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Ion Chromatography – Basics & Applications in Food Safety

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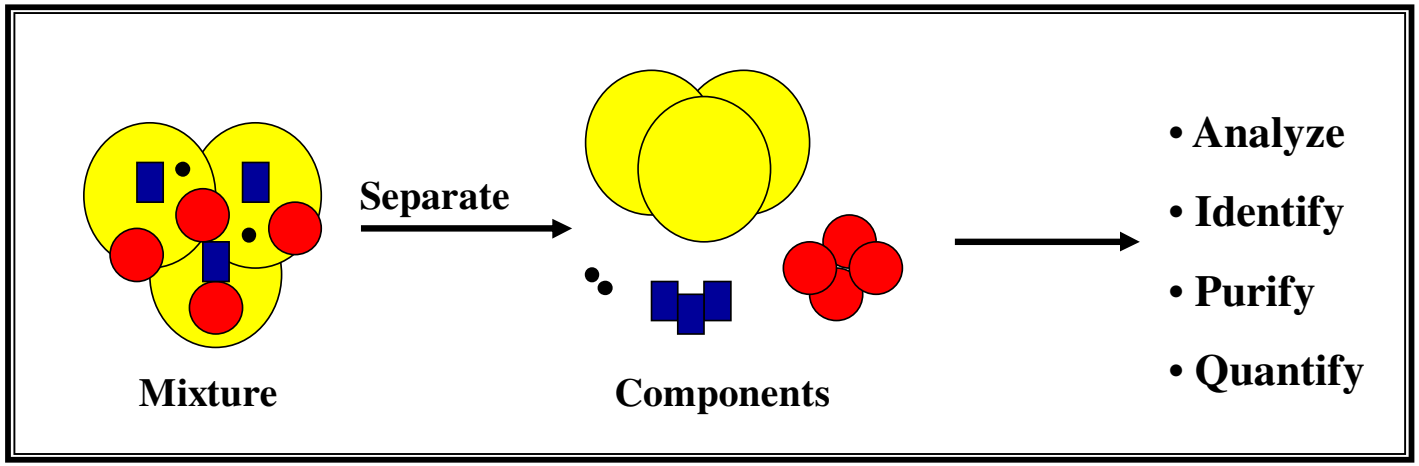
Applications – IC/SP

What is Chromatography?

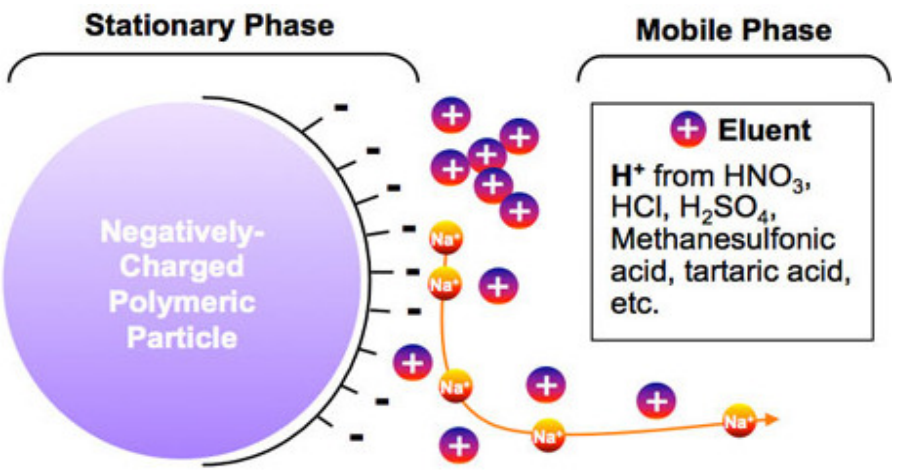
Chromatography is a technique for separating mixtures into their components in order to analyze, identify, purify, and/or quantify the mixture or components.



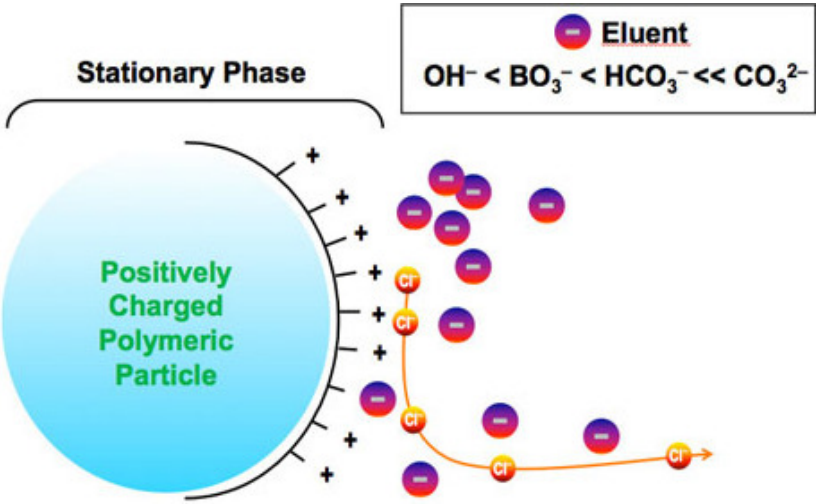
What is Ion Chromatography

1) Ion Chromatography is a liquid chromatographic technique, with which ionic and strongly polar species can be separated and detected
2) Ions can be either **Positive Charge (Cations)** or **Negative Charge (Anions)**
3) Separation takes place according to their affinity for Ion Exchange Stationary Phases.

Cation



Anion



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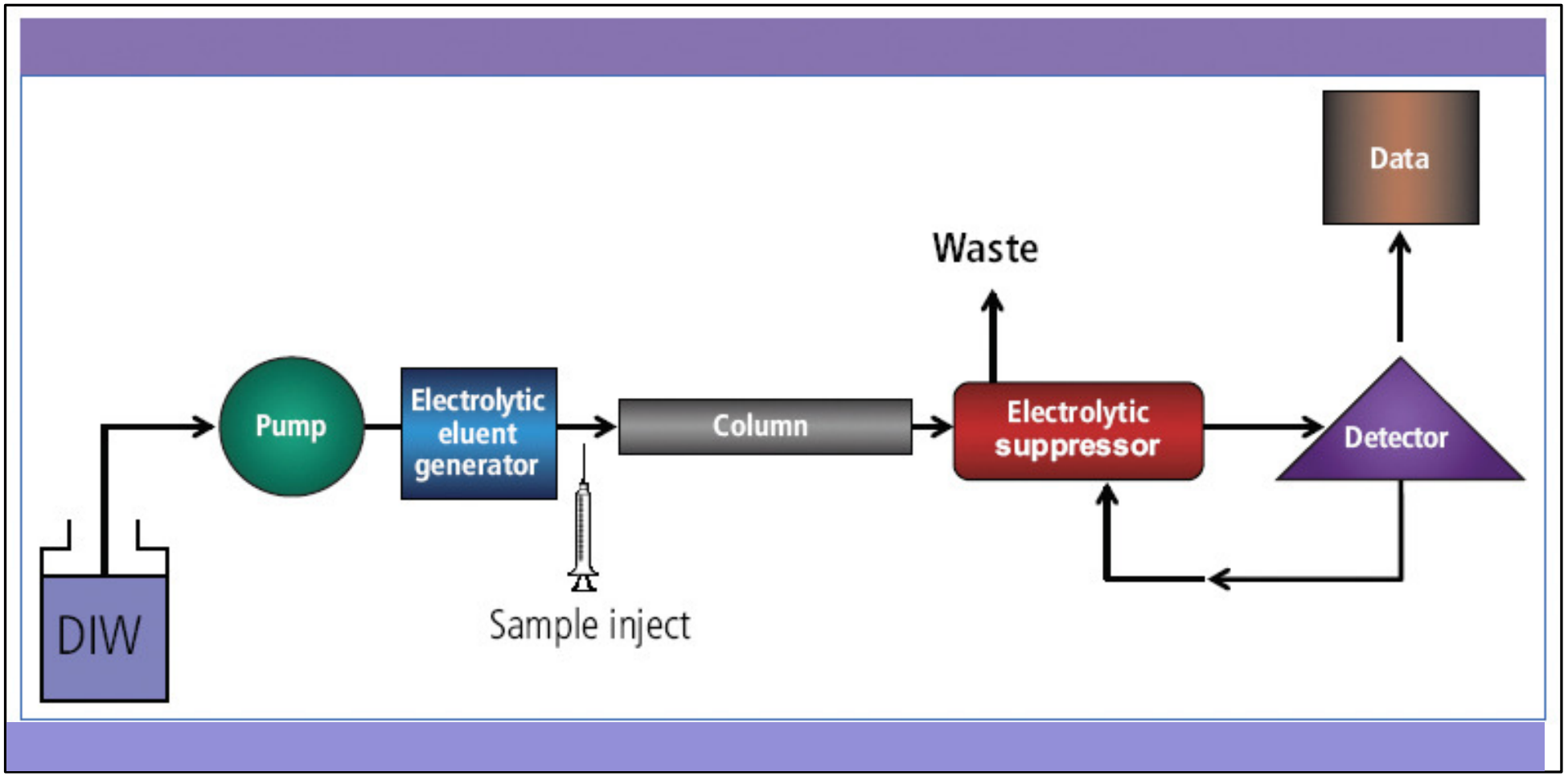
IC Flow path



