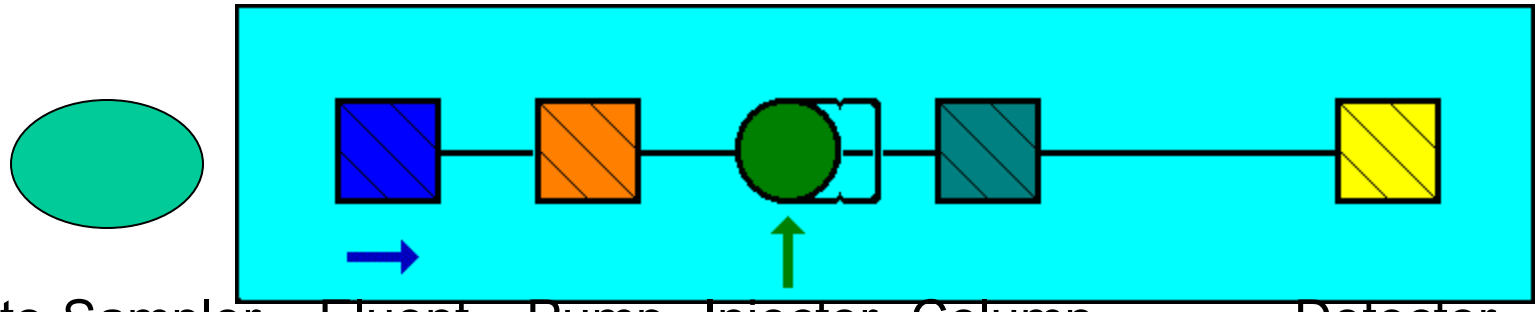
Sugar & Carbohydrate Analysis

A Practical Perspective

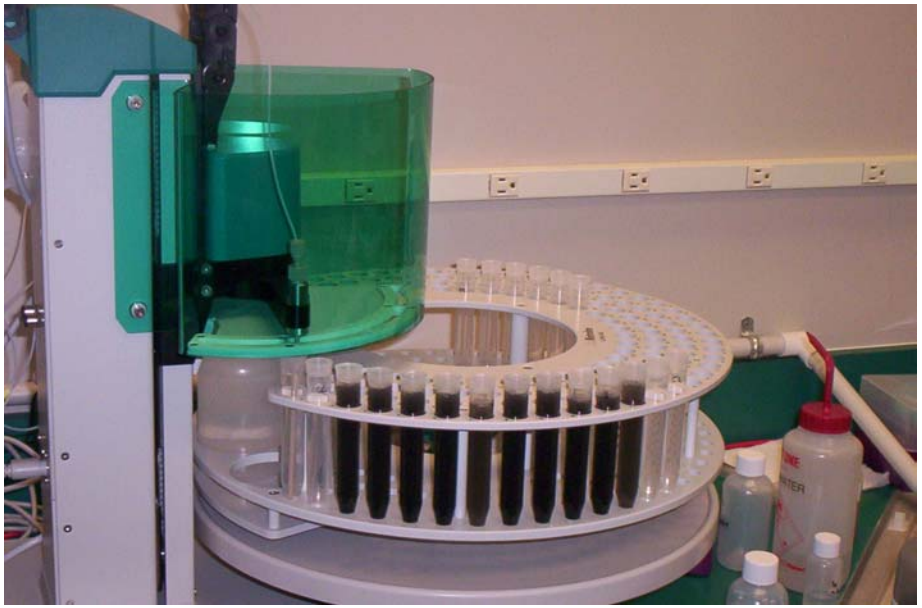
Liquid Chromatography System



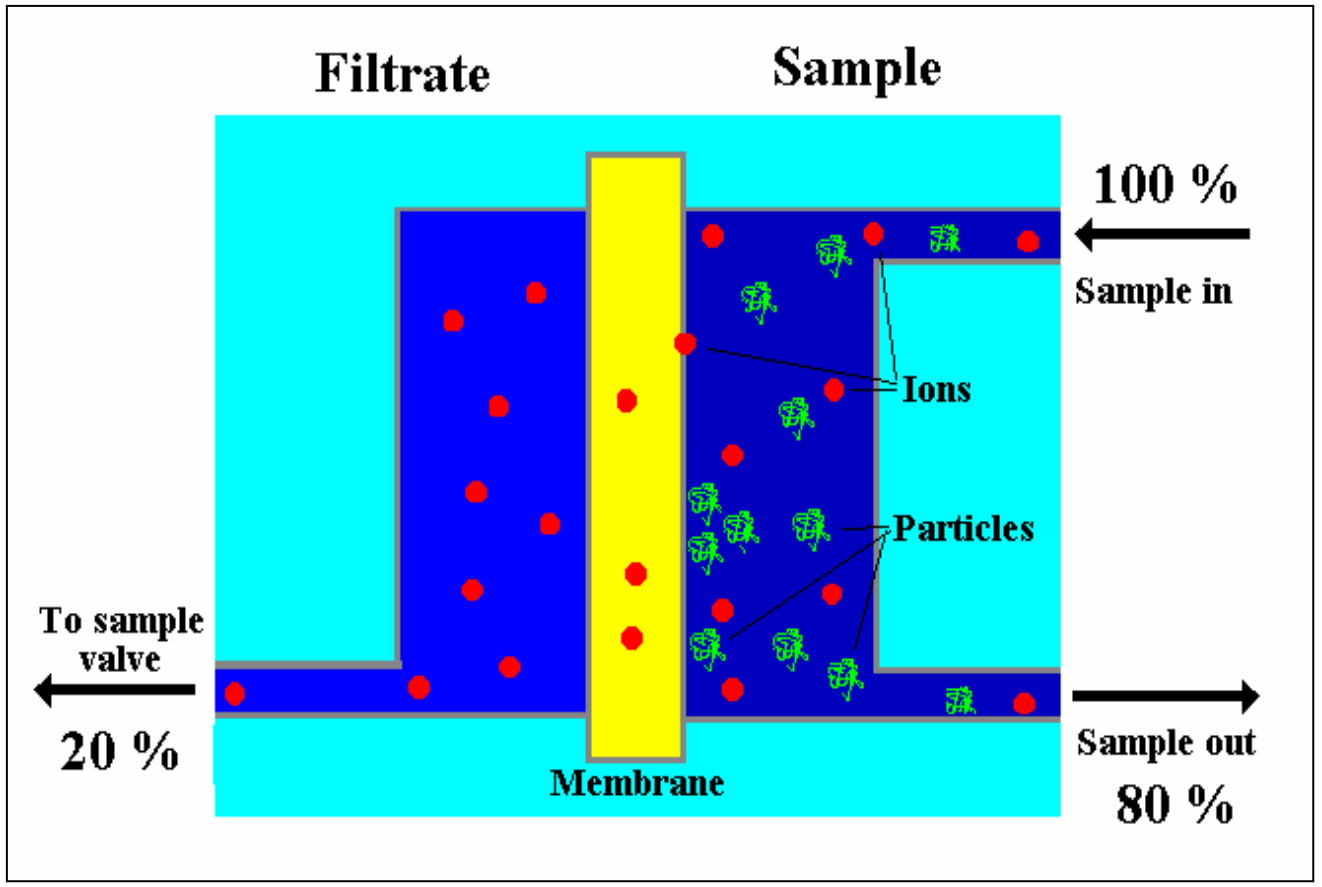
