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Carbohydrate Analysis: Column Chemistries and Detection

Joe Romano
Waters Corporation
Carbohydrates in Feeds Methodology Forum
AOAC 2007 Annual Meeting
Anaheim, CA
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Separation Modes for Carbohydrate Analysis

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- Reversed Phase
- Partition or Normal Phase
- Size Exclusion
- Ion Exclusion and Size Exclusion Combined
- Ligand Exchange and Size Exclusion Combined

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Detectors for Carbohydrate Analysis

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- Refractive Index Detection
- Evaporative Light Scattering Detection (ELSD)
- Pulsed Amperometric Detection (PAD)

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Reversed Phase Separation with Refractive Index Detection

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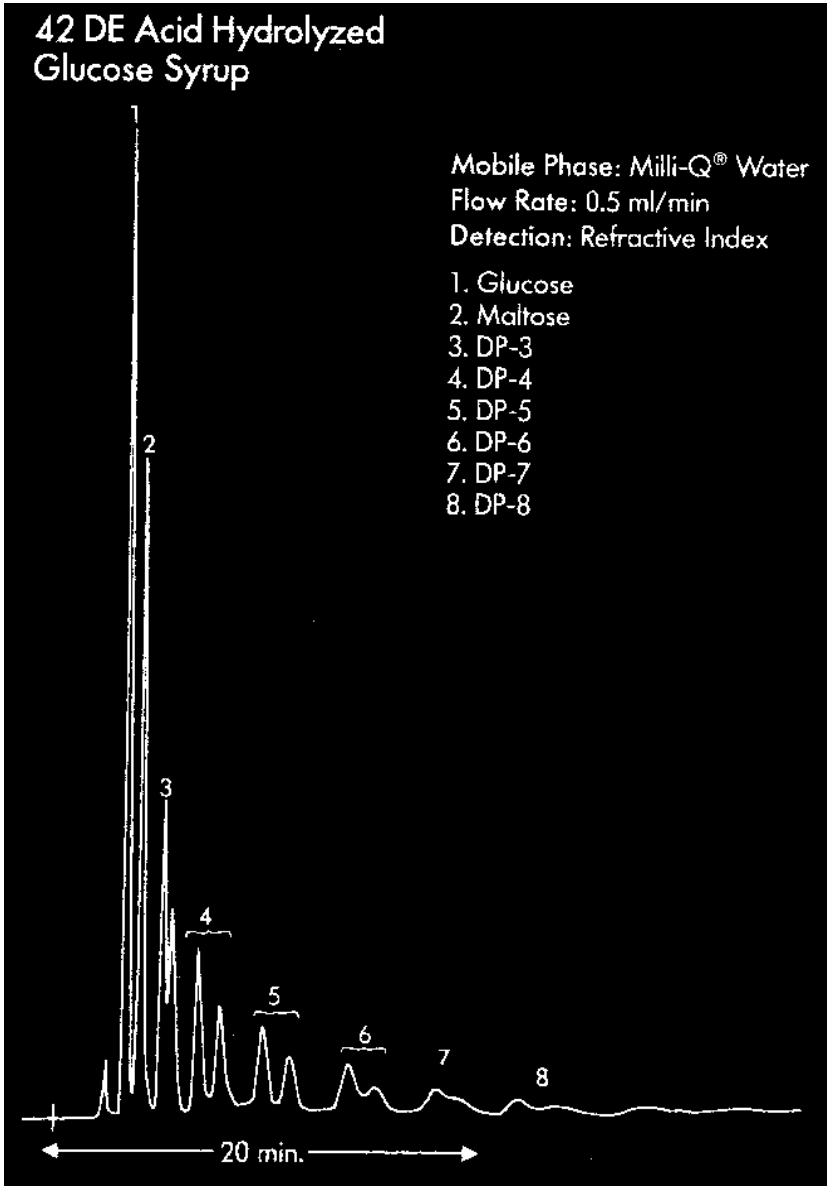

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Maltose (Alpha 1-4) Oligomers with Reversed Phase/ PAD

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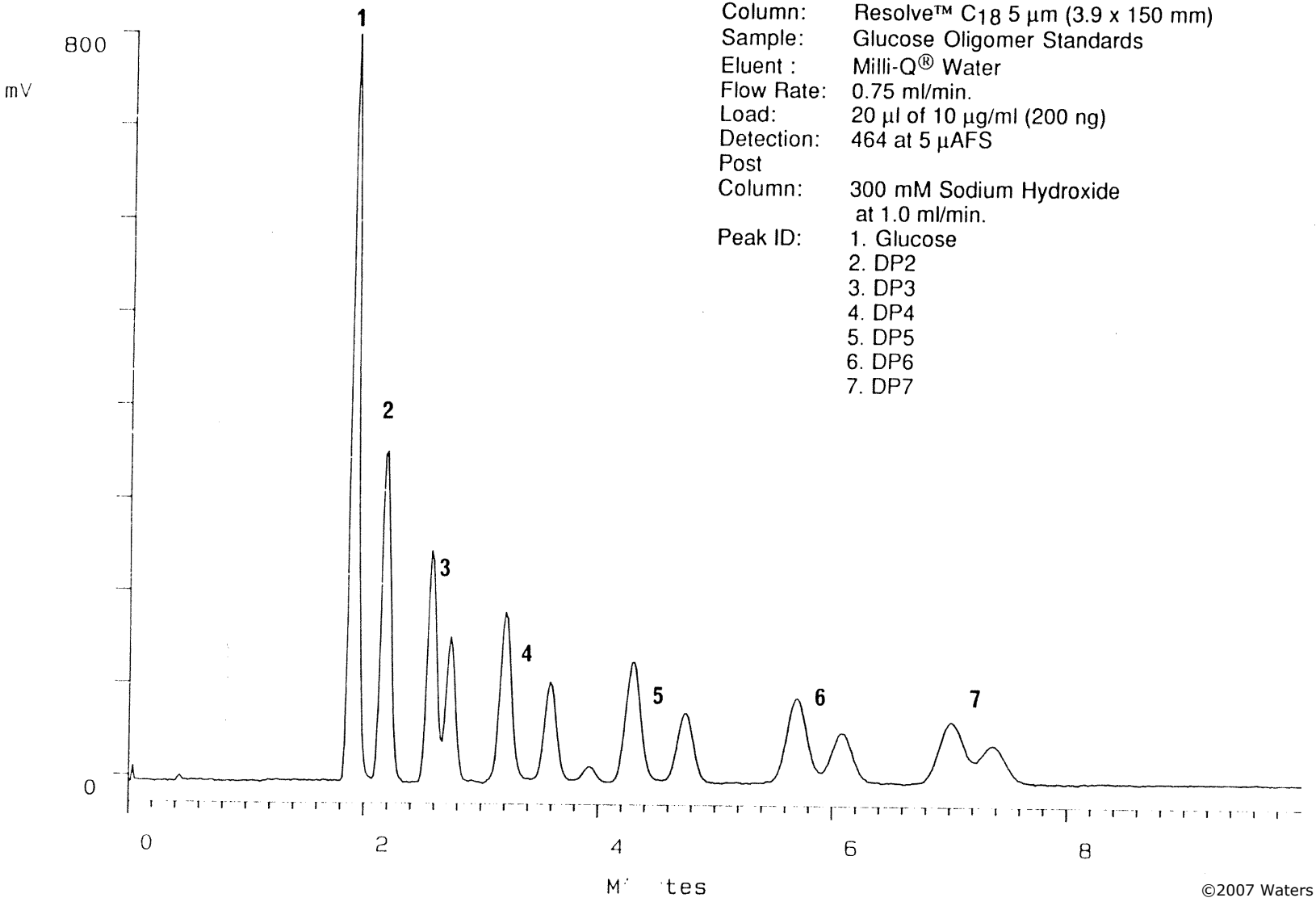

Column:	Resolve™ C_{18} 5 µm (3.9 x 150 mm)
Sample:	Glucose Oligomer Standards
Eluent :	Milli-Q® Water
Flow Rate:	0.75 ml/min.
Load:	20 µl of 10 µg/ml (200 ng)
Detection:	464 at 5 µAFS
Post Column:	300 mM Sodium Hydroxide at 1.0 ml/min.
Peak ID:	1. Glucose 2. DP2 3. DP3 4. DP4 5. DP5 6. DP6 7. DP7

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Laminarin (Beta 1-3) Oligomers with Reversed Phase (Resolve C18)/ ELSD