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Distinguish between HPLC and IC

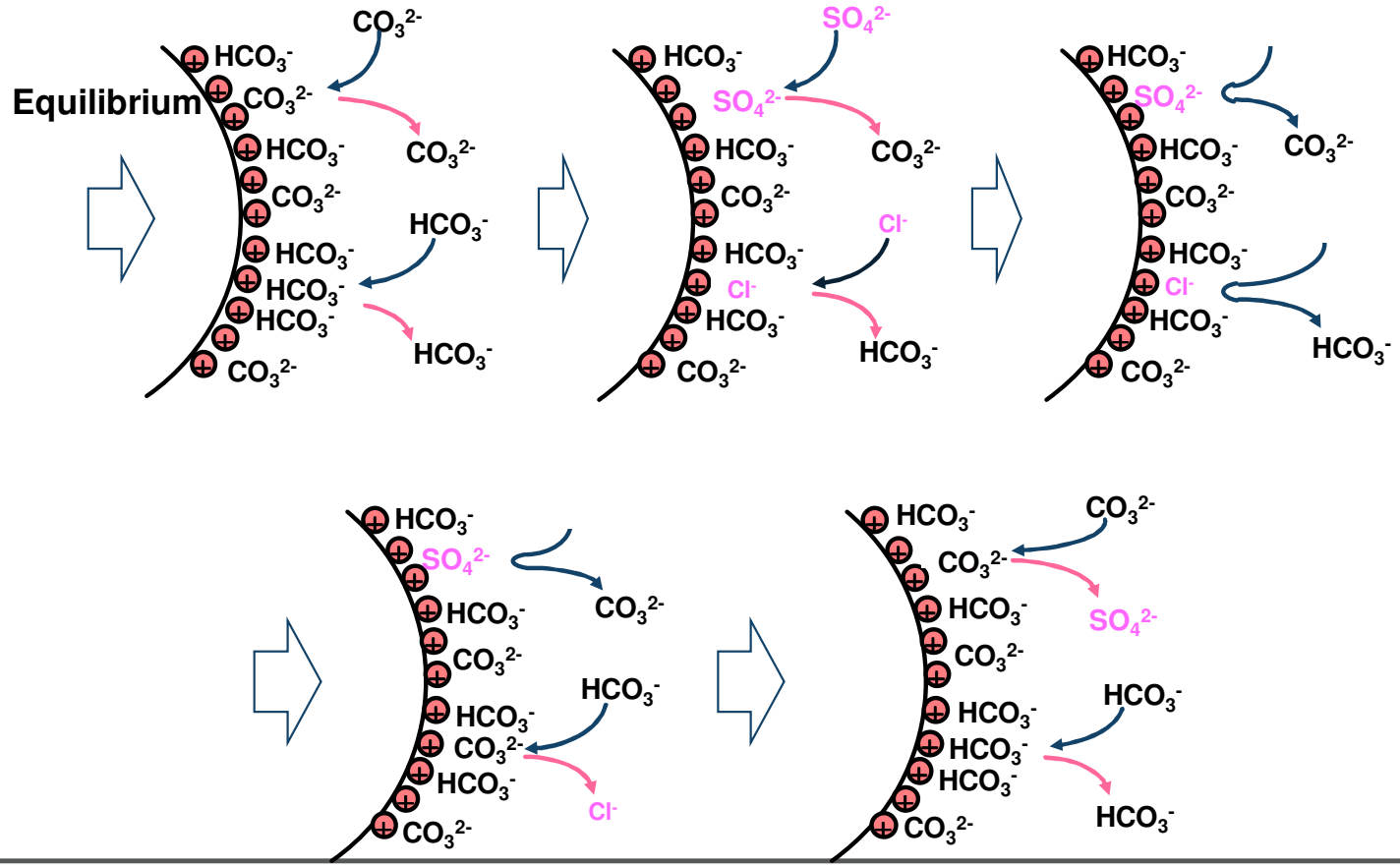
	HPLC	IC
1	The 'hardware' of HPLC is made up of SS	The 'hardware' of IC is made up of PEEK material.(Poly Ether Ether Ketone)
2	Generally non ionic mobile phase is use.	Ionic mobile phase is required
3	Stationary phase used in HPLC is mostly bonded Silica	Polystyrene base ion exchange resin copolymer with styrene and dvb is generally used as stationary phase in IC.
4	Only molecule is determined by HPLC	Charged species as well as some molecule can be determined.
5	Absorbance is measured in HPLC	Primarily Conductance is measured in IC
6	Can not used as IC.	Can be used as RP- HPLC.

Principle of Separation on Ion Chromatography (Anions)

Anions (- ve Charge)
Eluent should give free OH-/ $CO3^{-2}$/$HCO3^{-1}$
Strong Bases like NaOH/ KOH and Sodium Carbonate and Bicarbonate
Anion Column will be having Positive sites on Stationary Phase like Quaternary Ammonium Groups with Positive Charge.
At the time of System Equilibration Negative ions from the Eluent will get adsorbed on Positive charge of the column towards Neutrality
Negatively charged analytes will compete with Negative charged Hydroxide or Carbonate or Bicarbonate Eluent Ions for Positive sites on the column.
The anions will get attached and again get displaced from positive sites of stationary phase according to their Charge and Size.
Elution Order: F < Cl < Br < SO4< PO3

Ion Exchange Separation – Na_2CO_3 and $NaHCO_3$ Eluent

In case of NaOH or KOH Eluent "OH" will be the available ions for Interaction



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Principle of Separation on Ion Chromatography (Cations)