Sample Preparation

Sample Matrix

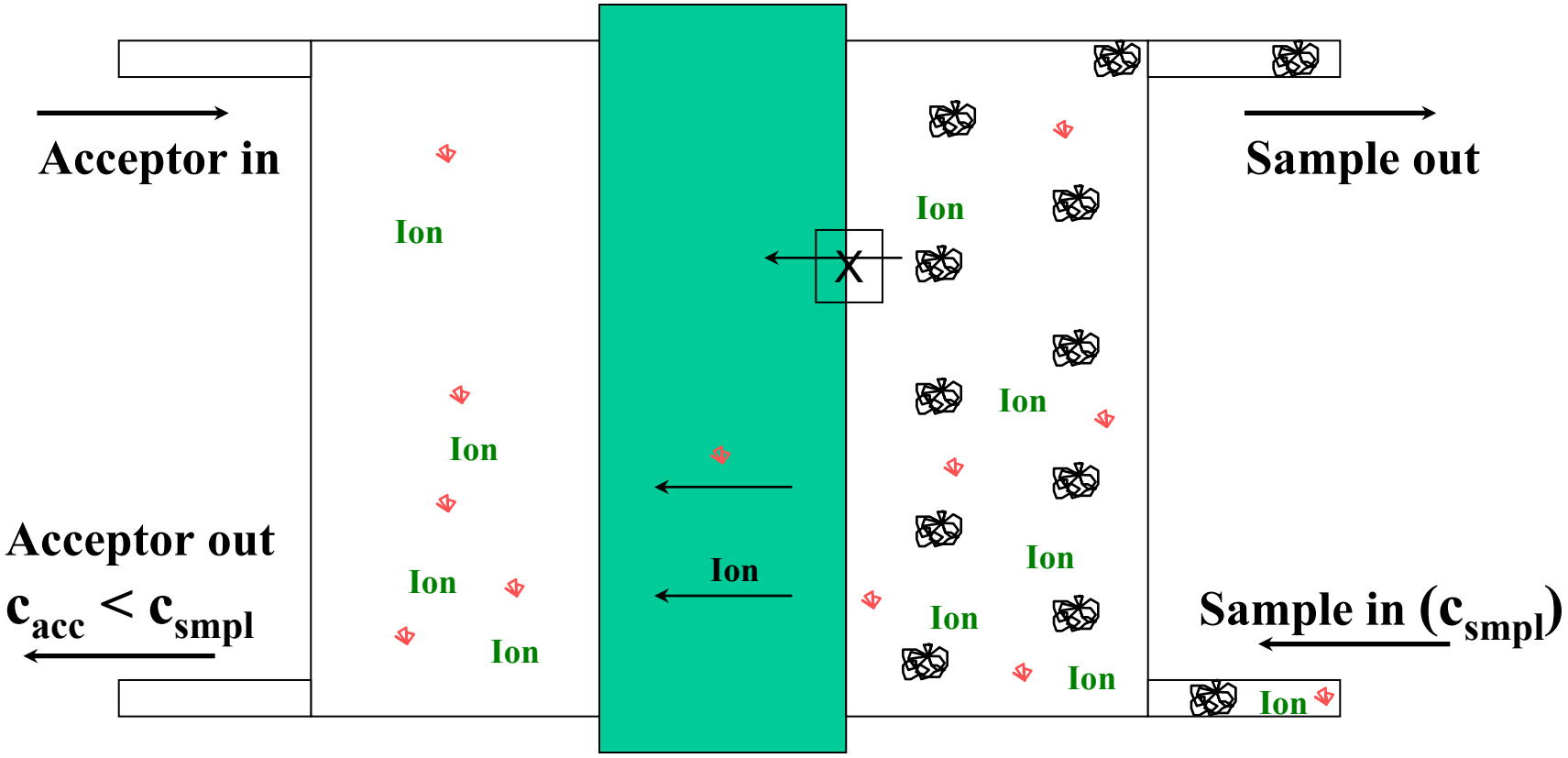
- Grind
- Extract
- Particles – Filtration
- Matrix elimination – extraction, dialysis, centrifuge, etc…