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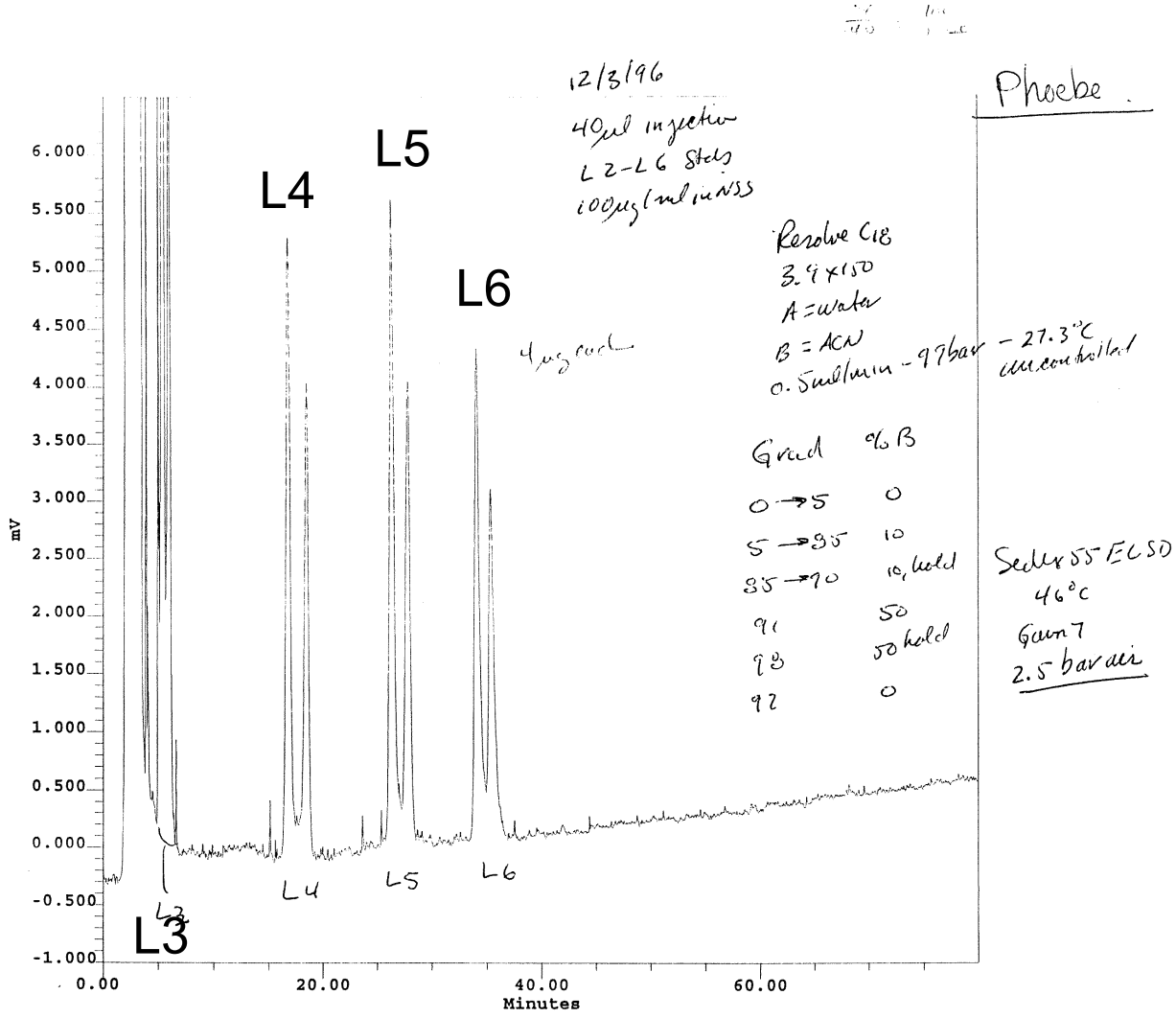

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Laminarin (Beta 1-3) Oligomers with Reversed Phase (Hypercarb)/ ELSD

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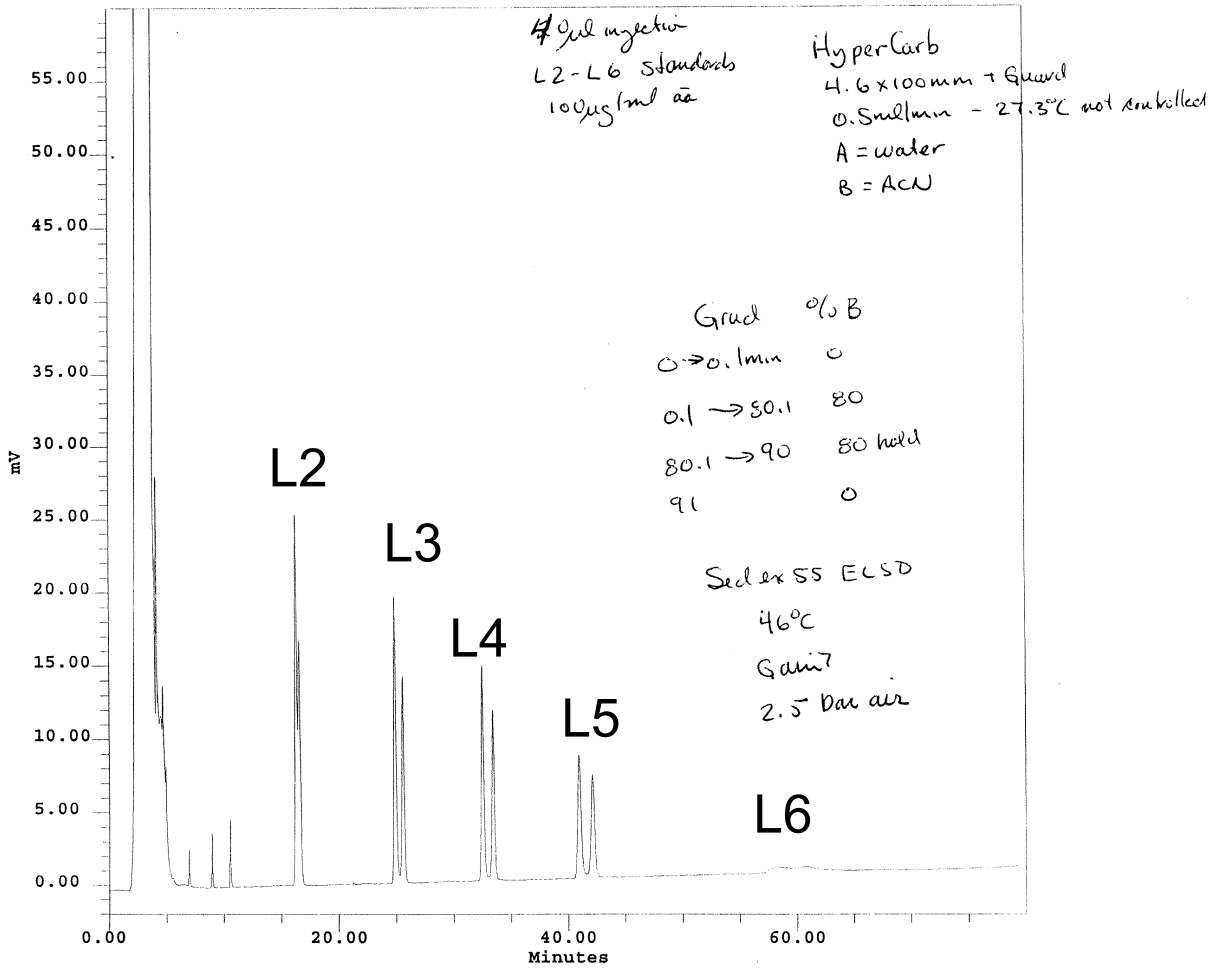

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Separation Modes for Carbohydrate Analysis

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- Ion Exclusion and Size Exclusion Combined
- Ligand Exchange and Size Exclusion Combined

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Food Mono- and Disaccharides: Normal Phase/ Partition/ HILIC

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- Challenge: Trying to resolve carbohydrates of:
  - Different MW's
  - Different isomers within a MW
- Options: Column Chemistries
  - Base particle: silica or polymer
  - Bonded phase: amine, diol, amide, polyamine
  - Amine-modified (coated) silica: spermine (SAM 1) or guanidine carbonate (SAM2), triethylamine

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Food & Beverage

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- Nutritional labeling of food products requires listing of sugar and total carbohydrate content
- Common sugars defined as:
  - Monosaccharides: fructose & glucose
  - Disaccharides: sucrose, maltose & lactose
- AOAC LC Methods recommend the use of propyl amine functional columns for analysis of mono & disaccharides in food products
- Current AOAC LC Methods (amino-based columns)
  - 977.20 Honey
  - 980.20 Chocolate
  - 982.14 Presweetened cereal
  - 984.17 Licorice extracts
  - 984.22 Purity of lactose