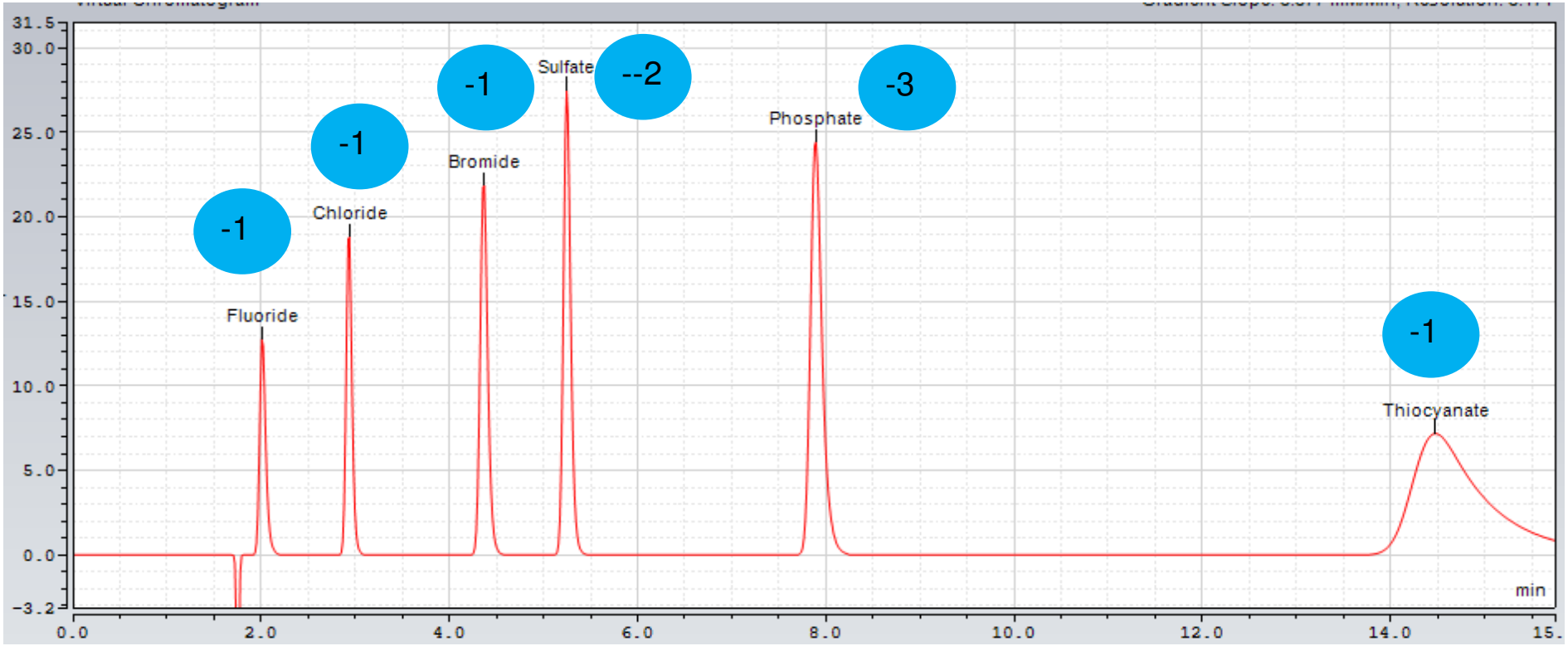
Cations (+ ve Charge)
Eluent should give free H+
Strong Acids like Methane Sulfonic acid or Sulfuric Acid
Cation Column will be having Negative sites on Stationary Phase like Sulfonates and Carbonates Groups with Negative Charge.
At the time of System Equilibration Positive ions from the Eluent (H+) will get adsorbed on Negative charge of the column towards Neutrality.
Positively charged analytes (NaCl, KCl) will compete with Positive charged Hydrogen Ions for Negative sites on the column.
The Cations will get attached and again get displaced from Negative sites of stationary phase according to their Charge and Size.
Elution Order: Li < Na < K < Mg< Ca

Retention Determining Parameters

Charge

Size

Polarizability



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Instrumentation: Pump, Autosampler

I) Eluent Reservoir – It is nothing but mobile phase container. This can be made up of good quality of glass or polypropylene depending upon the nature of mobile phase.

II) Pump – For optimal system performance it is essential that the pump provide smooth, accurate, and precise eluent delivery .The pump material as well as fluid path in the system is made up of Peek (Poly ether ether ketone) material. This peek material is compatible with 0 to 14 PH and with most of organic solvents.

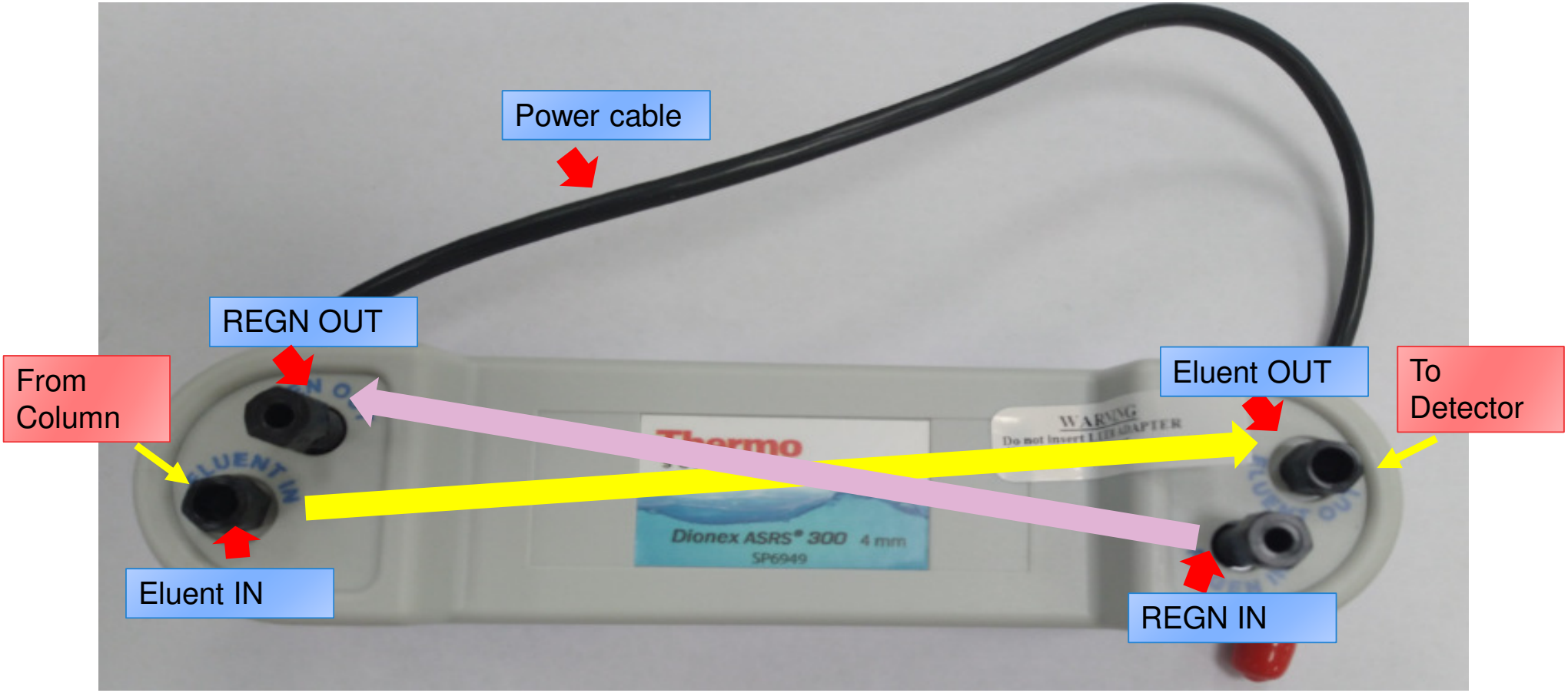
III)Sample Injector – This can be manual or auto to load the fixed amount of standard as well as sample in to the column.