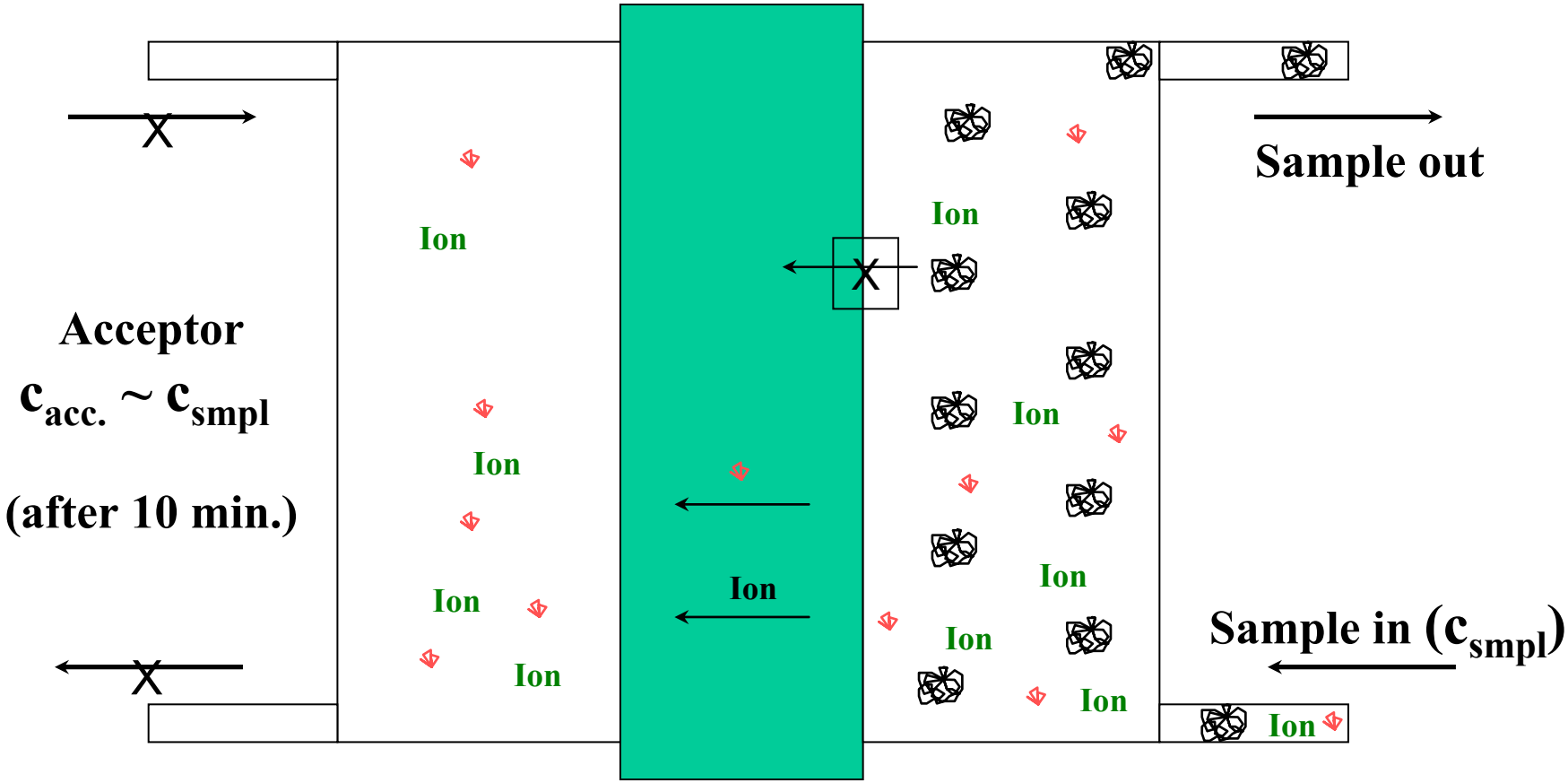
Filtration


Filtrate
Sample
100 %
Sample in
Ions
Particles
To sample valve
20 %
Membrane
Sample out
80 %

Dialysis


Acceptor in
Sample out
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Acceptor out
$c_{acc} < c_{smpl}$
Sample in (c_{smpl})

Dialysis


Sample out
Acceptor
$c_{acc.} \sim c_{smpl}$
(after 10 min.)
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Ion
Sample in (c_{smpl})

