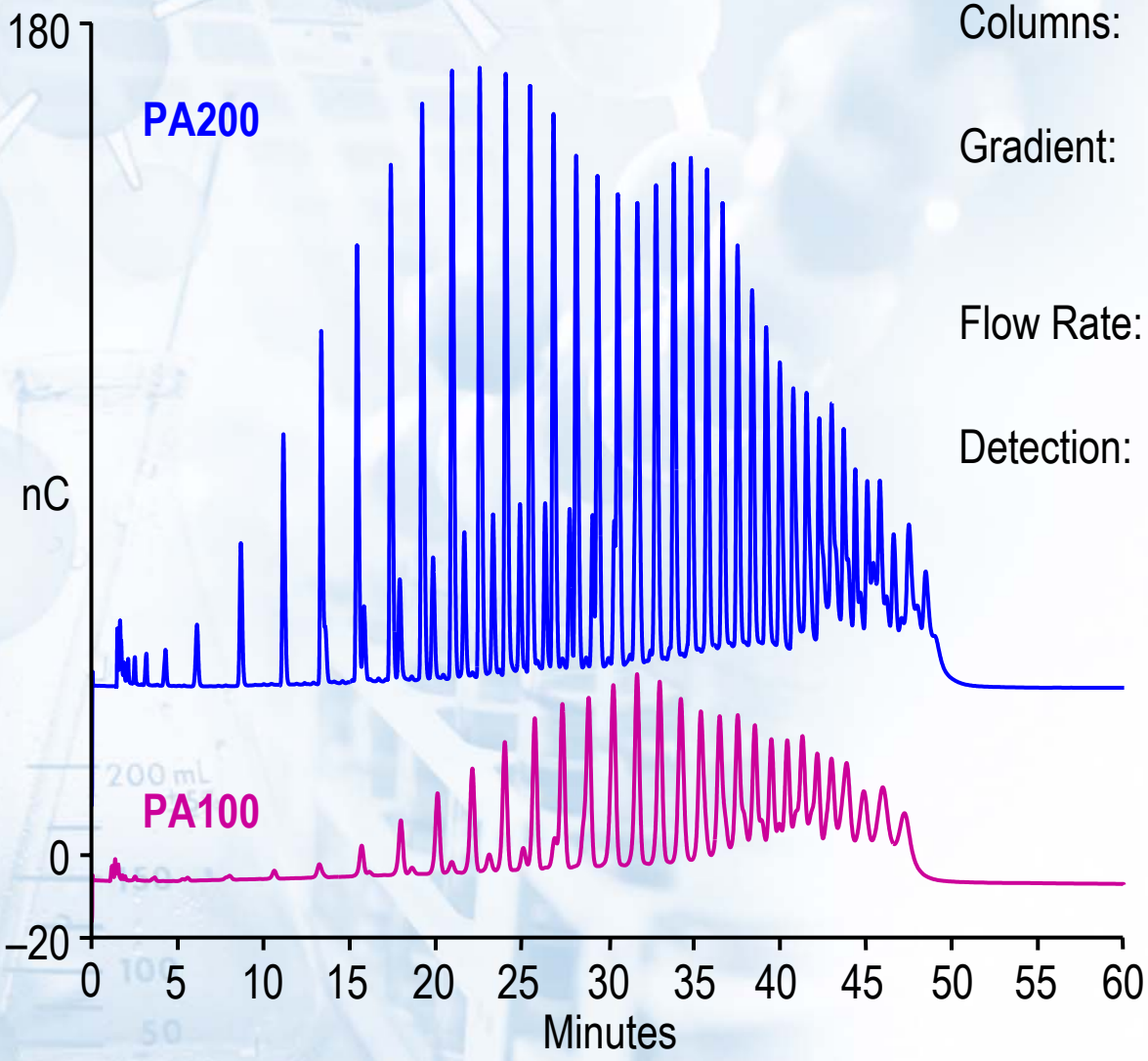
Maltrin is a trademark of Grain Processing Corp.