Instrumentation: Guard and Separator Column

- **I) Guard Column – generally guard column is made up of same material as analytical column. Guard column prevents direct shock to main analytical column. AG –Anion Guard.**

- **II) Analytical Column – The column is the heart of ion chromatography. Physically, it consists of a chemically inert tube packed with a polymeric resin. IC columns are available in different sizes and packed with different resins depending upon the application and desired mode of separation. AS – Anion Separator**

Working Principle of Suppressor



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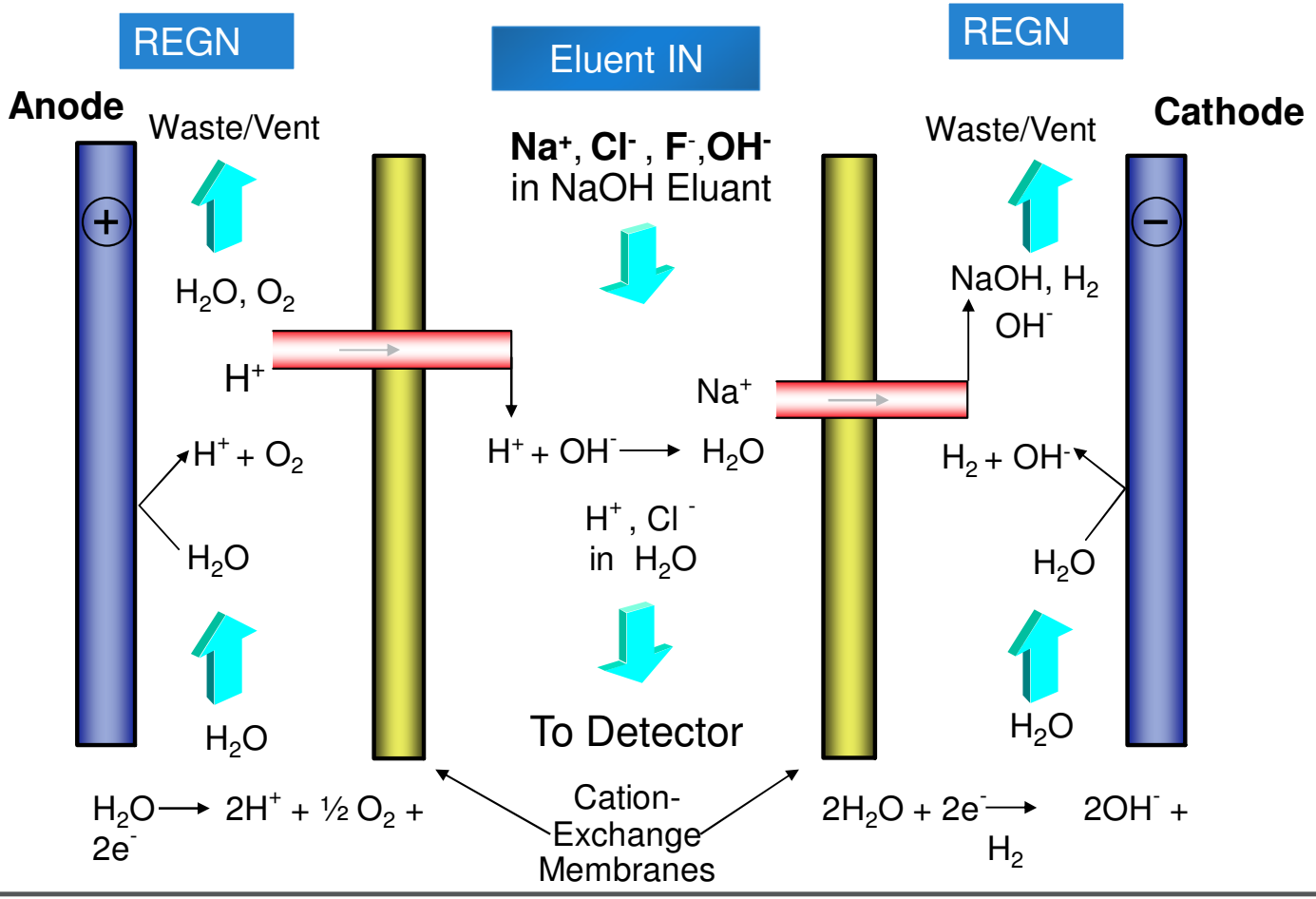
Chemistry and Ion Movement in an Anion SRS®



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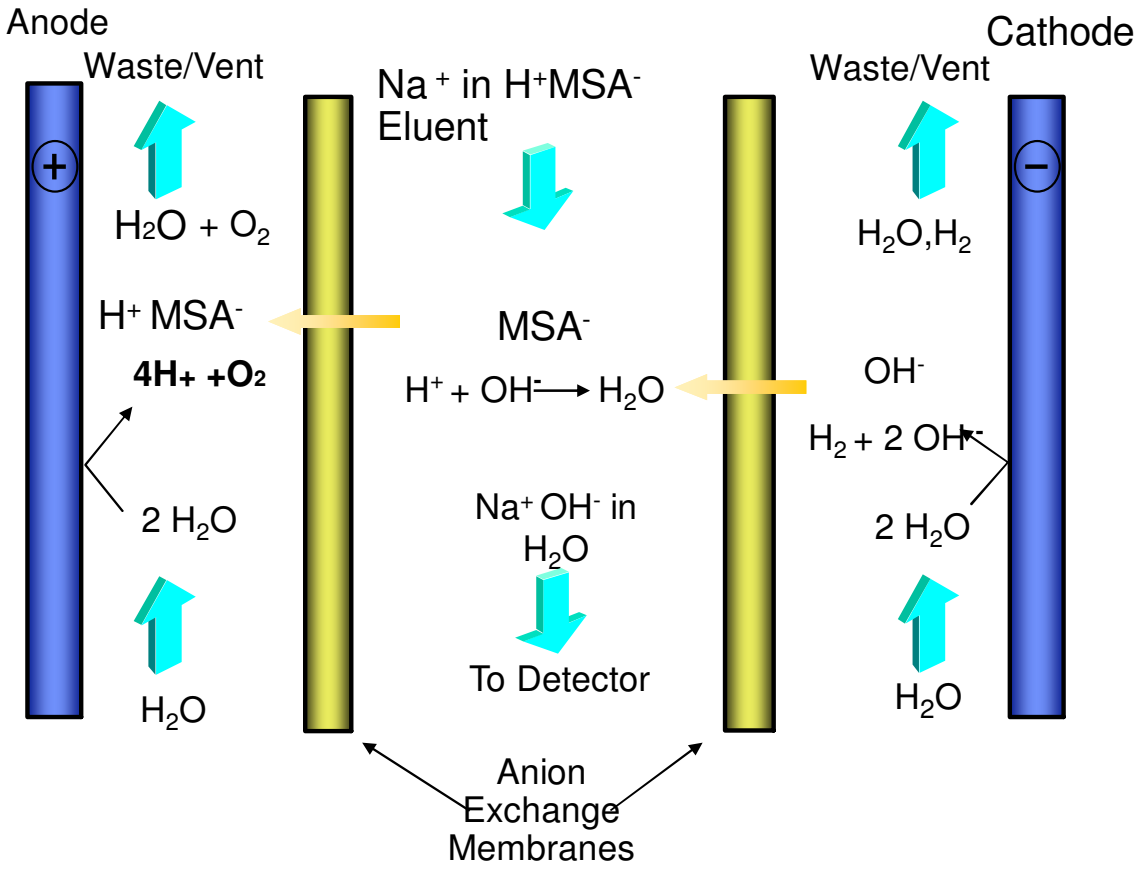
Chemistry and Ion Movement in Cation SRS®