Dialysis

- particles
- high molecular weights
- high organic load
- oils

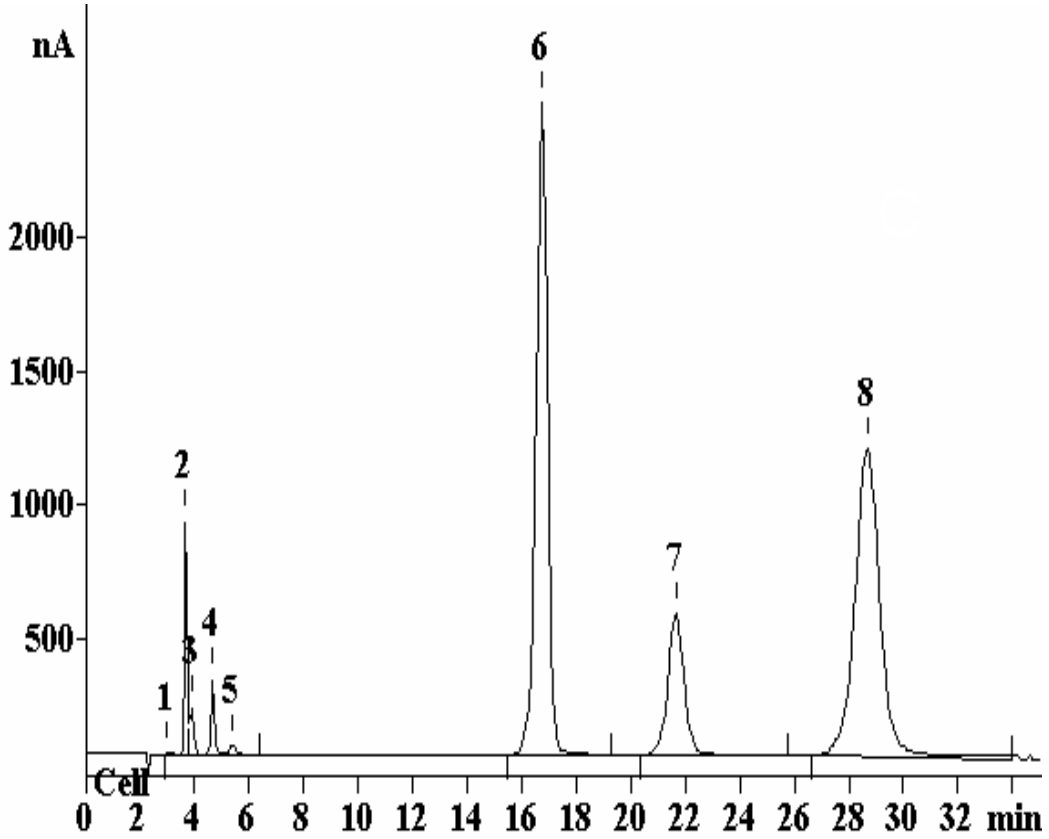
- fats
- proteins
- dyestuffs

Carbohydrates: orange juice

Metrosep Carb1 – 250		ppm
1	n.d.	
2	Inositol	1107
3	n.d.	
4	n.d.	
5	n.d.	
6	Glucose	19214
7	Fructose	18820
8	Sucrose	26155