Distribution of Chain Length of Amylopectins by HPAE-PAD



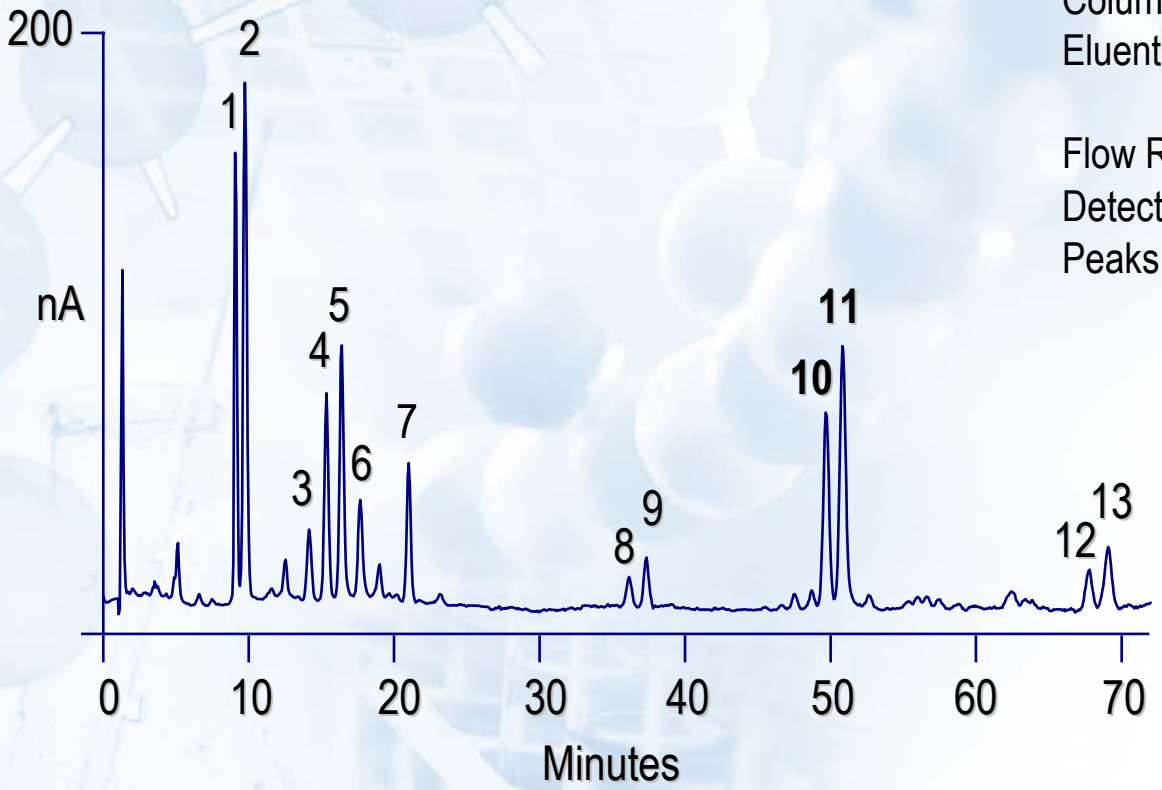
From: Koizumi, K., Fukuda, M., Hizukuri, S.. *J. Chromatography*. **1991**, *585,* 233–238

Improved Chain-Length Resolution of Inulin Polymers



Columns:	CarboPac™ PA200 (3 x 250 mm) CarboPac PA100 (4 x 250 mm)
Gradient:	120- to 320-mM NaOAc in 100 mM NaOH over 40 min
Flow Rate:	PA200: 0.5 mL/min PA100: 1.0 mL/min
Detection:	Pulsed amperometry, quadruple waveform, gold electrode

Separation of N-Linked Oligosaccharides



Column: CarboPac™ PA-100
Eluent: 0 to 250 mM NaOAc over 110 min in 100 mM NaOH
Flow Rate: 1 mL/min
Detector: PAD (Gold)
Peaks:
1. Fucosylated $Man_3GlcNAc_2$
2. $Man_3GlcNAc_2$
3. Asialo, agalacto bi, core fuc
4. Asialo, agalacto bi
5. Asialo bi, core fuc
6. Asialo bi
7. $Man_9GlcNAc_2$
8, 9. Disialylated tri (reduced)
10, 11. Trisialylated tri (reduced)
12, 13. Tetrasialylated tri (reduced)

NANA — Gal — GlcNAc — Man…
α2–6

Peak 10

NANA — Gal — GlcNAc — Man…
α2–3

Peak 11

Official Food Methods Using IC/PAD

Analysis	CarboPac™ Column	Official Method
Sugars in Molasses	CarboPac PA1	AOAC Method 996.04 ICUMSA Method Approval 1994
Carbohydrates in Soluble Coffee	CarboPac PA1	AOAC Method 995.13 ISO 11292 Approved
Fructans in Food and Food Products	CarboPac PA1	AOAC Method 997.08
Polydextrose	CarboPac PA1	AOAC Method 2000.11
Transgalacto-oligosaccharides	CarboPac PA1	AOAC Method 2001.02
Low-Level Glucose and Fructose in Raw and Refined Sugar	CarboPac PA1	AOAC Method 2000.17

Column Technology

High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)