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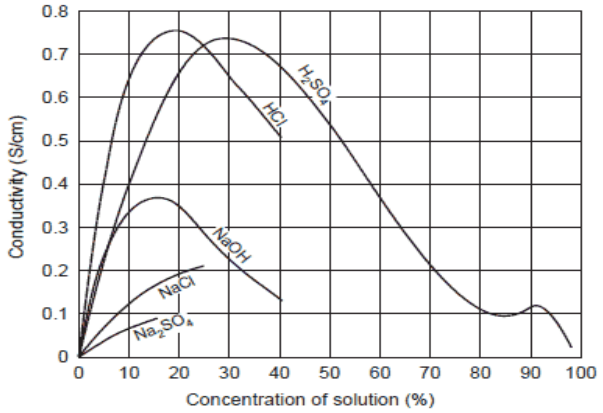
Equivalent Conductance's of Common Ions

Anions		Cations	
Ion	**l_0(µS cm^{-1})**	**Ion**	**l_0(µS cm^{-1})**
OH^-	198.6	H^+	349.8
Cl^-	76.4	Na^+	50.1
SO_4^{2-}	80.0	K^+	73.5
NO_3^-	71.5		
MSA^{-*}	48.8		

*MSA^-: Methanesulfonate



Relationship between Concentration of Solutions and Conductivity (at 18°C)

Advantages of Suppressor

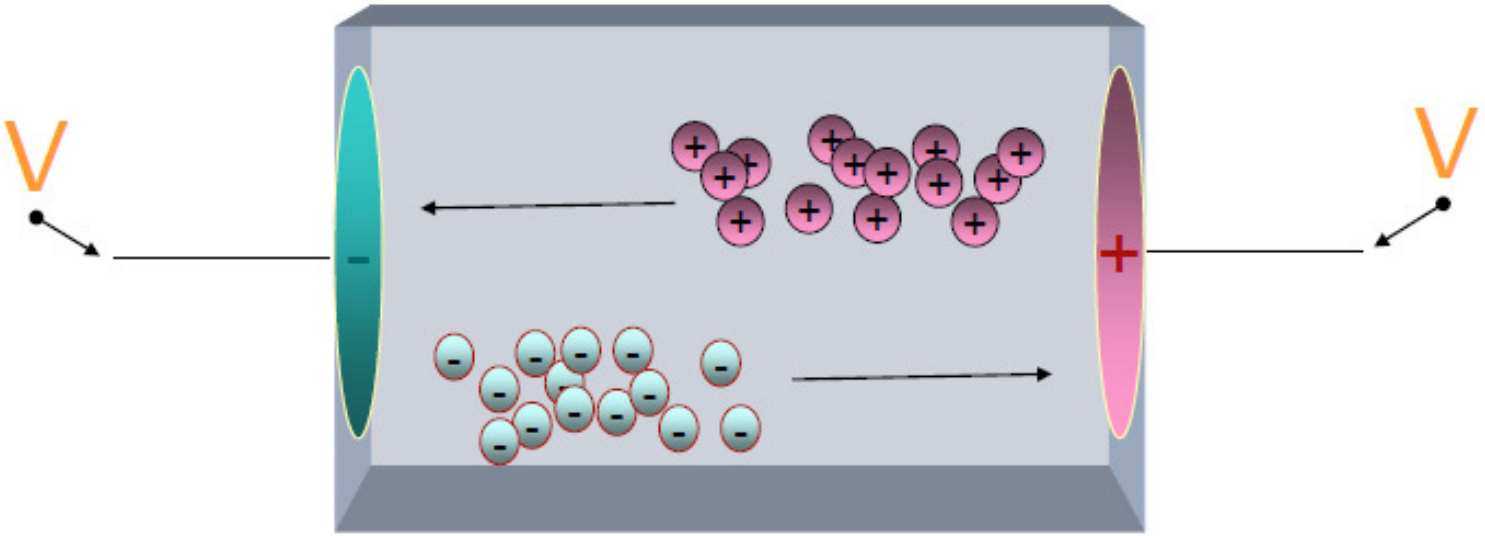
1. It reduces the background signal of Conductivity Detector. Increases the sensitivity of analysis.

2. It converts analyte in to highly conducting dilute acid and base and hence increases the response of the analyte and hence the sensitivity.

3. It removes all the counter ions from Eluent as well as standard salts and hence the analysis become more selective.

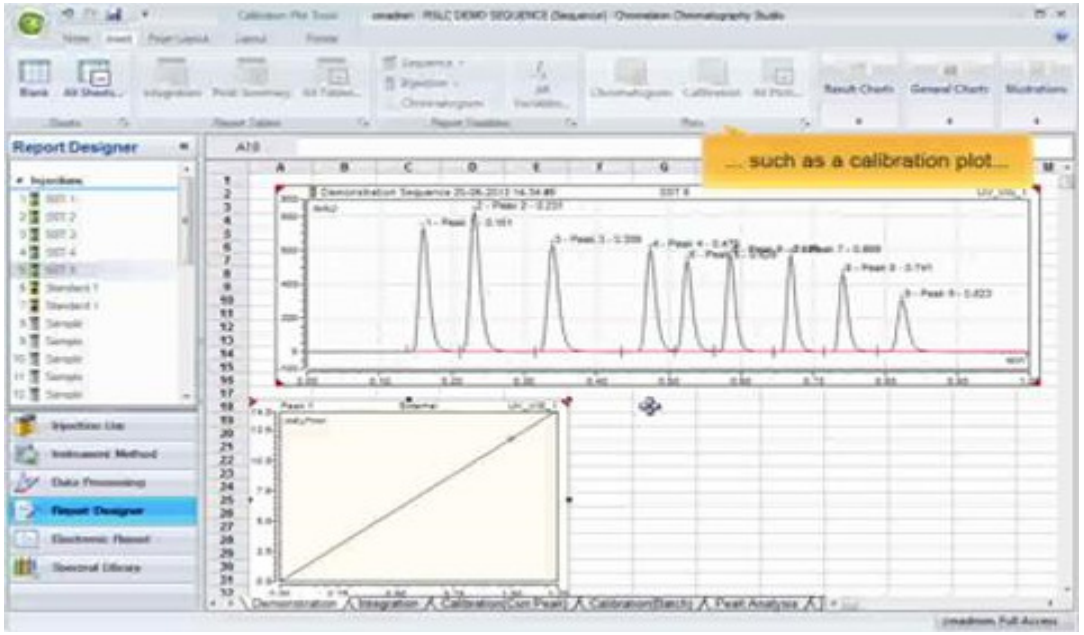
3) Detection mode :- Conductivity Detector

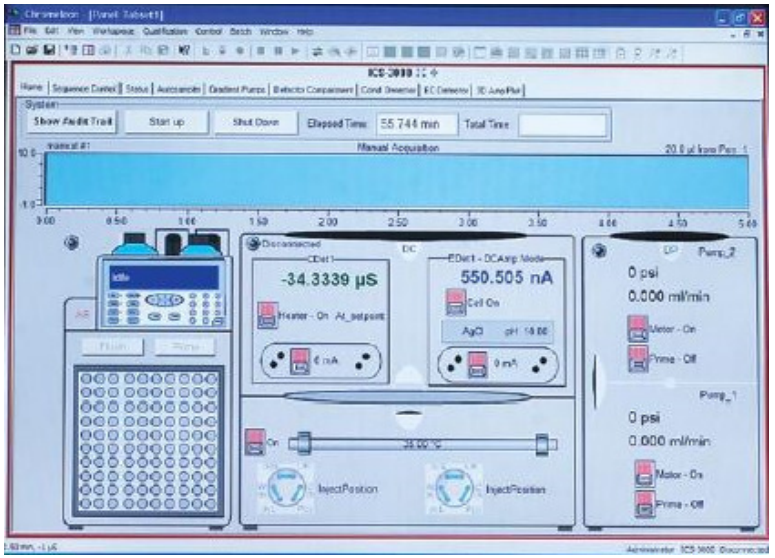
- **The conductivity of a solution is measured by applying an alternating voltage between two electrodes in a conductivity cell.**
- **At any instant in time, negatively charged anions migrate towards positive electrode and positively charged cations migrate towards negative electrode**