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Typical Samples

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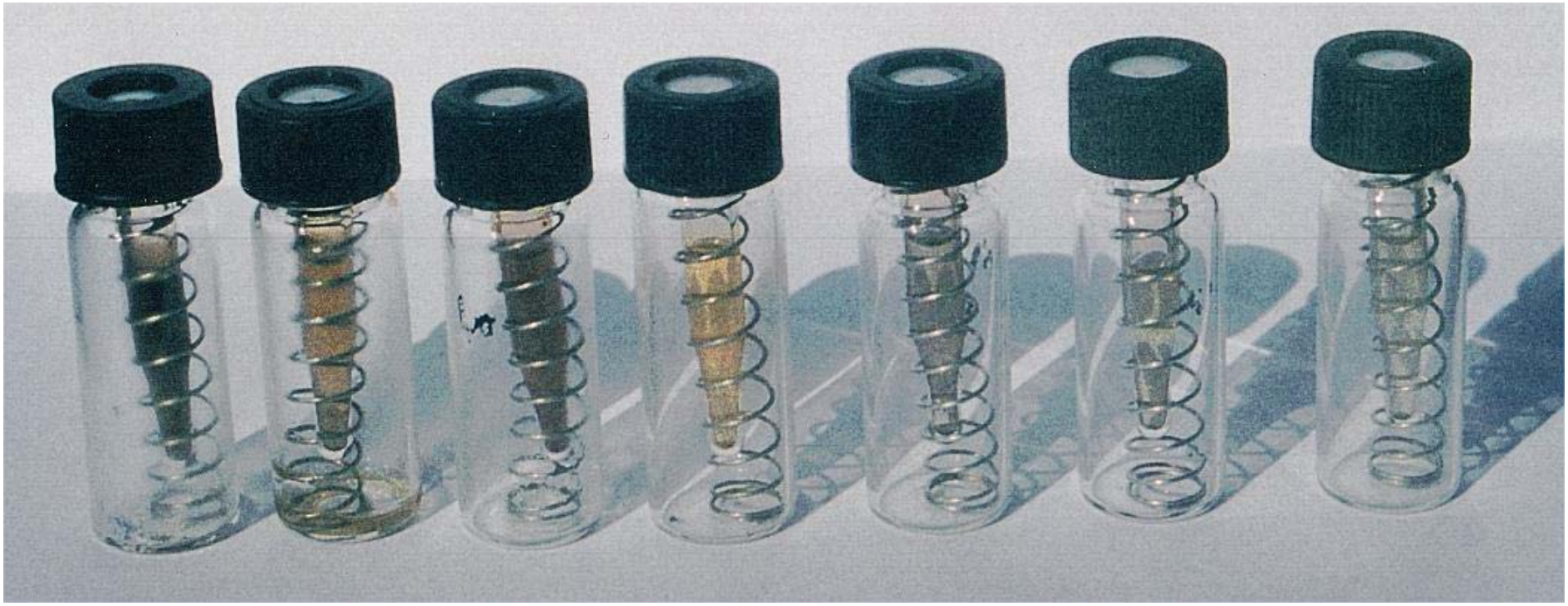

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Mono- and Disaccharide Analysis Partition or HILIC Chromatography

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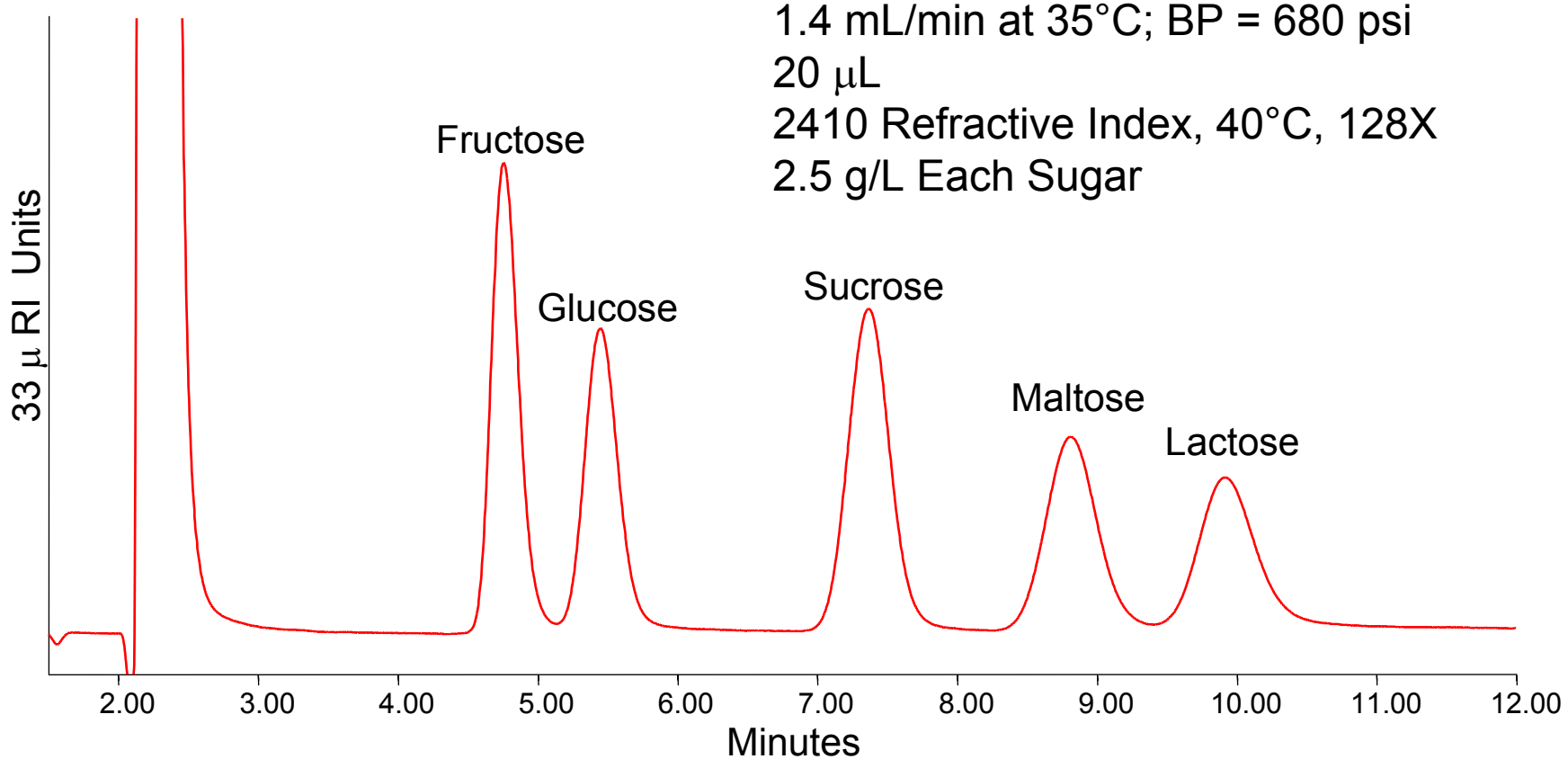
HP Carbohydrate Column, 4.6 mm x 25 cm
80% ACN / 20% Water
1.4 mL/min at 35°C; BP = 680 psi
20 µL
2410 Refractive Index, 40°C, 128X
2.5 g/L Each Sugar



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Sucralose & Sugars with ELSD