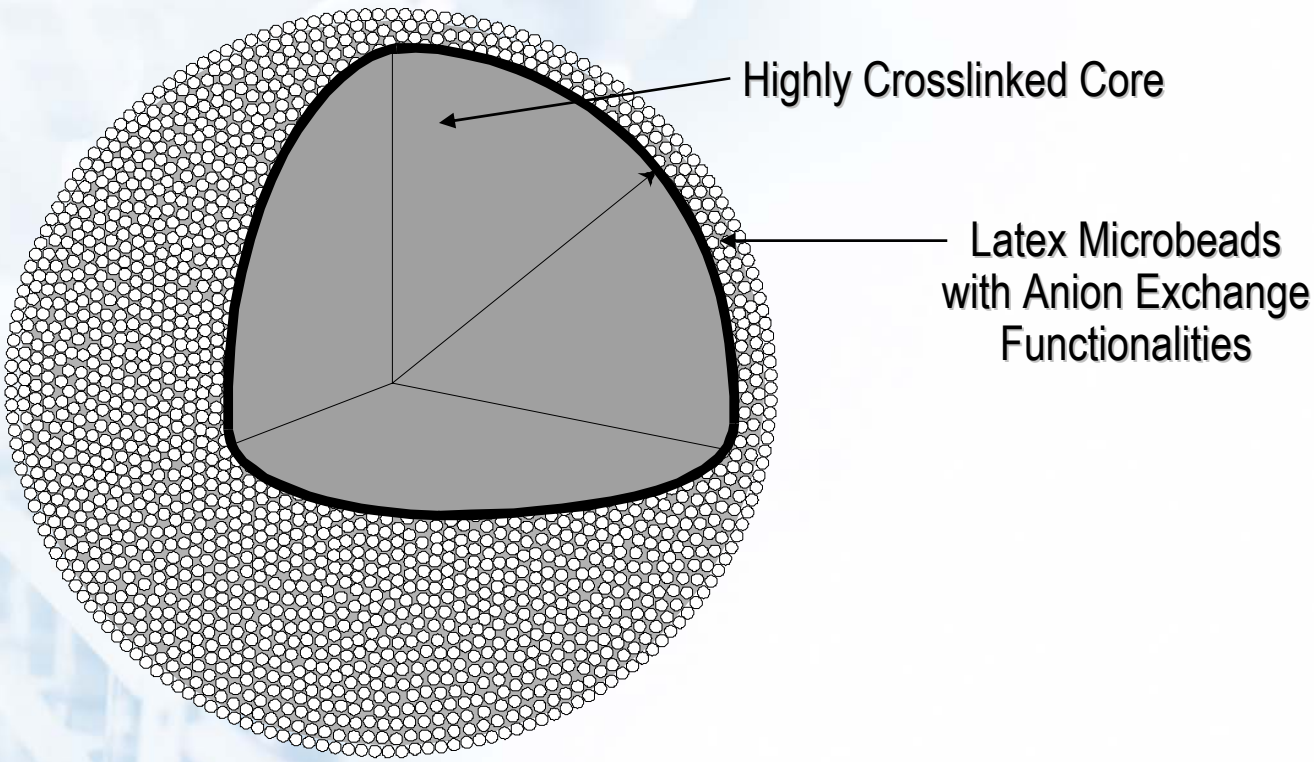
- Separates neutral and charged saccharides *without* derivatization
- Separates on the basis of:
  - Charge, size and composition
  - Branching, linkage isomerism

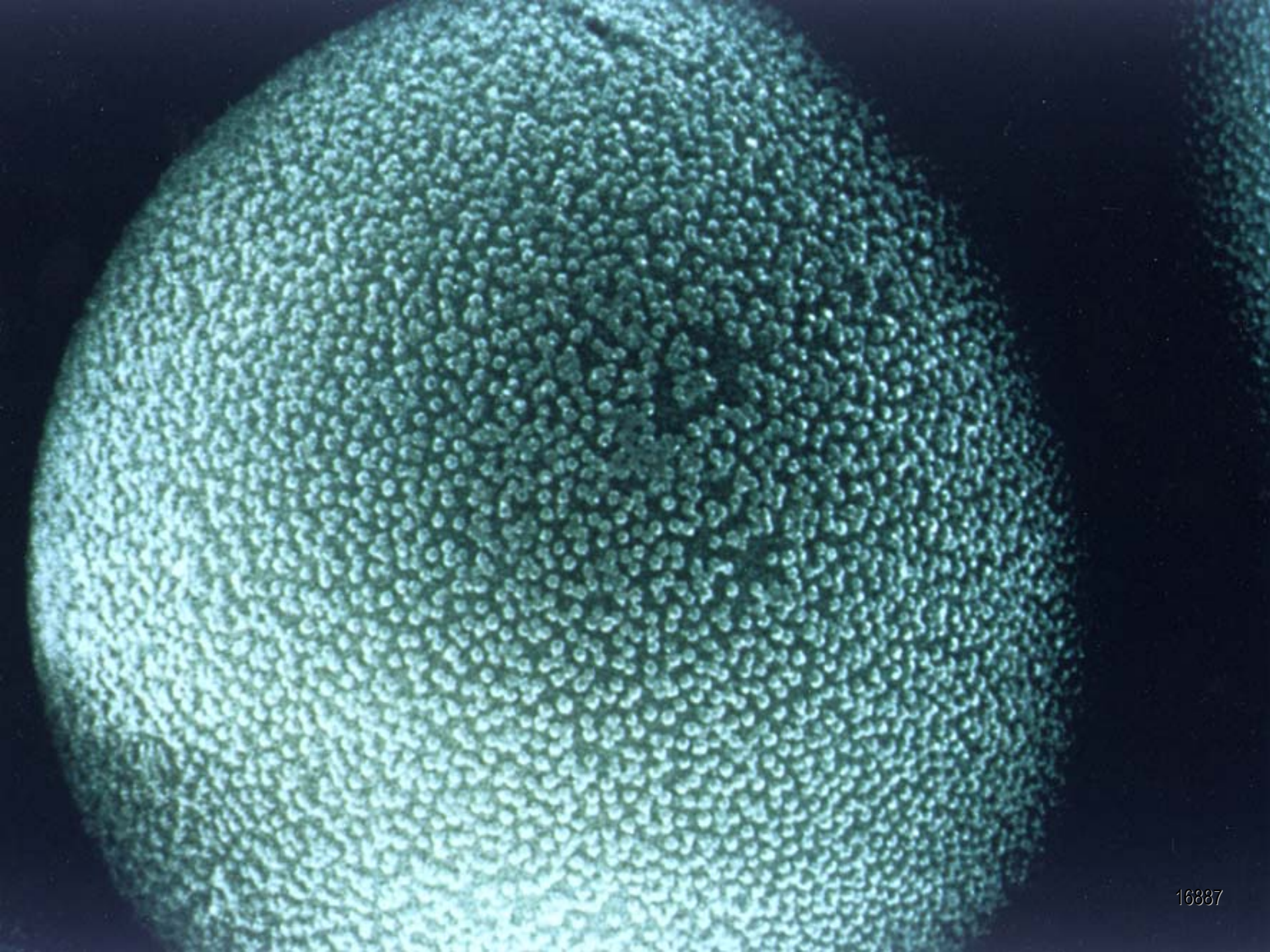
Dissociation Constants of Some Common Carbohydrates