Dialysis, dilution 1:100

100 mmol/L NaOH

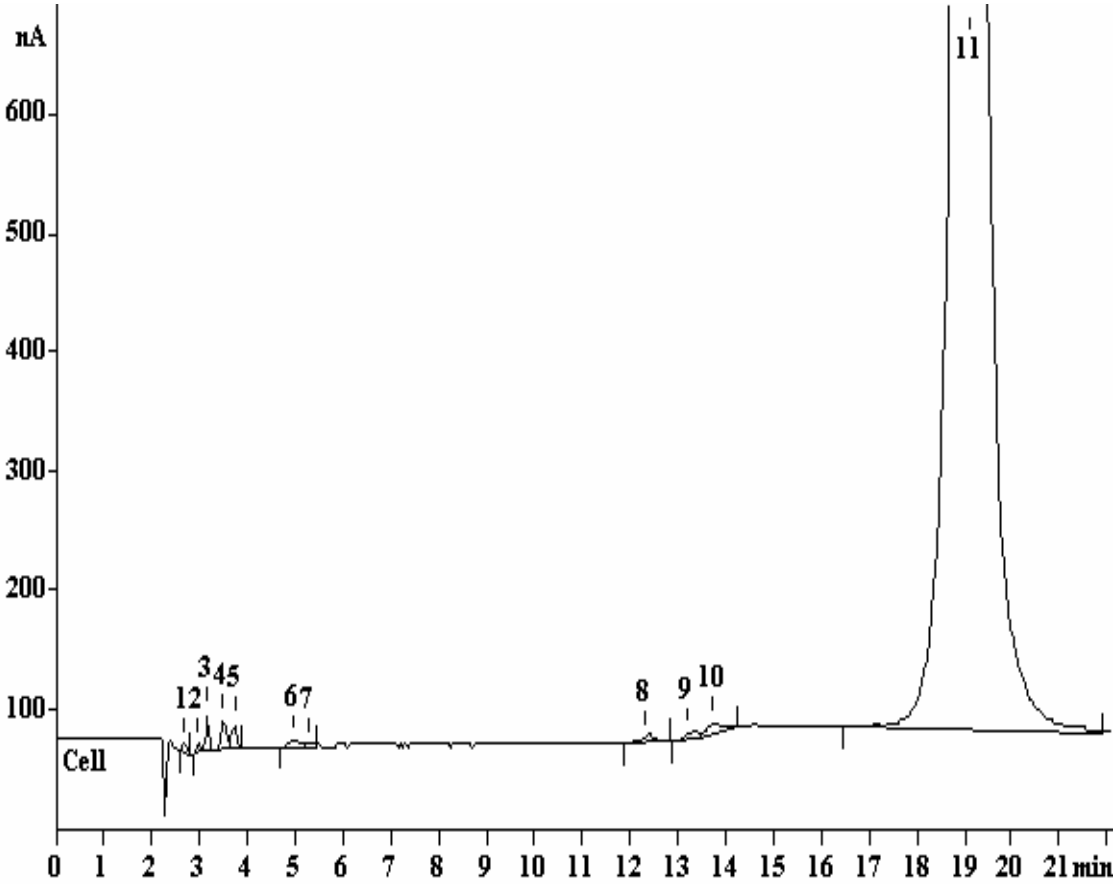


Carbohydrates: milk

Metrosep Carb1 – 250		ppm
1-3	Glycerin	52
4	Inositol	35
5	Glycerin	28
6	Xylitol	21
7	Arabitol	6
8	Arabinose	51
9	Mannose	47
10	Glucose	53
11	Lactose	35625

Dilution 1:1000, dialysis

100 mmol/L NaOH



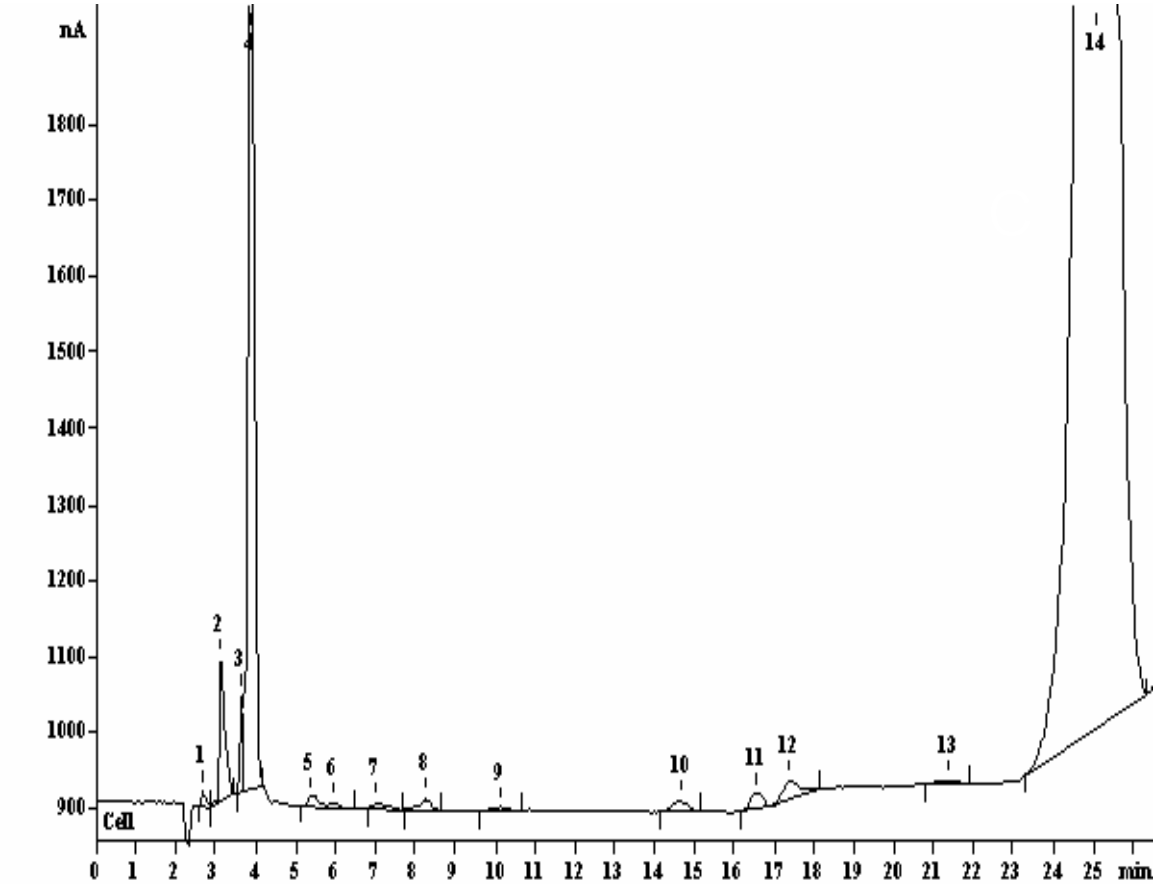
Anions, pulsed amperometric detectio

Carbohydrates: milk chocolate

Metrosep Carb1 – 250		
ppm		
3	Inositol	1.50
6	Arabitol	0.26
7	Sorbitol	0.40
8	Mannitol	0.77
10	Arabinose	1.29
11	Glucose	1.37
12	Xylose	2.09
13	Fructose	1.60
14	Lactose	838.96

Dilution 1:10, dialysis

100 mmol/L NaOH



Auto-Sampler

- Sugars and Carbohydrates fuel biological processes
- Reduce temperature in autosampler to slow / prevent change in sample
- Peltier cooling or external cooling