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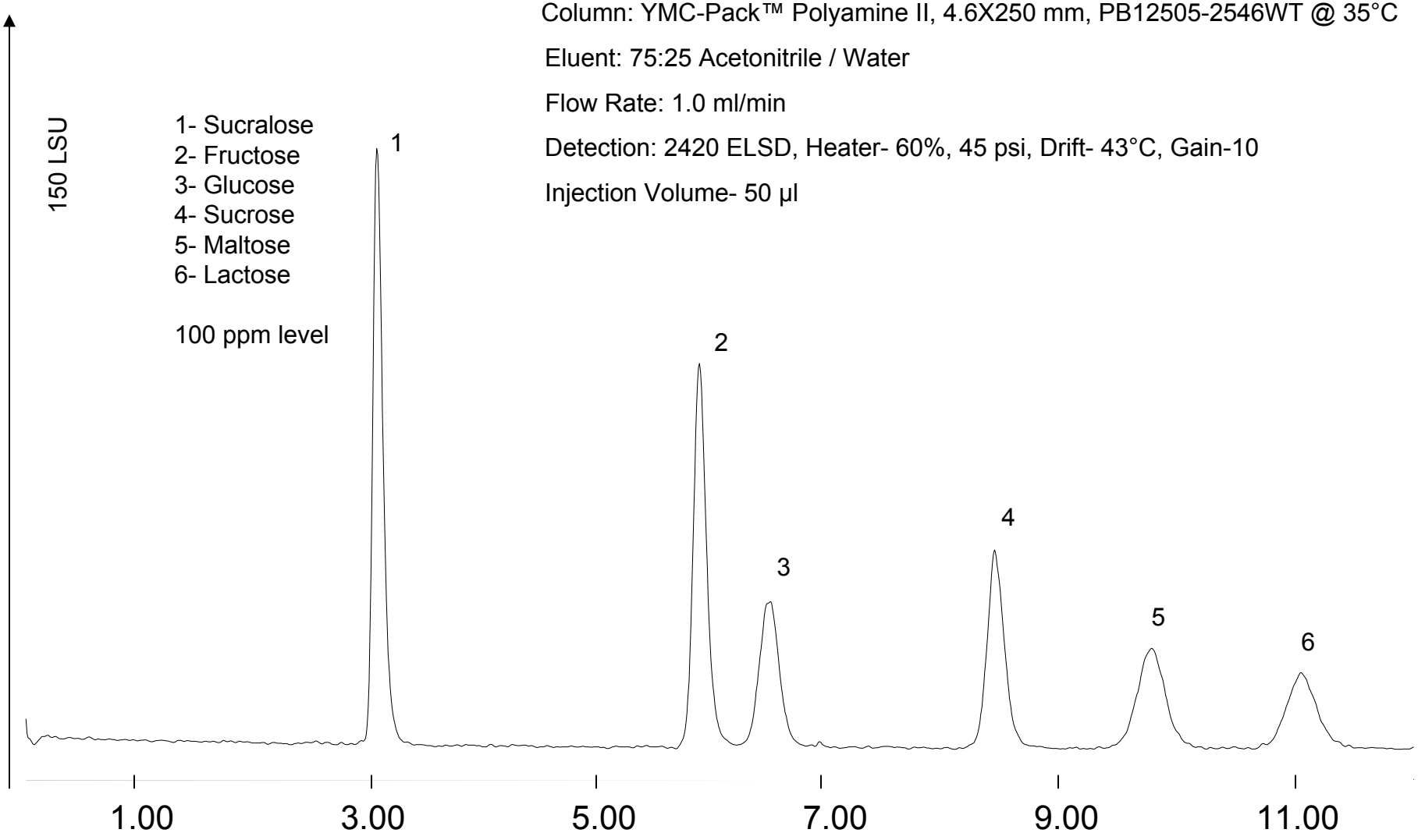
Column: YMC-Pack™ Polyamine II, 4.6X250 mm, PB12505-2546WT @ 35°C

Eluent: 75:25 Acetonitrile / Water

Flow Rate: 1.0 ml/min

Detection: 2420 ELSD, Heater- 60%, 45 psi, Drift- 43°C, Gain-10

Injection Volume- 50 µl



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Effect of Acetonitrile Concentration on Elution Time

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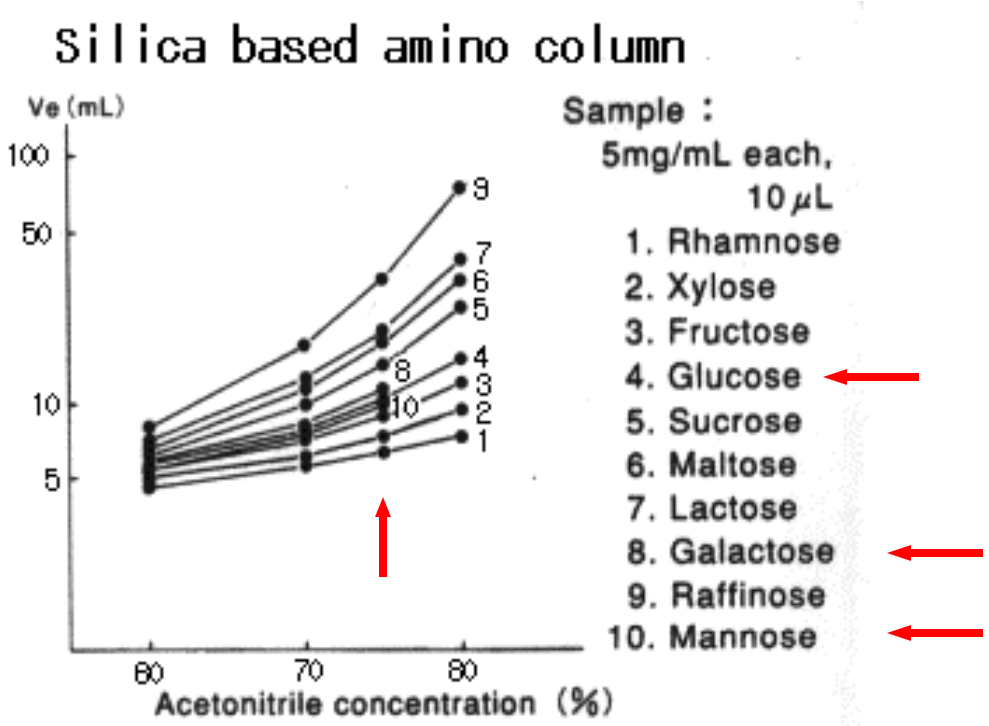

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Carbohydrates by Partition Chromatography

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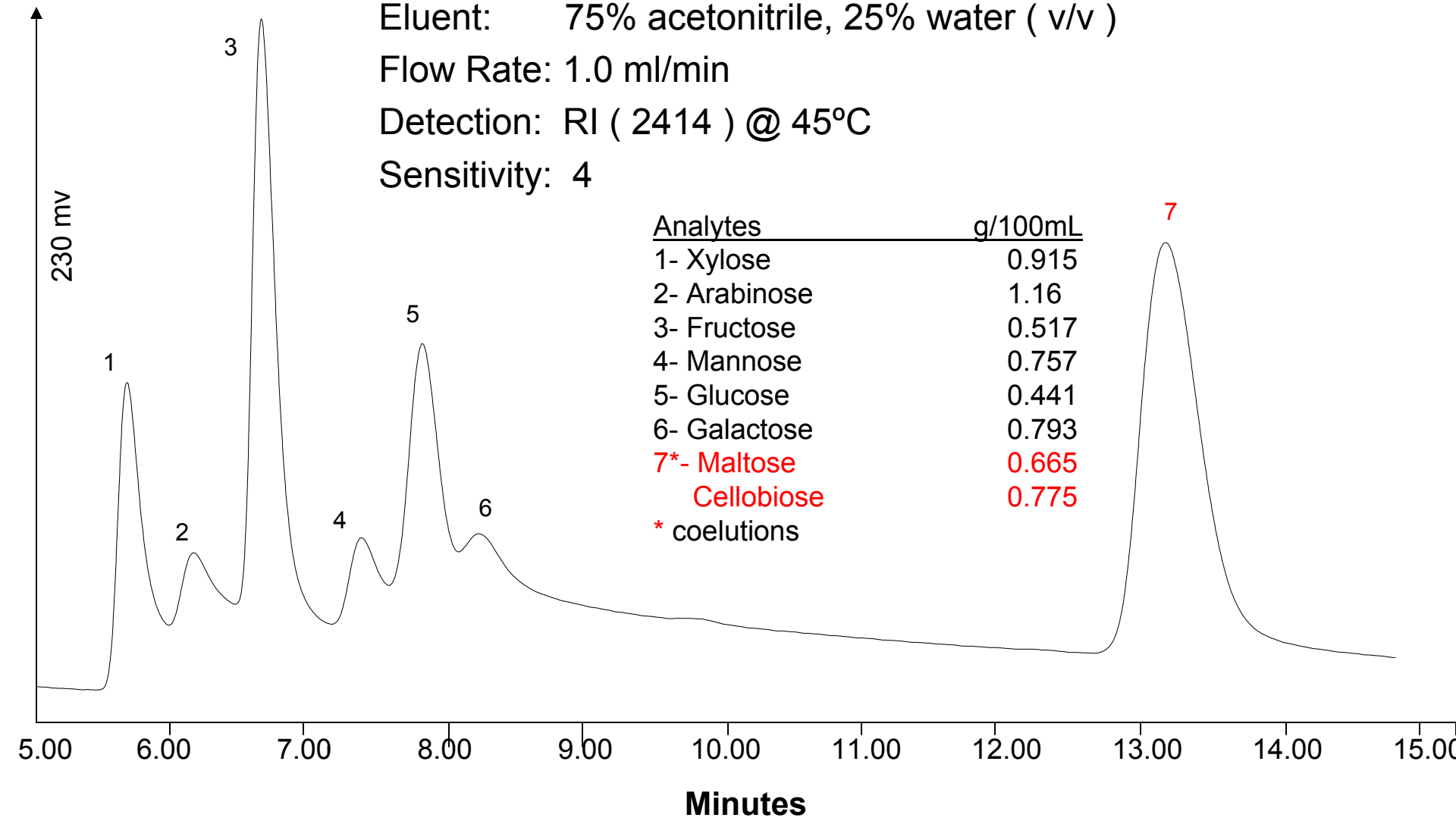
Column: Waters Carbohydrate Analysis, 4.6X250 mm @ 35ºC
Eluent: 75% acetonitrile, 25% water (v/v)
Flow Rate: 1.0 ml/min
Detection: RI (2414) @ 45ºC
Sensitivity: 4