(in Water at 25 °C)

Sugar	pK_a
Fructose	12.03
Mannose	12.08
Xylose	12.15
Glucose	12.28
Galactose	12.39
Dulcitol	13.43
Sorbitol	13.60
α-Methyl glucoside	13.71

Polymeric Nonporous ("Pellicular") Packing

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Electrochemical Detection

Pulsed Amperomerty Detection

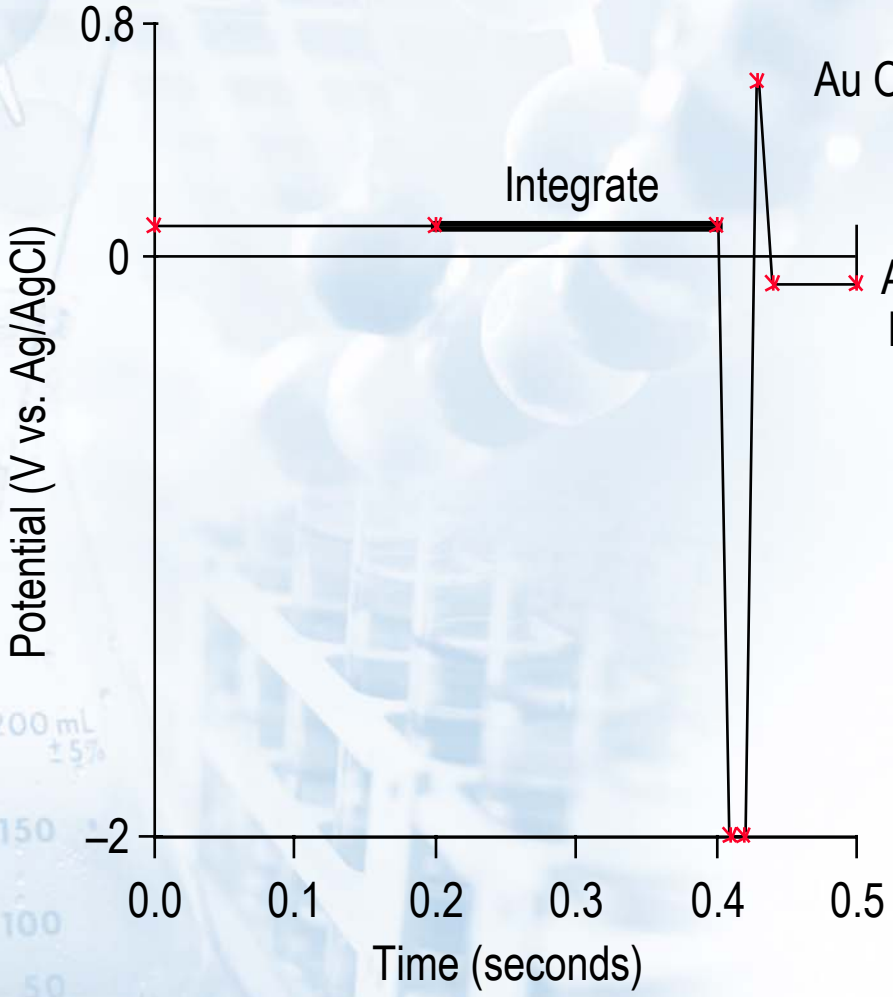
- **Advantages of PAD for Carbohydrate Detection**
  - No derivatization is necessary
  - Low detection limits: low picomole to high femtomole
  - Detection is linear over at least 4 orders of magnitude
  - PAD is a non-destructive method and can thus be used preparatively
  - Highly specific
  - Using optimized pulse settings, PAD is highly specific for carbohydrates
  - Versatile: isocratic or gradient elution, high pH or neutral pH (with post column base addition)
  - Specific for electroactive species
  - Both amino acids and carbohydrates can be detected in a single run