4) Data Mode :- Chromeleon Software

- **Signal generated by detector is transmitted to Recorder or directly displayed as a chromatogram on computer. The concentration of ionic analytes are automatically determined and tabulated.**





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Ion Chromatography Instruments (Gradient Compatible)

1. Aquion System

1. Isocratic Pump
2. Max Pressure limit 5000 psi
3. Only Conductivity and UV detector

Ion Chromatography Instruments (Gradient Compatible)

2. Integrion System

1. Isocratic Pump

2. Max pressure limit 5000 psi

3. Conductivity, Amperometry and UV detector (One at a time)

Ion Chromatography Instruments (Gradient Compatible)

3. ICS 6000 System

1. Quaternary gradient Pump

2. Max. pressure 6000 psi

3. Consumable Monitoring

3. Conductivity, Amperometry and UV detector (in series)

High End System

Ion-Exchange Chromatography Offers Simple, Direct, and Interference-Free Analyses

- **Sensitive Detection**
  Samples can be diluted to decrease the concentration of matrix components

- **Specific Detection**
  Low abundant analytes can be detected in presence of large concentration of matrix components

- **Analyte-Specific Separations**
  The selectivity of the separator columns is tailored for the specific requirements of the various analyte classes

Advantages of Ion Chromatography / 1

Speed

- Complete anion and cation profiles in about 15-20 minutes

Sensitivity

- Analyses in the lowest µg/L-range without pre-concentration
- Analyses in the lowest ng/L-range after pre-concentration

Selectivity

- Huge variety of stationary phases
- Specific detection (suppression, UV, fluorescence, MS, ICP)

Advantages of Ion Chromatography / 2

Simultaneity

- Simultaneous analysis of many sample components (In contrast to AAS, photometry, titration, etc.)

Costs

- Cost effective as Mobile phase like coustic or Acids in diluted form is required for analysis.

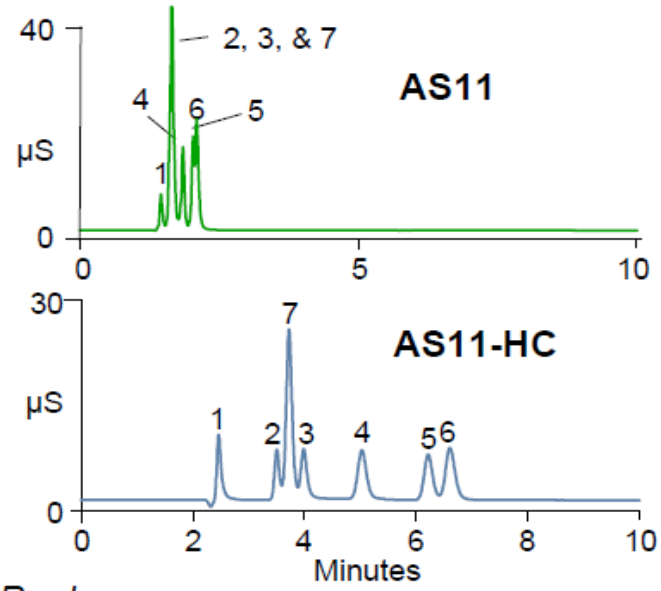
Robustness

- pH and solvent compatible separators allow a variety of applications
- Analysis of complex matrices such as wastewater, foods, body fluids, etc.

Example: Retention Time Change as per capacity

- Capacity

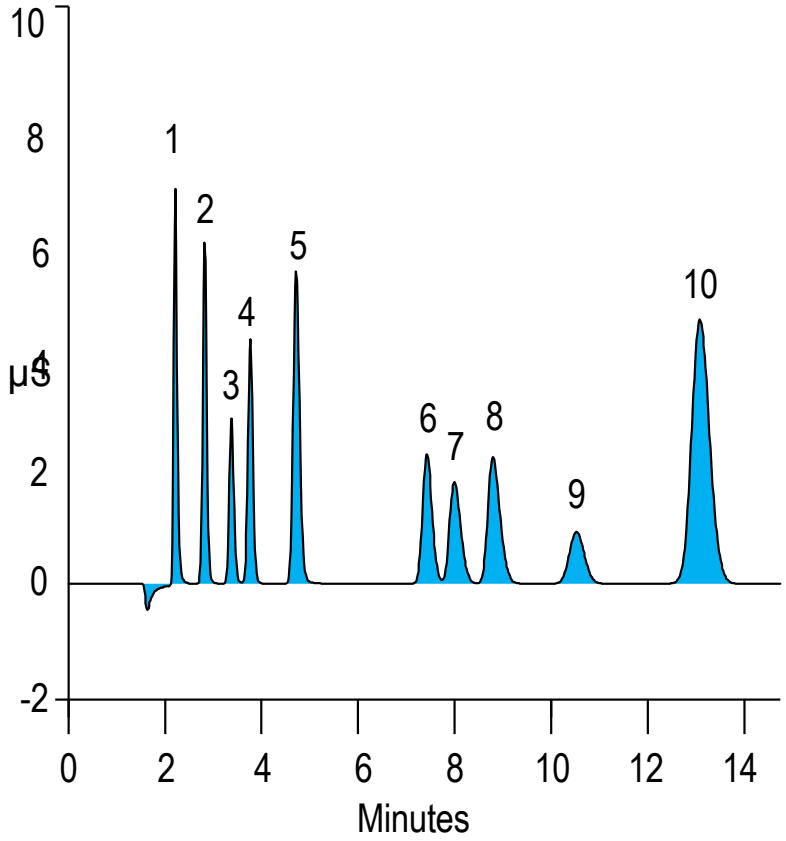
Number of ion exchange sites/weight equivalent of column packing.
The higher the capacity, the stronger the retention.



- Most ion exchange column have low capacity to improve detection and sensitivity
- Complex samples and measurement of trace levels in the presence of high level matrix ions require higher capacities

Peaks:
1. Fluoride 2. Chloride 3. Nitrite
4. Phosphate 5. Bromide 6. Nitrate 7. Sulfate

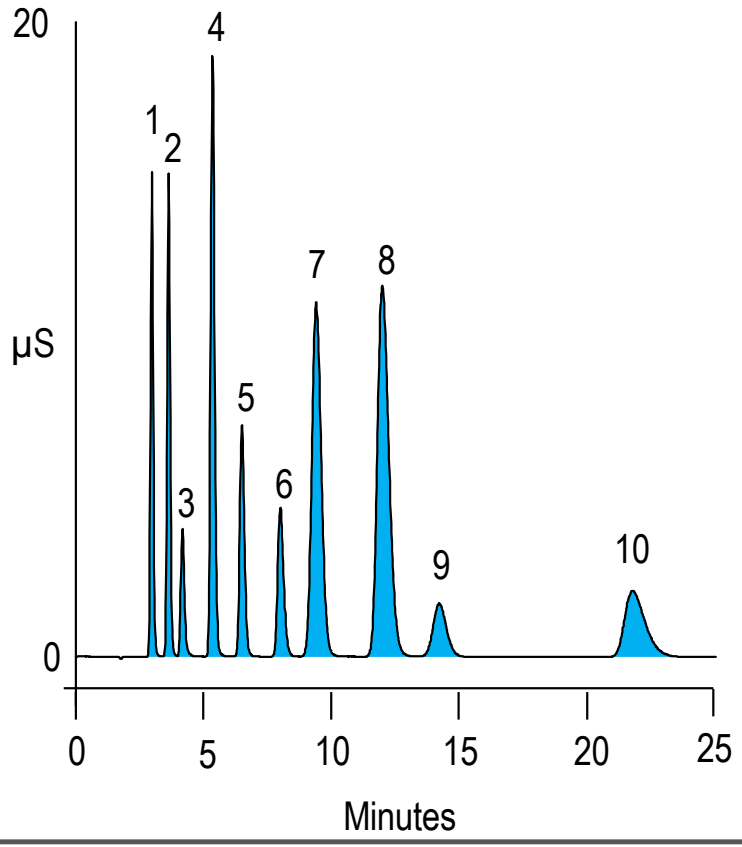
Anion Standard on IonPac AS12A



Column:	IonPac AS12A
Eluent:	0.3 mM Sodium bicarbonate 2.7 mM Sodium carbonate
Flow Rate:	1.5 mL/min.
Inj. Volume:	25 µL
Detection:	Suppressed Conductivity