Analytes	g/100mL
1- Xylose	0.915
2- Arabinose	1.16
3- Fructose	0.517
4- Mannose	0.757
5- Glucose	0.441
6- Galactose	0.793
7*- Maltose	0.665
Cellobiose	0.775

* coelutions



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Separation Modes for Carbohydrate Analysis

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- Reversed Phase
- Partition or Normal Phase
- Size Exclusion (Polymers)
- Ion Exclusion and Size Exclusion Combined
- Ligand Exchange and Size Exclusion Combined
- Anion Exchange

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Ethanol Fermentation Analysis Using Breeze™ HPLC System

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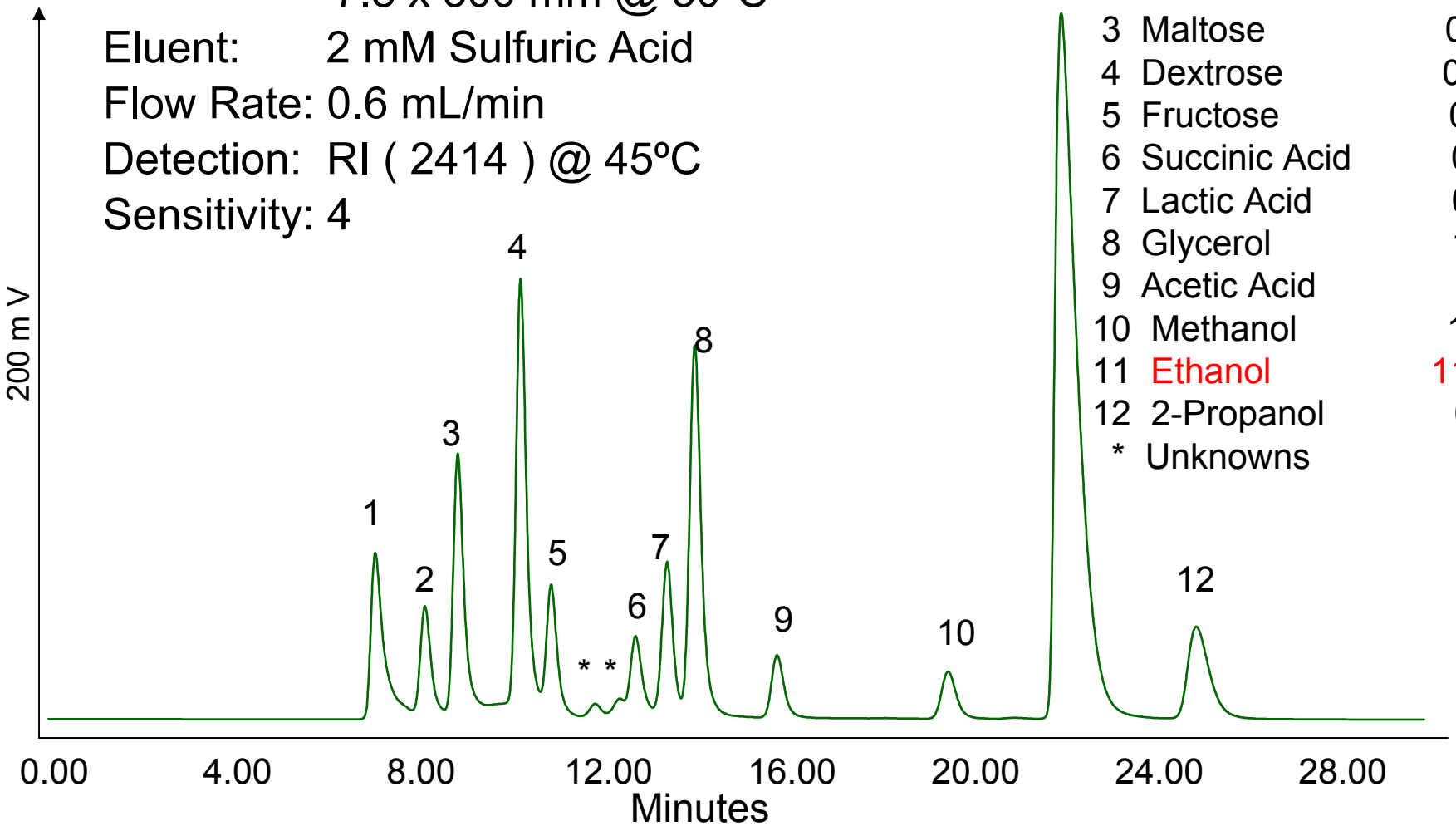
Column: Waters Ion Exclusion 7.8 x 300 mm @ 50ºC
Eluent: 2 mM Sulfuric Acid
Flow Rate: 0.6 mL/min
Detection: RI (2414) @ 45ºC
Sensitivity: 4

	Analytes	g/100 mL
1	Dextrin	0.71
2	Maltotriose	0.25
3	Maltose	0.55
4	Dextrose	0.97
5	Fructose	0.24
6	Succinic Acid	0.28
7	Lactic Acid	0.83
8	Glycerol	1.11
9	Acetic Acid	0.40
10	Methanol	1.19
11	Ethanol	11.85
12	2-Propanol	0.75
*	Unknowns	



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Ethanol Fermentation Analytes

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Current Analytes

1. Dextrin (>DP4)
2. Maltotriose
3. Maltose (alpha 1-4)
4. Glucose (dextrose)
5. Fructose
6. Glycerol
7. Propanol
8. Ethanol
9. Methanol
10. Lactic Acid
11. Succinic Acid
12. Acetic Acid

Biomass Analytes

1. Dextrin (>DP4)
2. Maltotriose
3. Maltose (alpha 1-4)
4. Cellobiose (beta 1-4)
5. Glucose (dextrose)
6. Fructose
7. Galactose
8. Mannose
9. Arabinose
10. Xylose
11. Glycerol
12. Ethanol
13. Methanol
14. Lactic Acid
15. Succinic Acid
16. Acetic Acid

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16 Biomass Carbohydrates by Ion Exclusion/ SEC