- **Disadvantages of PAD for Carbohydrate Detection *****
  - Each sugar gives a different signal, thus calibration curves must be run for quantitative results

  - *** True for every quantitative analysis

Principal of Pulsed Amperometric Detection (PAD)

Quadruple Potential Waveform for Carbohydrates



Time	Pot.	Integ.
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	

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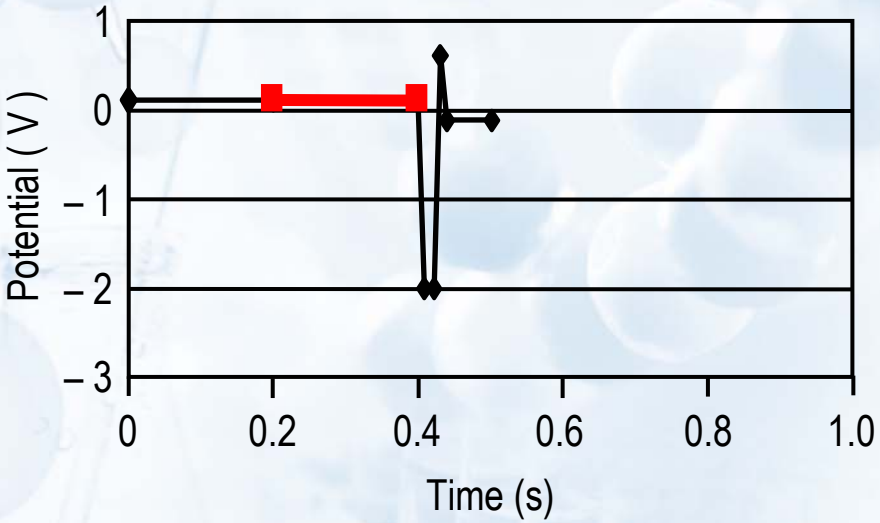
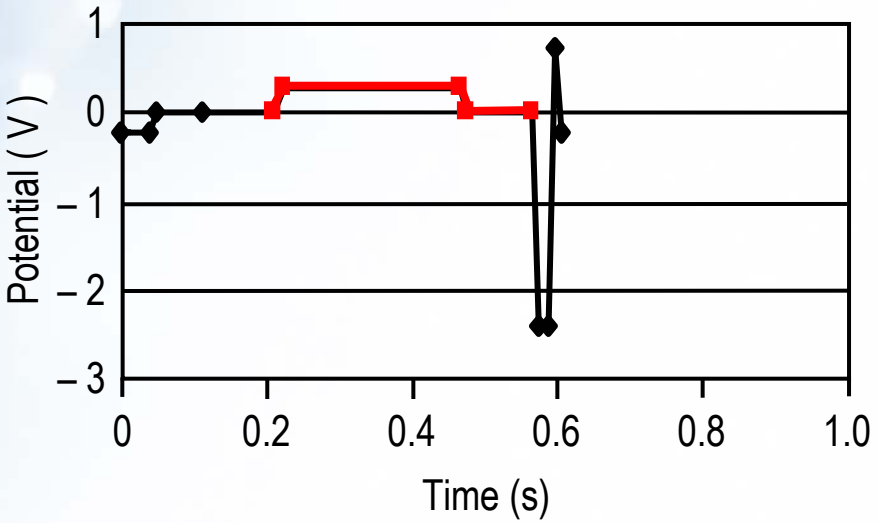
Pulsed Amperometric Detection (PAD)