Peaks:	mg/L
1. Fluoride	3
2. Chlorite	10
3. Bromate	10
4. Chloride	4
5. Nitrite	10
6. Bromide	10
7. Chlorate	10
8. Nitrate	10
9. Orthophosphate	10
10. Sulfate	20

Separation of Alkali and Alkaline-Earth Metals and Ammonium



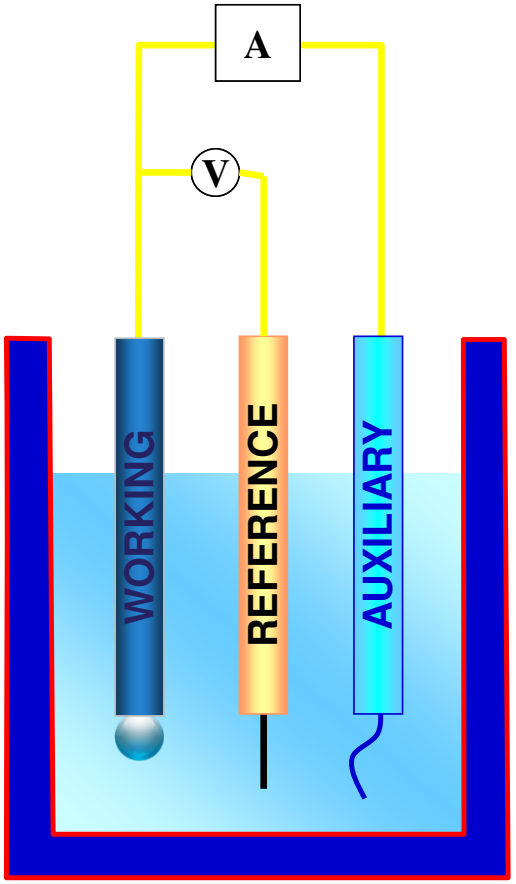
Separator:	IonPac CS12A	
Eluant:	18 mmol/L MSA	
Flow rate:	1 mL/min	
Inj. volume:	25 µL	
Detection:	Suppressed conductivity, Recycle Mode	
Peaks:	1. Lithium	1 mg/L
	2. Sodium	4
	3. Ammonium	5
	4. Potassium	10
	5. Rubidium	10
	6. Cesium	10
	7. Magnesium	5
	8. Calcium	10
	9. Strontium	10
	10. Barium	10

ELECTRO CHEMICAL DETECTION

ELECTROCHEMICAL DETECTION

The electrochemical detector requires three electrodes:

- Working Electrode (where the oxidation or reduction takes place)
- Auxiliary Electrode (counter-electrode, cell body)
- Reference Electrode (compensates for any changes in the background signal of the mobile phase)



Ampermetic Detector Consist of three part :

1. Counter electrode
2. Working electrode
3. Reference electrode

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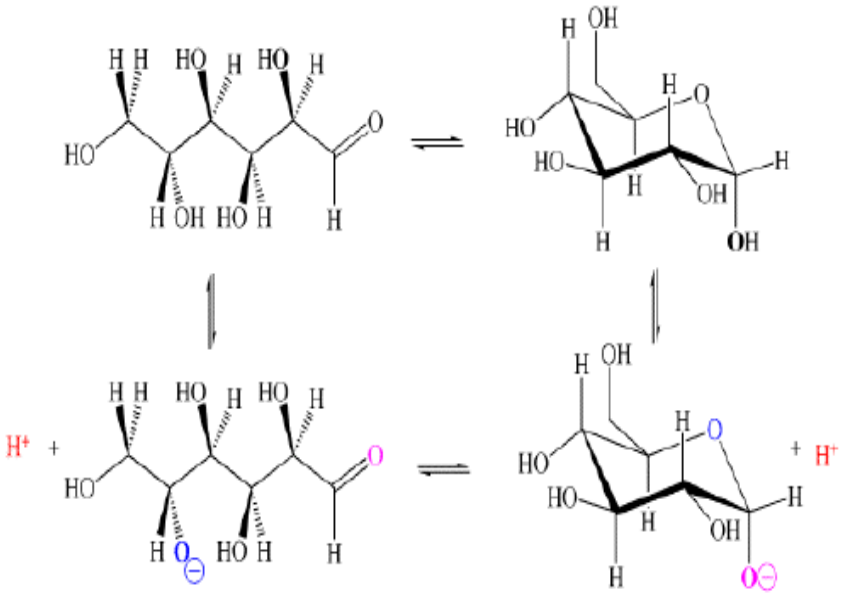
Principle of **Separation** on Ion Chromatography (Carbohydrates)

Carbohydrates (with neutral charge)
Eluent comprises of High Strength Hydroxide Phase with sodium acetate for highly retaining analytes
The carboPac series of columns with Pellicular Anion exchange resin bed.
Carbohydrates molecules get ionized at High pH of Eluent forms Oxy-Anions.
These Oxy-Anions retains on Anion exchange columns according to their pKa value.
Higher the pKa value, Lesser the retention
Sodium acetate accelerates the elution of strongly bound species without compromising selectivity and without interfering with pulsed ampereometric detection.

Principle of **Separation** on Ion Chromatography (Carbohydrates)