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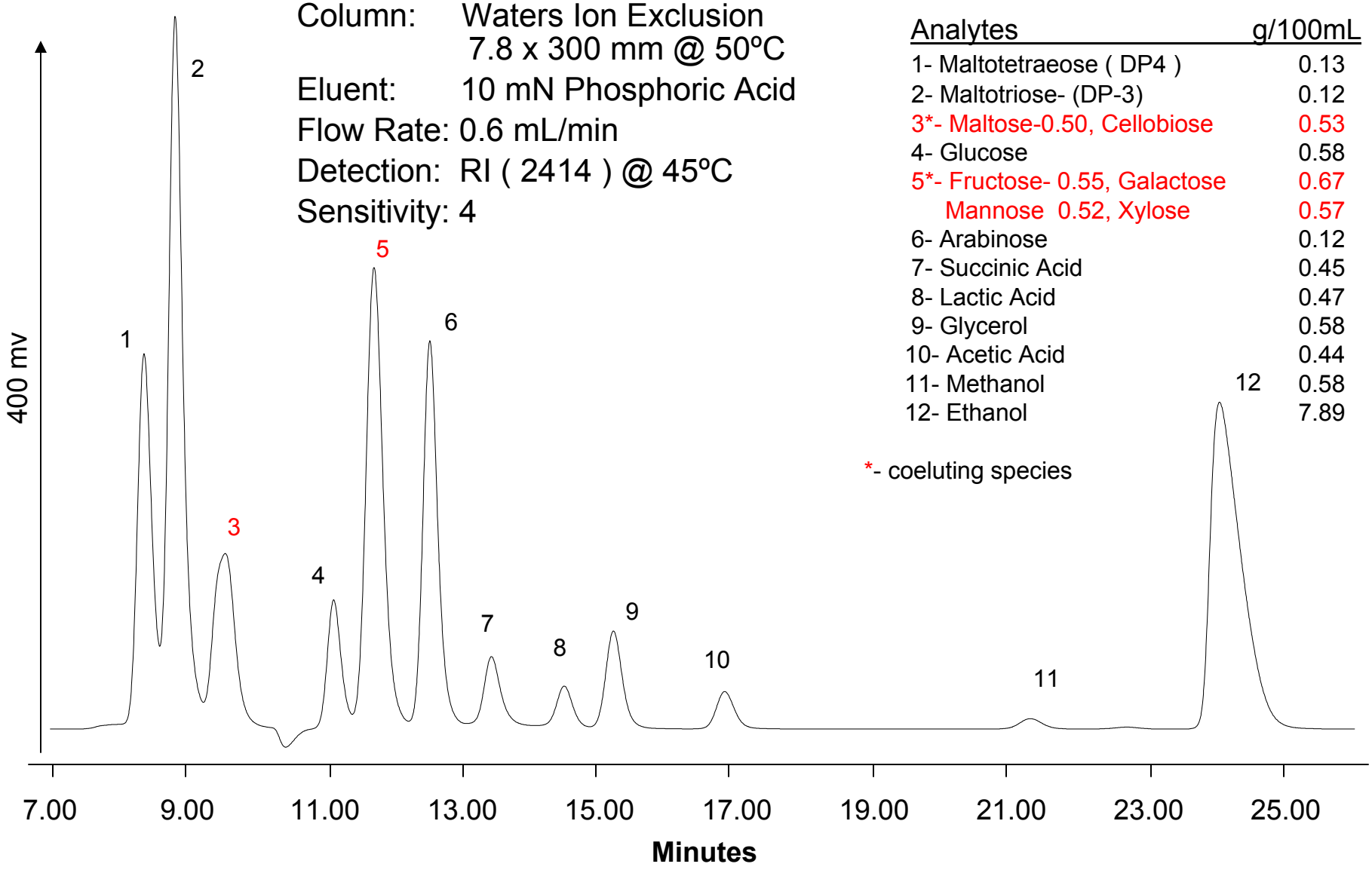
Column: Waters Ion Exclusion 7.8 x 300 mm @ 50ºC
Eluent: 10 mN Phosphoric Acid
Flow Rate: 0.6 mL/min
Detection: RI (2414) @ 45ºC
Sensitivity: 4

Analytes	g/100mL
1- Maltotetraeose (DP4)	0.13
2- Maltotriose- (DP-3)	0.12
3*- Maltose-0.50, Cellobiose	0.53
4- Glucose	0.58
5*- Fructose- 0.55, Galactose	0.67
Mannose 0.52, Xylose	0.57
6- Arabinose	0.12
7- Succinic Acid	0.45
8- Lactic Acid	0.47
9- Glycerol	0.58
10- Acetic Acid	0.44
11- Methanol	0.58
12- Ethanol	7.89

*- coeluting species



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Separation Modes for Carbohydrate Analysis

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- Ion Exclusion and Size Exclusion Combined
- Ligand Exchange and Size Exclusion Combined

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Ligand Exchange and Size Exclusion Combined

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- Cation exchange columns
- Separation based first on size exclusion mode (largest carbohydrate elutes first from column)
- Separation based second on ligand exchange mode (interaction between hydroxyls and metal counter ions)
  - Ca++ and Pb++ (strongest separation)
  - Na+ (weaker separations)

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Explaination of Ligand Exchange Mode

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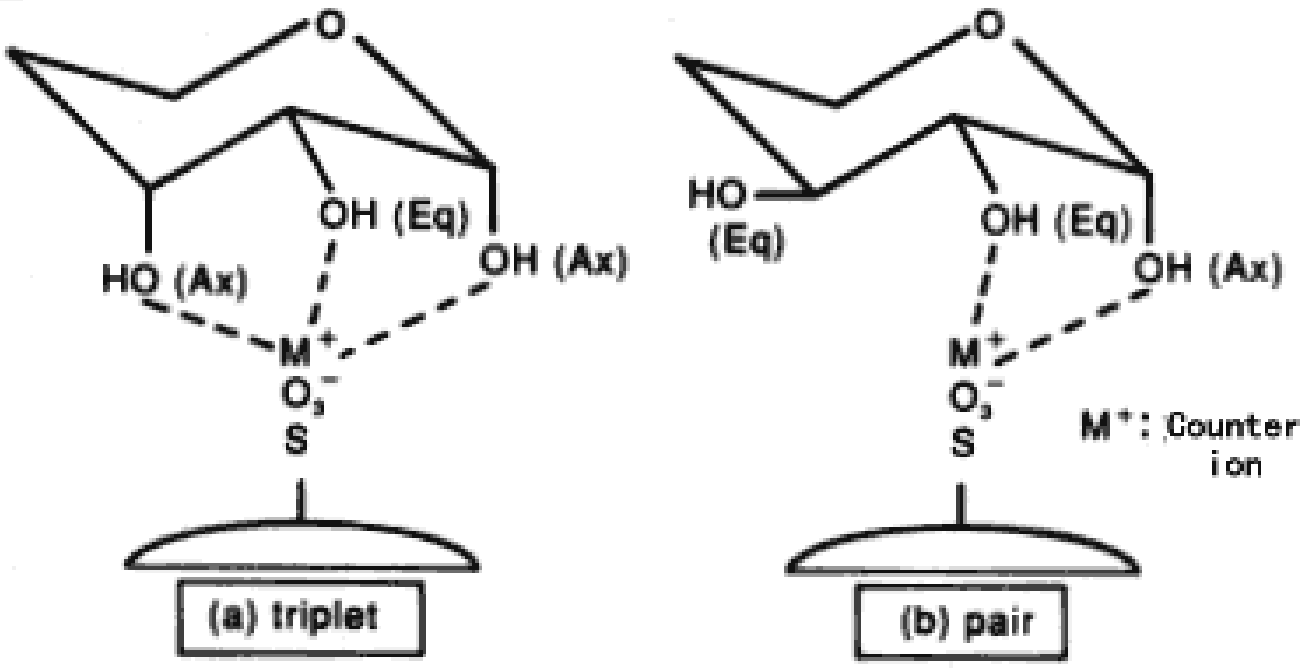

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Separation of Anomers
Ca $^{2+}$ Ligand Exchange/ SEC Column

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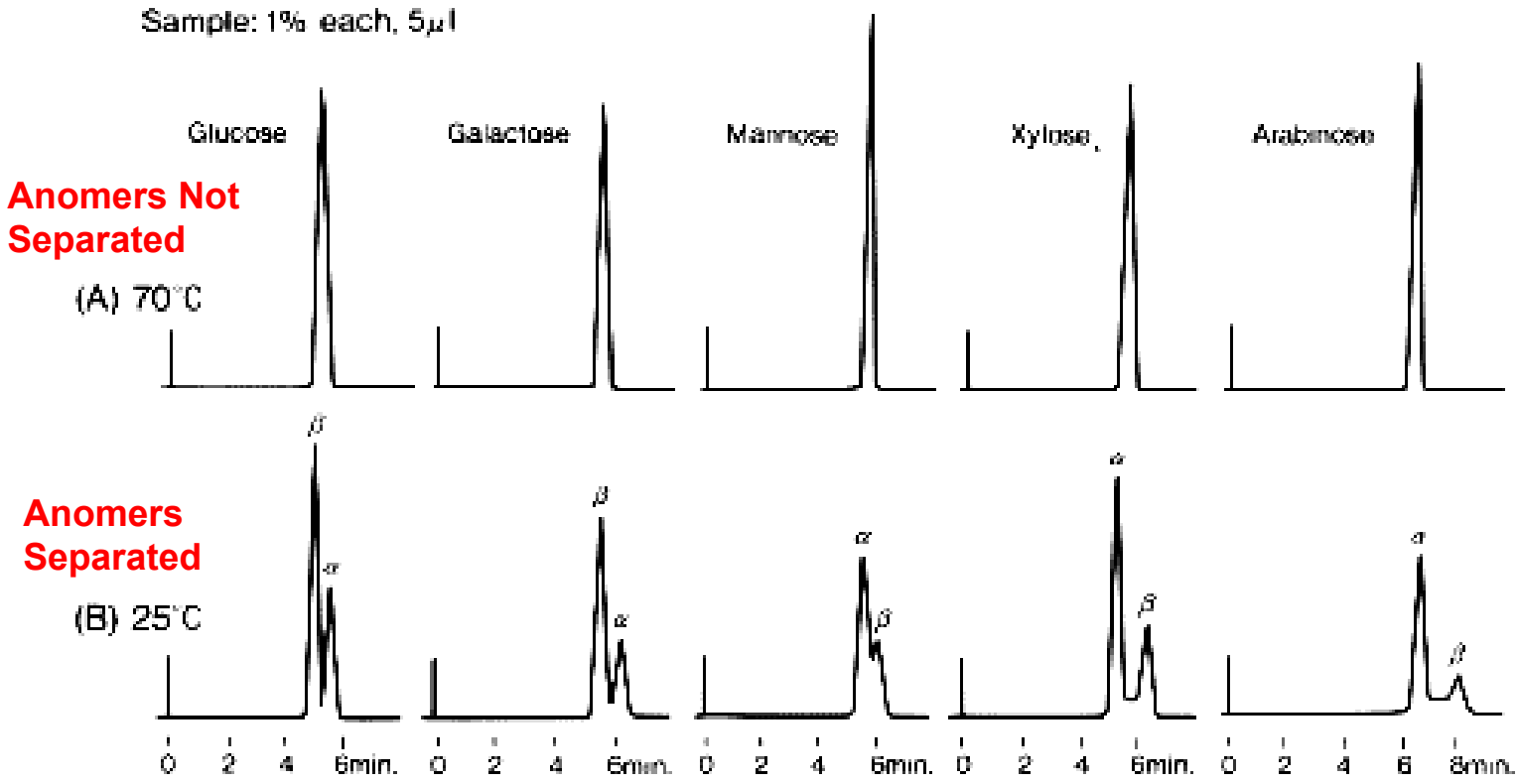
Columns must be run at temperatures between 70 and 90° C