Waveform and Integration Period



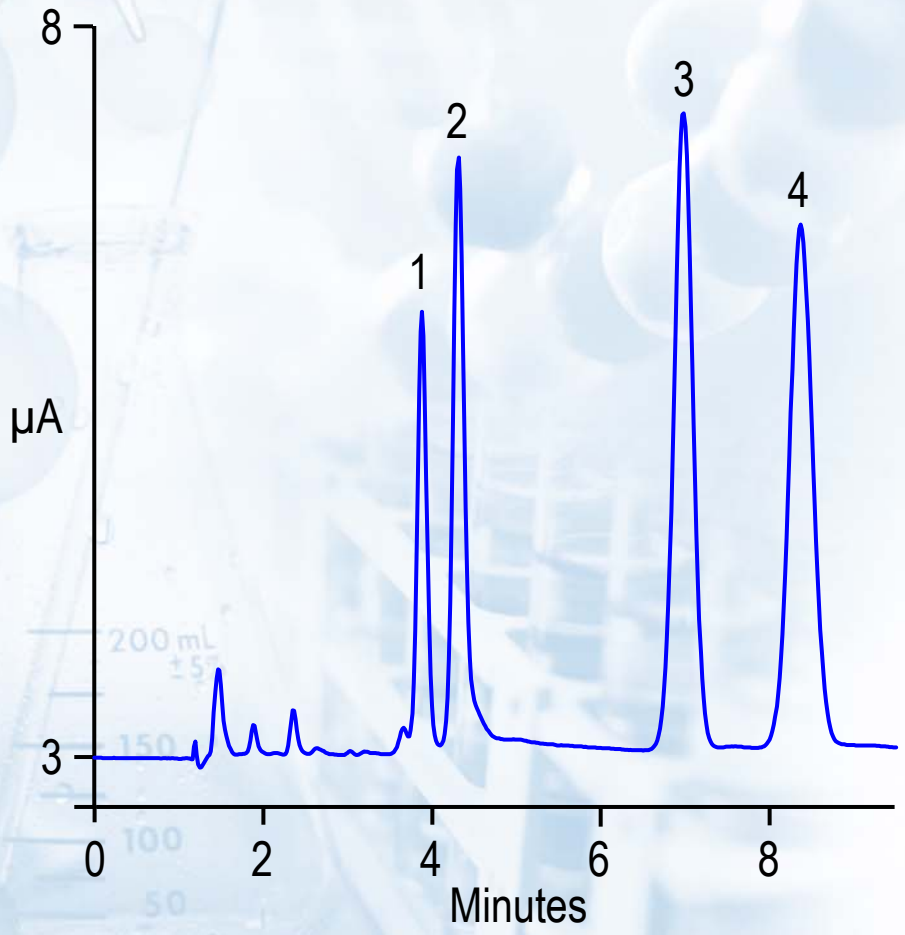
Carbohydrate Waveform



Amino Acid Waveform

Integration Period

Sugars in Sugar Cane Molasses



Column:	CarboPac™ PA1 and guard	
Eluent:	150 mM NaOH	
Flow Rate:	1.0 mL/min	
Inj. Volume:	50 µL	
Detection:	Pulsed amperometry	
Peaks:	1. Glucose	4.39%
	2. Fructose	6.67
	3. Lactose (int. std.)	–
	4. Sucrose	30.8

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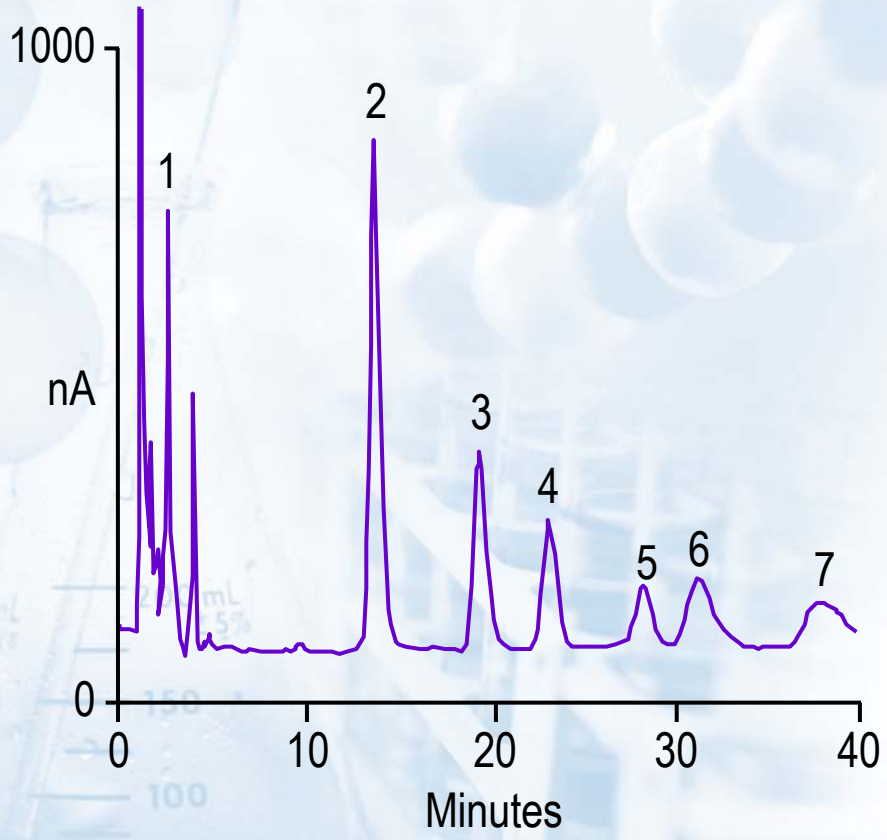
Carbohydrates in Instant Coffee