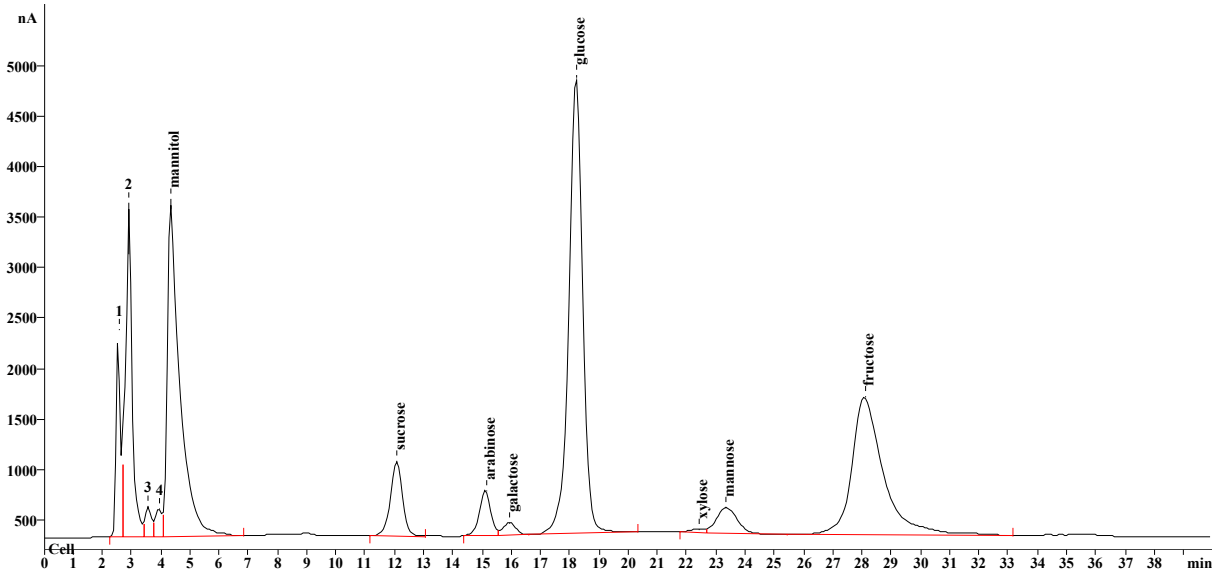
Eluent

- **Specific for column and separation**
- **Isocratic for simple sugars**
- **Gradient for complex mixtures**
- **Follow manufacturer recommendations**

Column:METROSEP Carb 1 4X250mm

Eluent: 2.5 mmol/L sodium hydroxide + 0.65 mmol/L sodium acetate in ultrapure water

Post column reagent: 300 mmol/L sodium hydroxide in ultra pure water



Columns

- Choice of vendors
- Selectivity/separation needed
- Isocratic and gradient separations

Column Selection

Sugar Class	Separation Mode
	Ligand Exchange +GFC
Monosacharides Disacharides	Ligand Exchange +Reversed Phase
	Normal Phase
Sacharides & Sugar Alcohols	Ligand Exchange +GFC
	Ligand Exchange + Normal Phase
Oligosacharides	Ligand Exchange +GFC
Amino Sugars	Ligand Exchange +GFC
	Normal Phase
Acidic Sugars	Ion Exclusion
	Ion Exchange
Sacharides & Organic Acids	Ion Exclusion+GFC
Sugar Alcohols & Organic Acids	Ion Exclusion+GFC