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Effect of Temperature
Ca $^{2+}$ Ligand Exchange/ SEC Column

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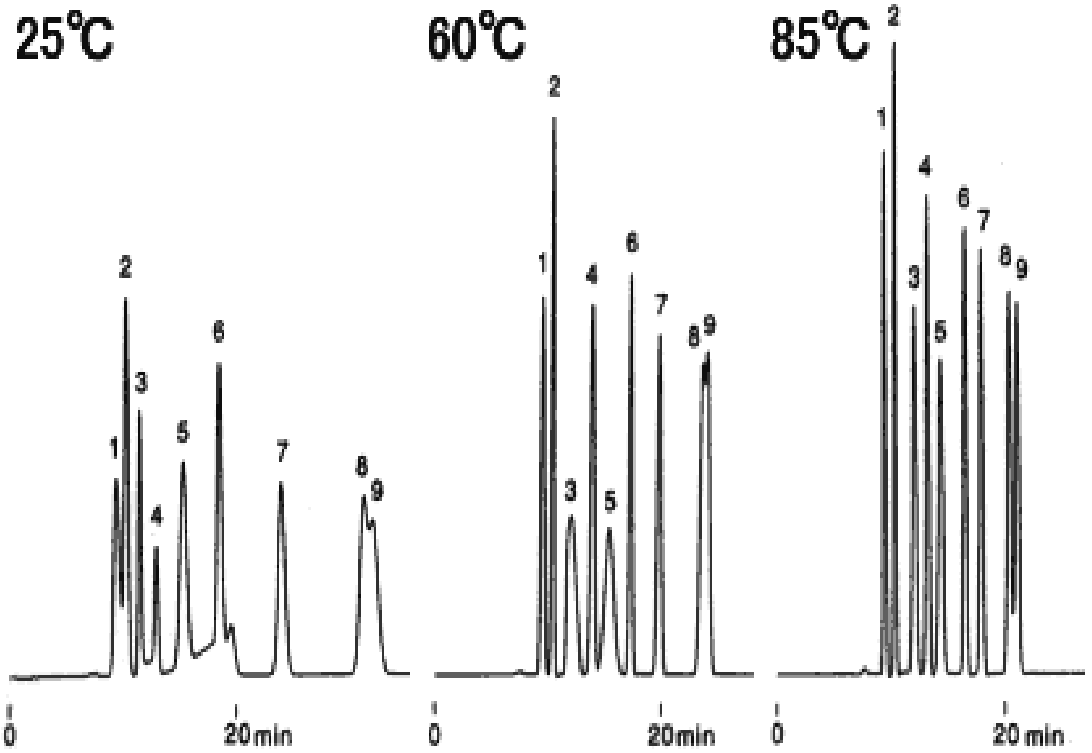

Sample :
0.5% each, 5 μL
1. Isomaltose
2. Sucrose
3. Glucose
4. Lactitol
5. Fructose
6. meso-Erythritol
7. Mannitol
8. Du;citol
9. Xylitol

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Comparison of Ligand Exchange/SEC Columns with Different Counter Ions

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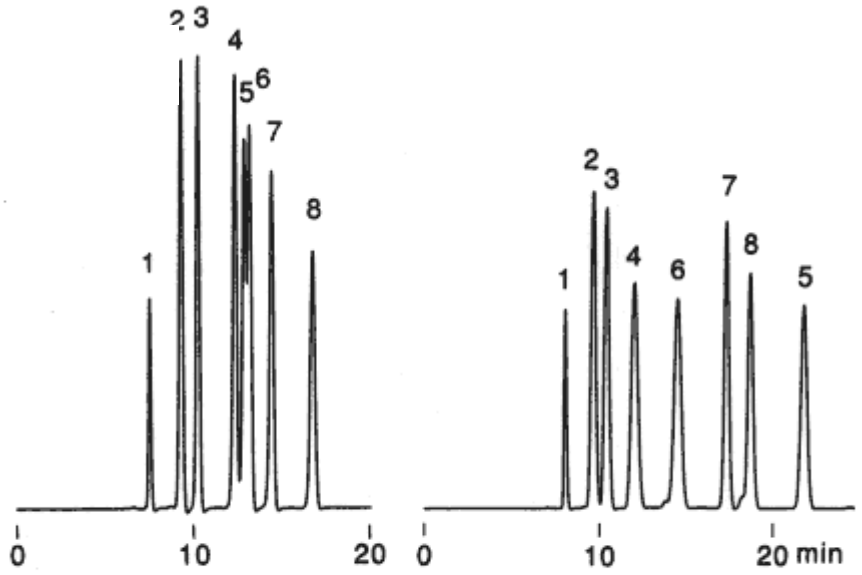
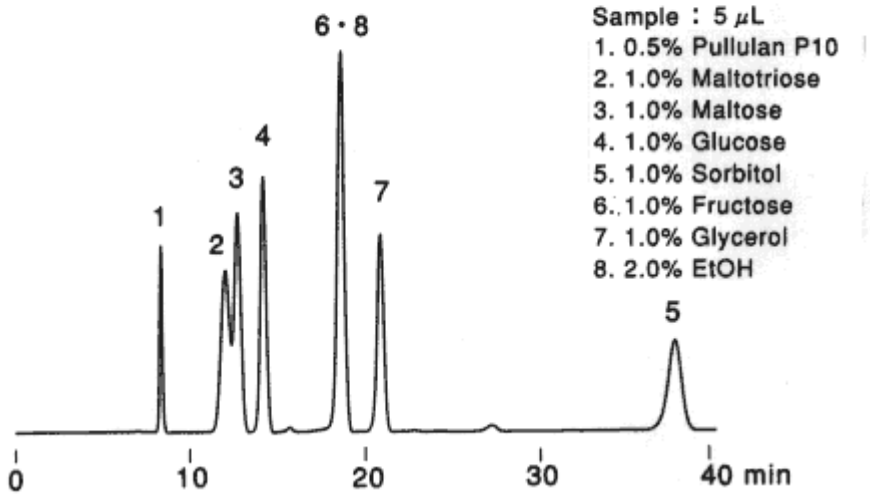
Na^+



Ca^{2+}

Pb^{2+}



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Monosaccharides and Disaccharides
Ca^{2+} Ligand Exchange/SEC