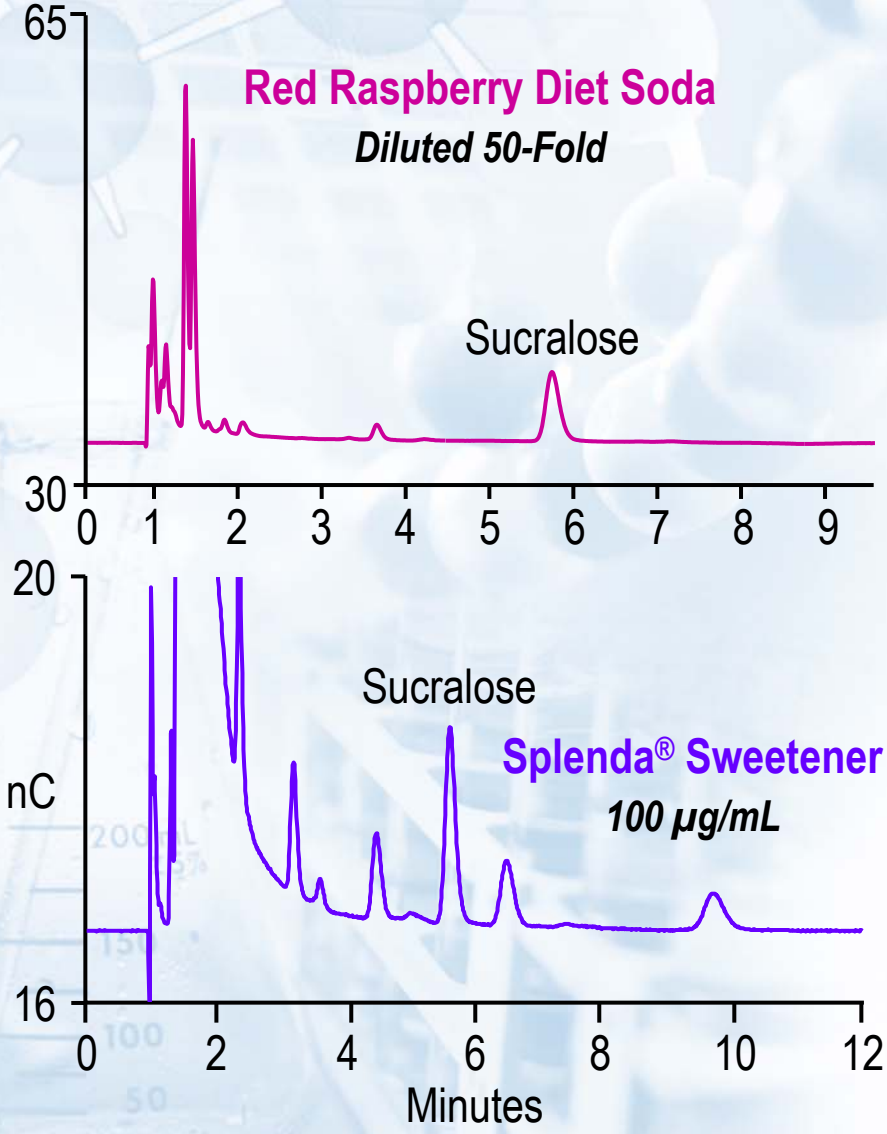
Column:	CarboPac™ PA1
Eluent:	150 mM sodium hydroxide/ deionized water gradient
Inj. Volume:	25 µL of 10 g/L solution
Detector:	Pulsed amperometry, Au electrode; postcolumn addition of 0.3 M NaOH

Peaks:

1.	Mannitol	21	mg/L
2.	Arabinose	140	
3.	Galactose	76	
4.	Glucose	44	
5.	Xylose	26	
6.	Mannose	51	
7.	Fructose	93	

Sample Preparation: Phenolics removed with OnGuard® P cartridge

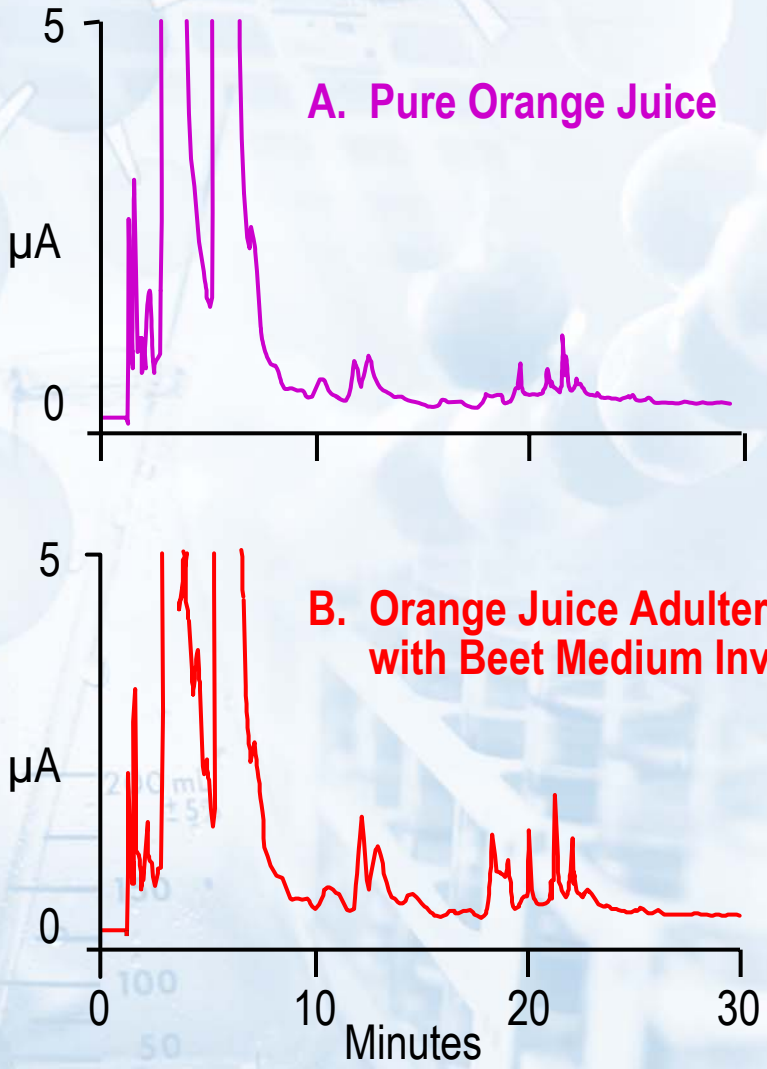
Sucralose in Diet Drink and Artificial Sweetener Product