Isocratic Separation

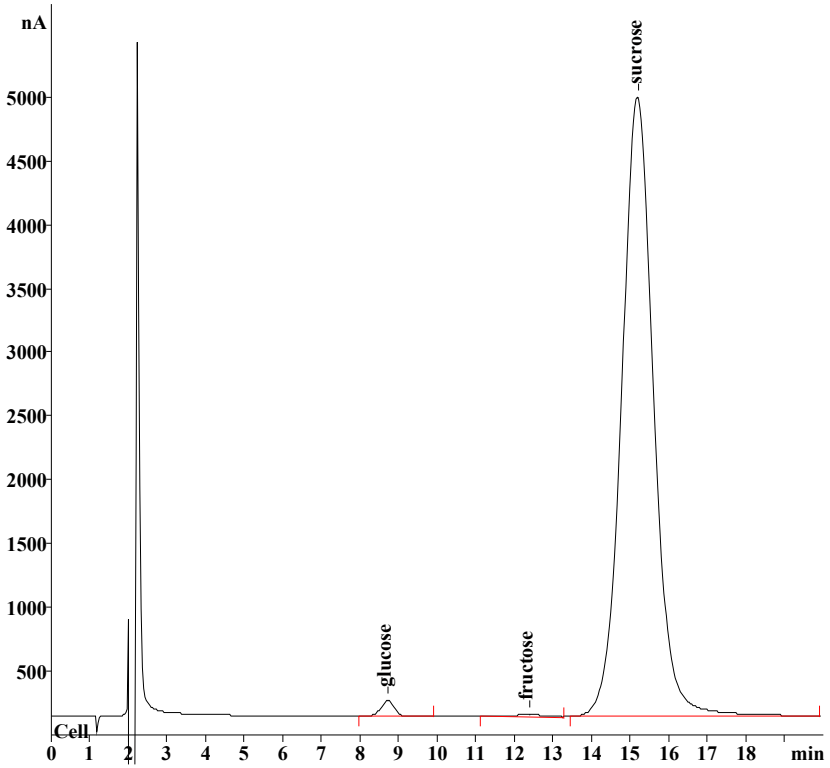
Sample: Vodka

Column: Metrosep Carb 1 4X150mm

Mobile Phase: 100mM NaOH

Loop: 20uL

Detector: Metrohm PAD



Separation

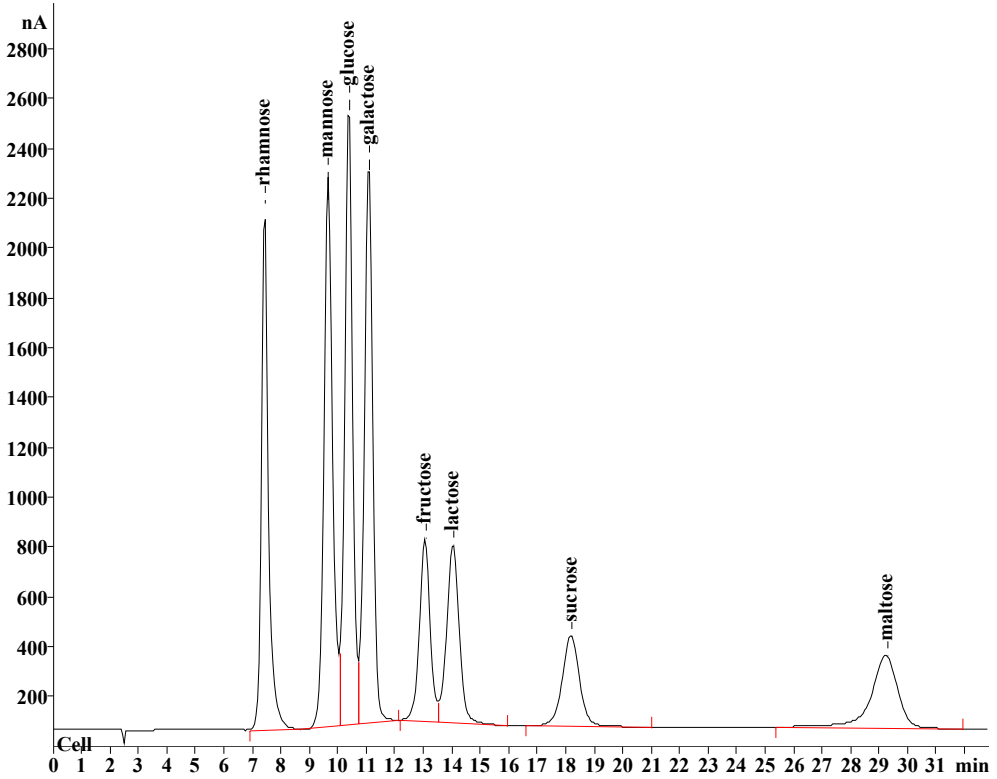
Sample: Sugar Standards

Column: Metrosep Carb 1 4x250mm

Mobile Phase: 200mM NaOH

Loop: 20uL

Detector: Metrohm PAD



Separation

VARIAN

APPLICATION NOTE

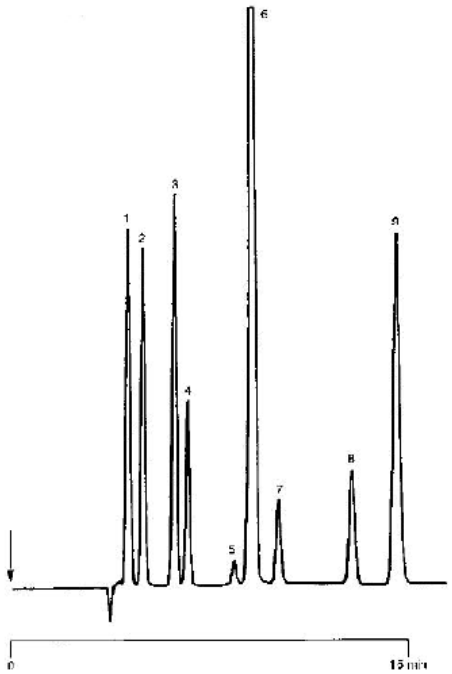
Organic acids and sugars Application 1186 LC

Fermentation standard

Technique	: HPLC
Column:	: Metacarb 67H , 300 x 6.5 mm Part. No. A5244
Mobile Phase:	: 0.025 N H_2SO_4
Flow Rate:	: 0.6 mL/min
Temperature:	: 65 °C
Detection:	: RI
Sample	: 20 µL

Peak Identification

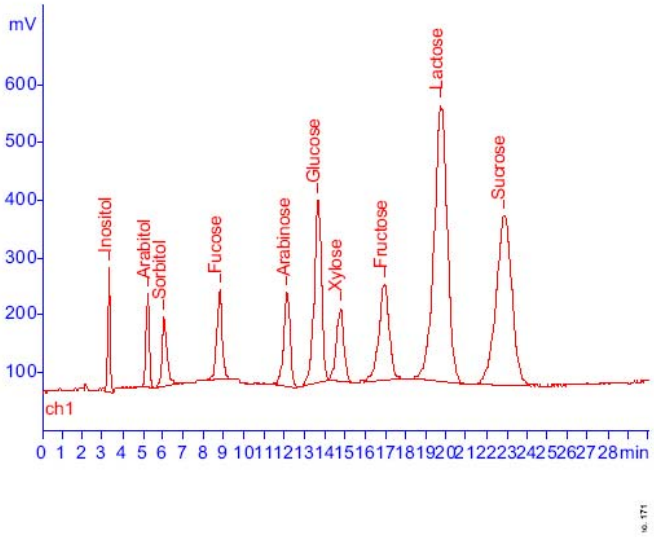
1 Matotriose
2 Maltose
3 Glucose
4 Fructose
5 Lactic acid
6 Glycerol
7 Acetic acid
8 Methanol
9 Ethanol