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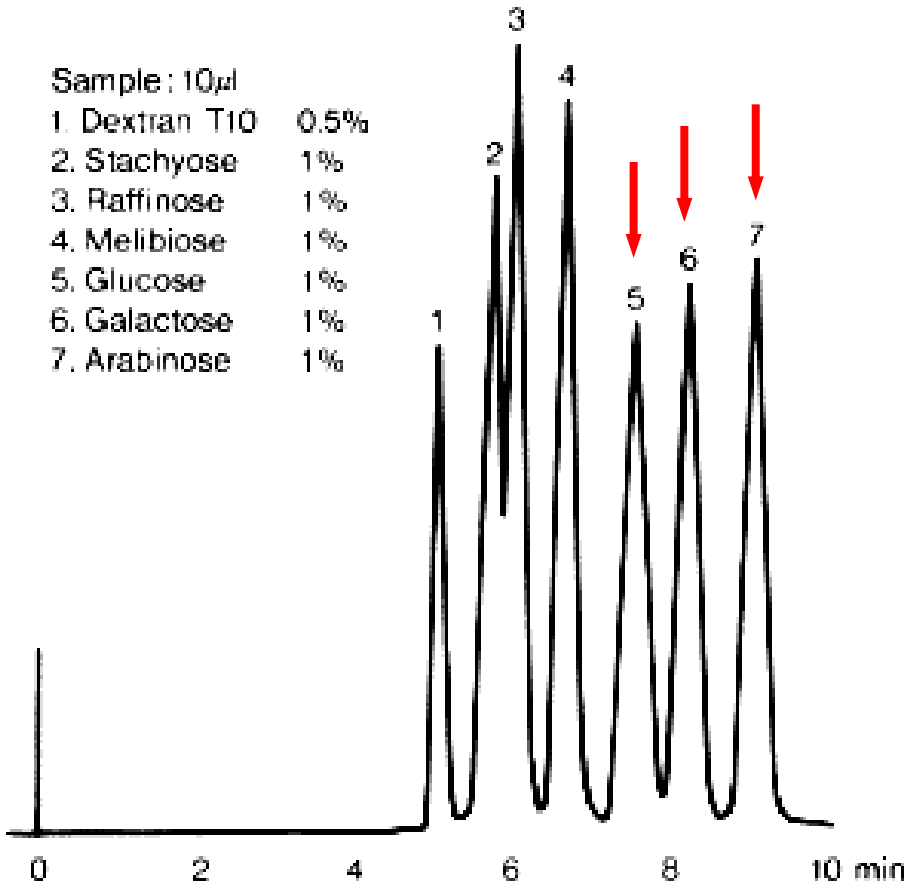

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Biomass Carbohydrates by Ca^{2+} Ligand Exchange/ SEC

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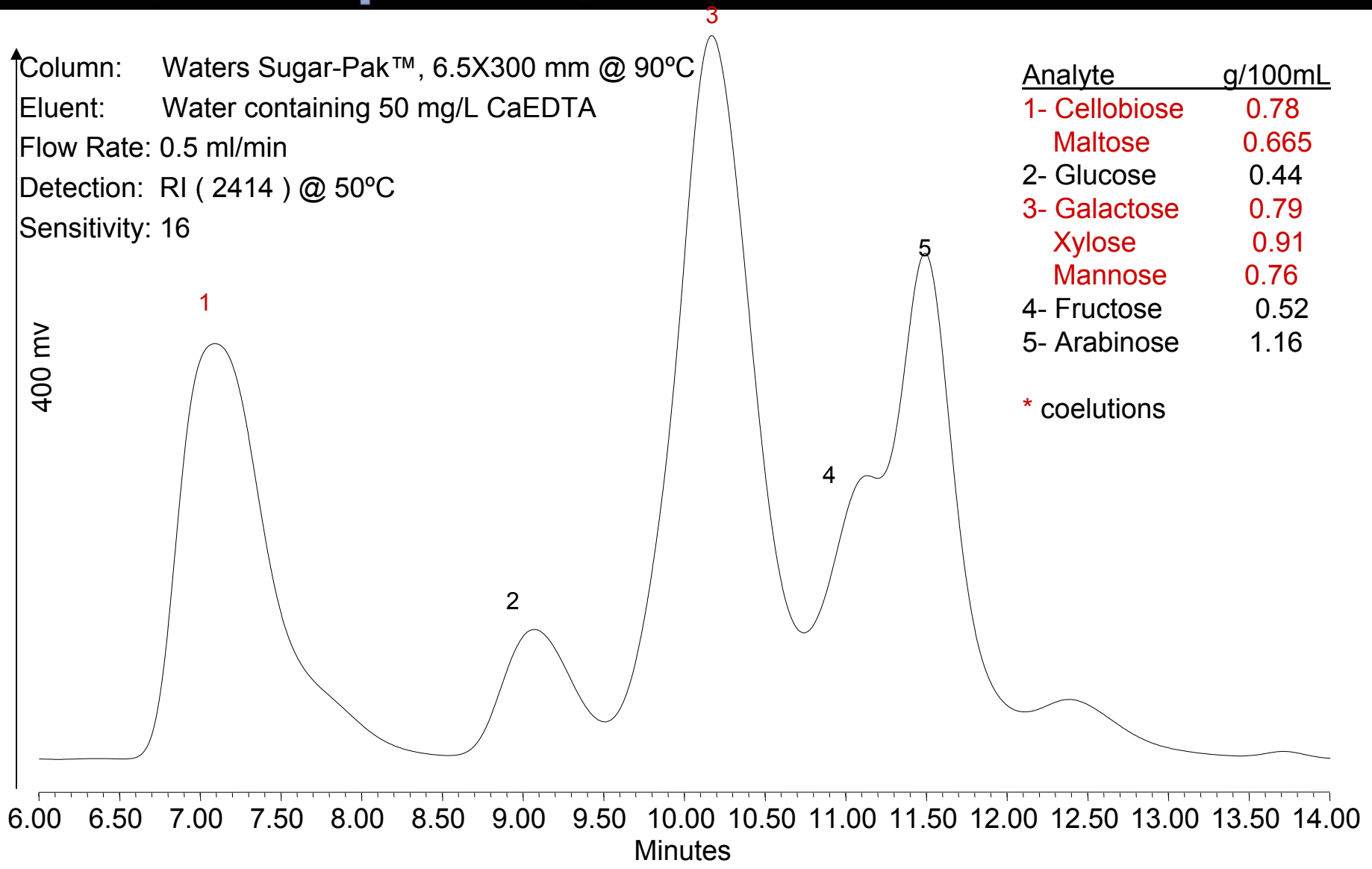

Analyte	g/100mL
1- Cellobiose	0.78
Maltose	0.665
2- Glucose	0.44
3- Galactose	0.79
Xylose	0.91
Mannose	0.76
4- Fructose	0.52
5- Arabinose	1.16

* coelutions

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Monosaccharides and Disaccharides
Pb $^{2+}$ Ligand Exchange/ SEC Column

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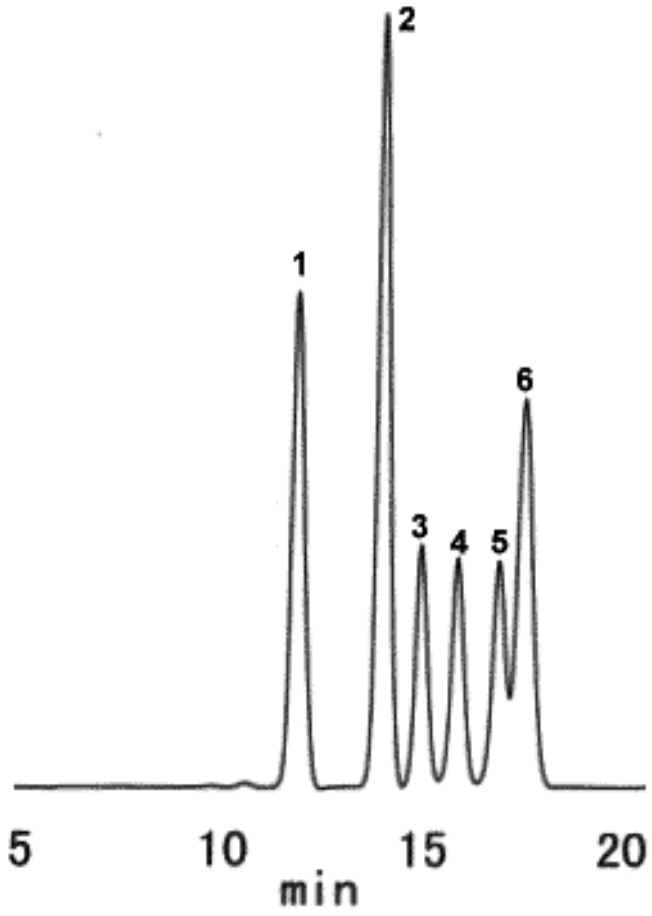

Slow flow rates: 0.6 ml/min

High Temperature: 80º C

Lead salt in mobile phase

Sample preparation required

Sample: 5micro-L
1.Cellobiose 1.0%
2.Glucose 1.5%
3.Xylose 0.5%
4.Galactose 0.5%
5.Arabinose 0.5%
6.Mannose 1.0%

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Summary: Separation Modes for Carbohydrate Analysis

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- Ligand Exchange and Size Exclusion Combined

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