Column:	CarboPac™ PA20 with guard
Eluents:	40 mM sodium hydroxide/ 75 mM sodium acetate
Temperature:	30 °C
Flow Rate:	0.5 mL/min, 10-g/L solution
Inj. Volume:	25 µL
Detection:	Pulsed amperometric detection, carbohydrate waveform, disposable gold working electrode

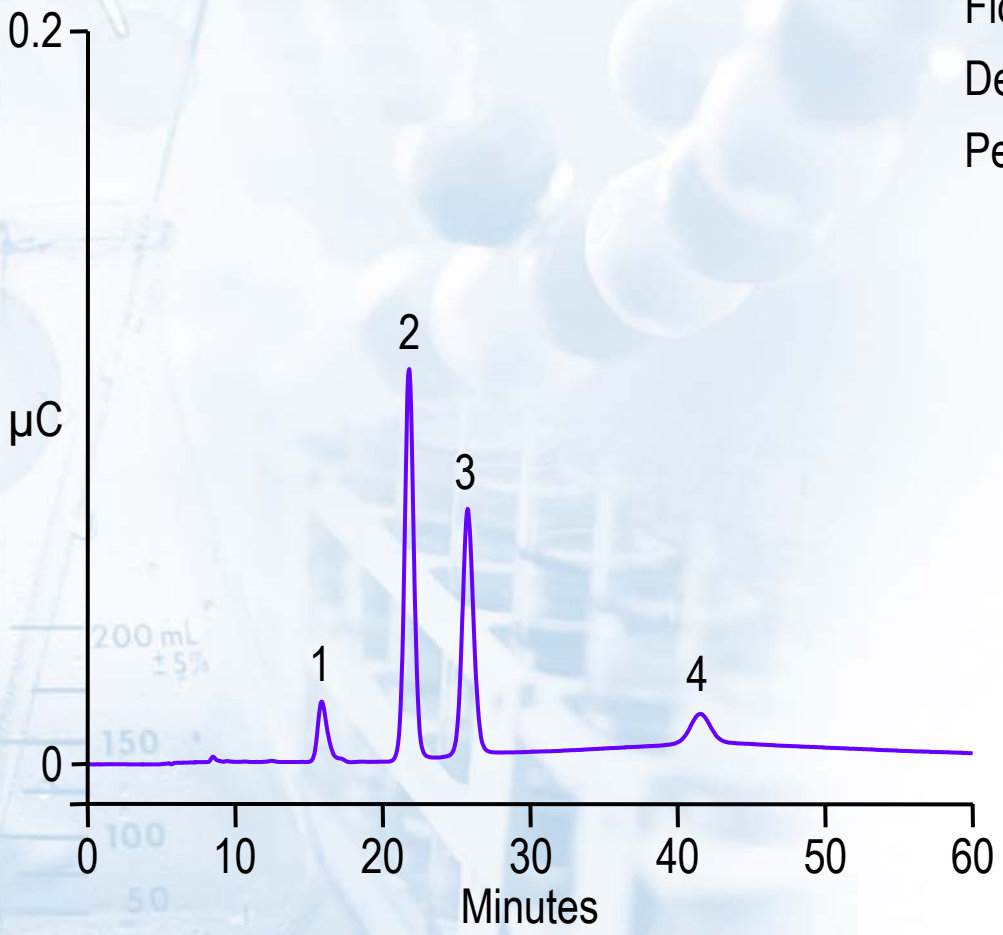
Splenda is a registered trademark of McNeil-PP

Oligosaccharide Profiles of Pure and Adulterated Orange Juice



Column:	CarboPac™ PA-100
Eluent:	Sodium hydroxide/ sodium acetate gradient
Flow Rate:	1 mL/min
Conc.:	2 mg/mL
Inj. Volume:	25 µL
Detection:	Pulsed amperometry, Au electrode

Apple Juice



Column:	CarboPac™ MA1
Eluent:	500 mM sodium hydroxide
Flow Rate:	0.4 mL/min
Detection:	Pulsed amperometry
Peaks:	1. Sorbitol 14 µg
	2. Glucose 130
	3. Fructose 36
	4. Sucrose 0.2

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