Separation

Sugaralcohols, Mono-and Disaccharides with BioQuant Carbohydrate

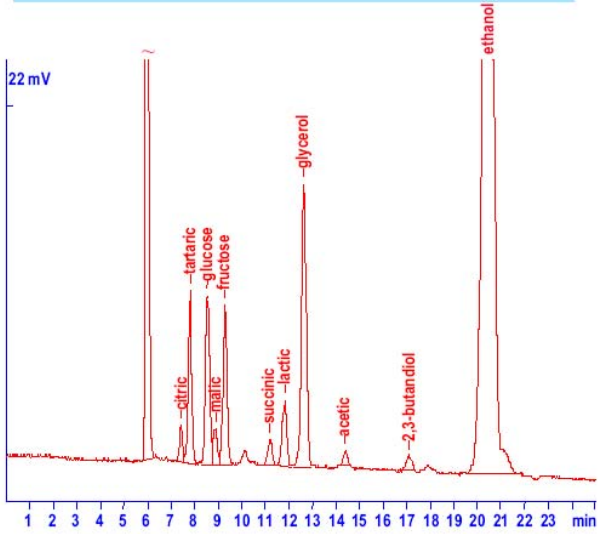
Column:	Sugar1
Dimension:	250 x 4.6 mm PEEK
Eluent:	100 mM NaOH
Flow:	0.9 ml/min
Detection:	BioQuant Carbohydrate
Temperature:	28 °C
Injection:	20 µl
Sample:	**1** Inositol, **2** Arabitol, **3** Sorbitol, **4** Fucose, **5** Arabinose, **6** Glucose, **7** Xylose, **8** Fructose **9** Lactose, **10** Sucrose (1- 50 mg/l)



Carbohydrates, Organic Acids and Alcohols in Wine

Carbohydrate H+

Part. Number:	00253776
Dimension:	300 x 7.8 mm
Eluent:	1.25 mM H_2SO_4
Flow:	0.6 ml/min
Detection:	RI
Temperature:	45 °C
Injection:	50 µl
Sample:	Wine Complett Standard



Detectors

- Refractive Index
- Pulsed Amperometric
- Mass Spec

Detectors

	Selectivity	Sensitivity	Cost	Separation
Refractive Index	Little	0.1 to 0.05%	Moderate	Isocratic
Pulsed Amperometric	Some	ng/g, ppb	Moderate	Isocratic, Gradient
Mass Spec	Most	ng/g, ppb	Expensive	Isocratic, Gradient

Pulsed Amperometric Detector

Measuring principle

A defined decent potential is applied to a three electrode system consisting of reference-, working- and auxiliary electrodes. If any oxidizable substance pass these electrodes an oxidation process takes place. A current corresponding to the concentration of the respective substance is measured.

- Simple connection to any IC- or HPLC-system
- Large variety of electrode materials (glassy carbon, carbon paste, silver, gold, etc.)
- Highly sensitive
- Very selective

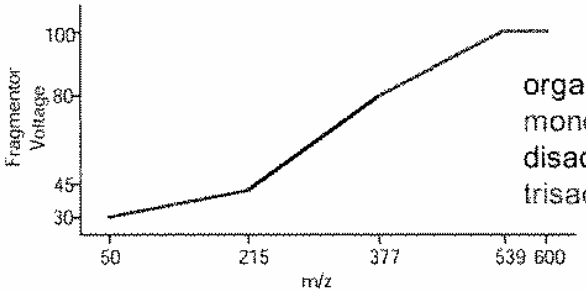
Mass Spec

- **Electrospray ionization**
  - Operating temp 250 to 300°C
  - Sugars oxidize
  - Form adduct with HCl
- **HCl Adduct formation**
  - HCl is volatile
  - Source of Cl^- for adduction
  - Post column addition

Chloride Adduction

- Carbohydrates chloride adduct and only these adducts are detectable in negative mode ESI.
- Chloride adducts of carbohydrates are collisionally stabilized in the API source.
- In carbohydrates, the chlorine appears to hydrogen bond to hydroxyl groups.
- Chloride adduction also occurs with alcohols such as glycerol, but the signal is weaker.
- Chloride does not adduct to organic acids and does not enhance the MS signal.

Optimizing MS Response with Ramped Fragmentor Voltage



	m/z	V
organic acids	50	30
monosaccharides	215	45
disaccharides	377	80
trisaccharides	539	100

- Fragmentor voltage effects both sensitivity and fragmentation and is compound class specific
- Fragmentor voltage can change as a function of the mass (ramped) during scan or selected ion monitoring
- Ramped fragmentor voltage can be used to optimize the response from both molecular and fragment ions

Summary

- You have choices in hardware and separation
- Choose the right tools for your application
- What do you need?

Thank You

Larry Tucker
Brinkmann Instruments, Inc
ltucker@brinkmann.com
281 330 9532