Sugar	pKa
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

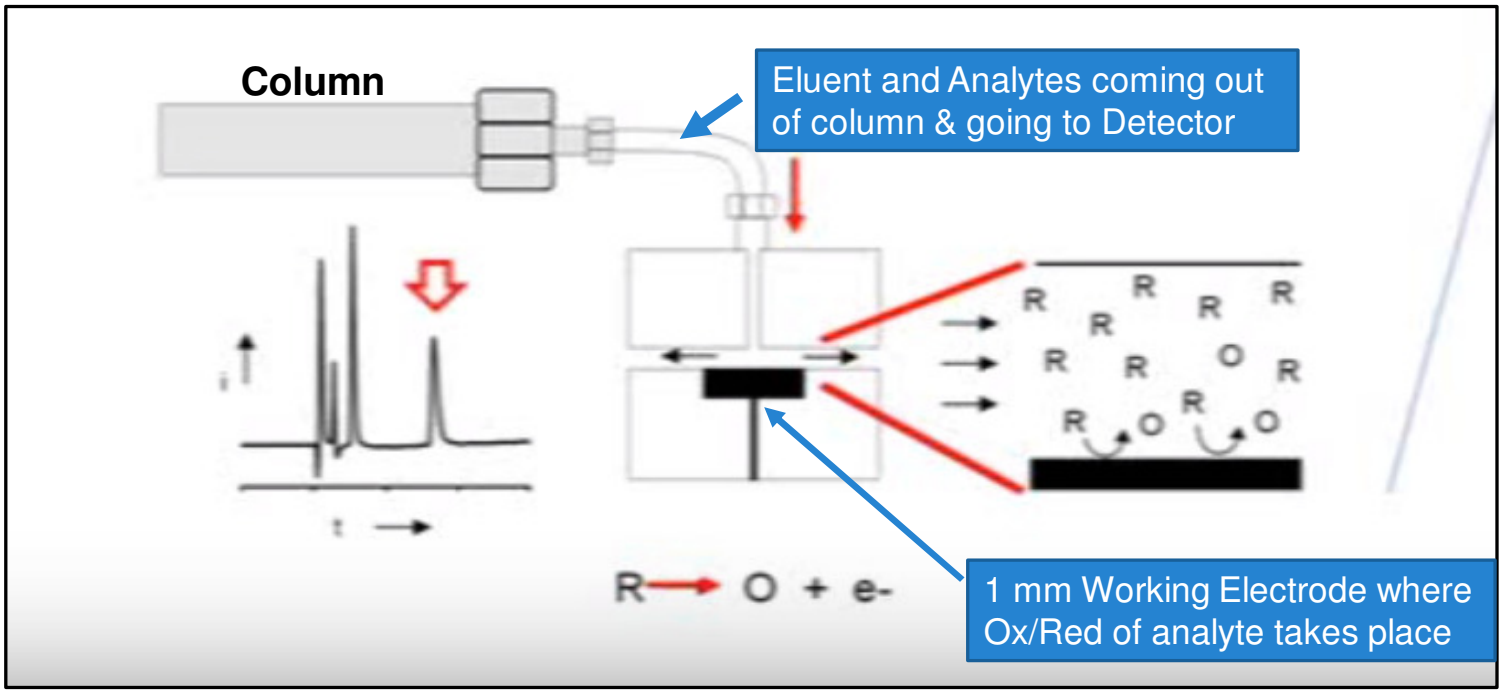
Dionex (now part of Thermo Scientific) Technical Note 20 Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). Sunnyvale, CA, 2004.

Principle of **Detection** on Ion Chromatography (Carbohydrates)

Carbohydrates
Single Potential (DC) or Waveform (series of potential) runs continuously on Working electrode.
After eluting out from column, the analyte comes in contact with Working electrode and get either oxidized or reduced at the detection potential.
After give and take electrons the current will be generated (recorded as peak), and it will be directly proportional to Analyte concentration.
Constant potential is always maintained between Working and Auxiliary electrode; while Reference electrode whose potential is already known measures and controls the constant potential among them.
In three electrode system, when working electrode works as cathode (Oxidative potential) similar magnitude reductive potential will be generated at other half cell i.e. Auxiliary electrode.

Principle of **Detection** on Ion Chromatography (Carbohydrates)

Eluent and analyte coming out from column and going to the working electrode surface where it get either oxidizes or reduced and current generated which shows the peak.



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Direct-Current (DC) Amperometry

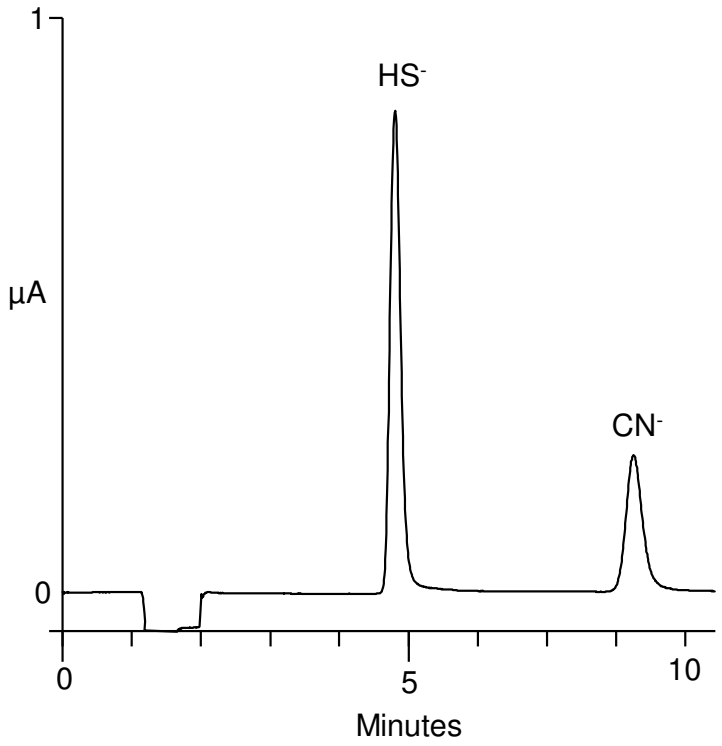
How it works:

- Constant Potential is applied to an electrode
- Redox of analyte generates current which is measured.

Applications:

- Catecholamines
- Some inorganic anions

Example: Analysis of Sulfide and Cyanide



Column:	CarboPac PA1 with Guard
Eluent:	0.1 mol/L NaOH + 0.5 mol/L NaOAc + 0.5 % (v/v) ethylenediamine
Flow rate:	1 mL/min
Detection:	DC Amperometry, Ag electrode, 0 V
Inj. volume:	50 µL
Sample preparation:	Samples have been acidified, distilled acc. DIN 38 405 D13 and absorbed in 0.1-1 mol/L NaOH.

Integrated Amperometry

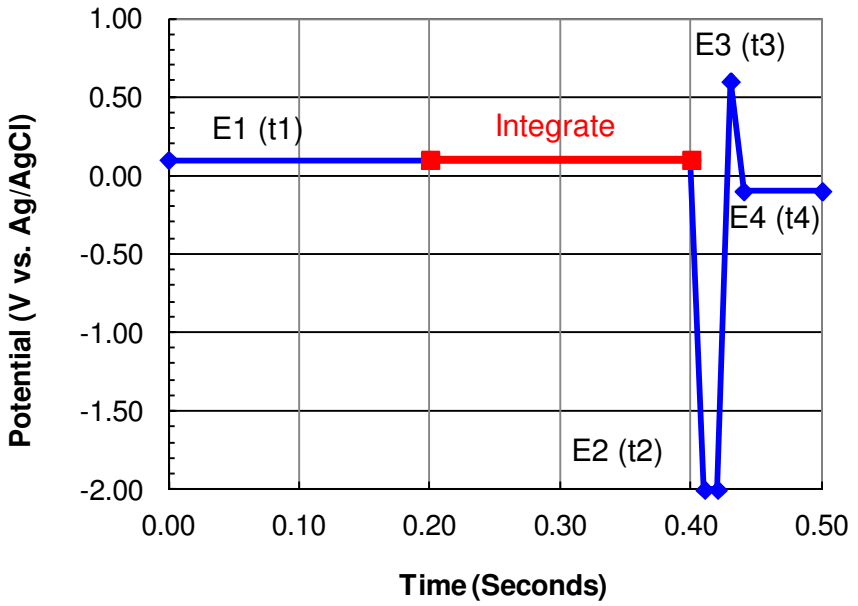
How it works:

- Rapidly repeating pattern of potentials (“waveform”) applied to electrode
- Current integrated at analytical potential
- Electrode continuously reconditioned

Applications:

- Carbohydrates
- Amino acids
- Alcohols
- Sulfur-containing compounds
- Many electroactive inorganic ions

Standard Quadruple Waveform for Carbohydrates



Time (s)	Potential (V)	Integration
0.00	0.10	
0.20	0.10	Start
0.40	0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

1. Detection potential (E1)
2. Duration time of E1 (t1)
3. Integration period (Integrate)
4. Reductive cleaning potential (E2)
5. Duration time of E2 (t2)
6. Oxidative cleaning potential (E3)
7. Duration time of E3 (t3)
8. Pre-detection (oxide reduction) potential (E4)
9. Duration time of E4 (t4)

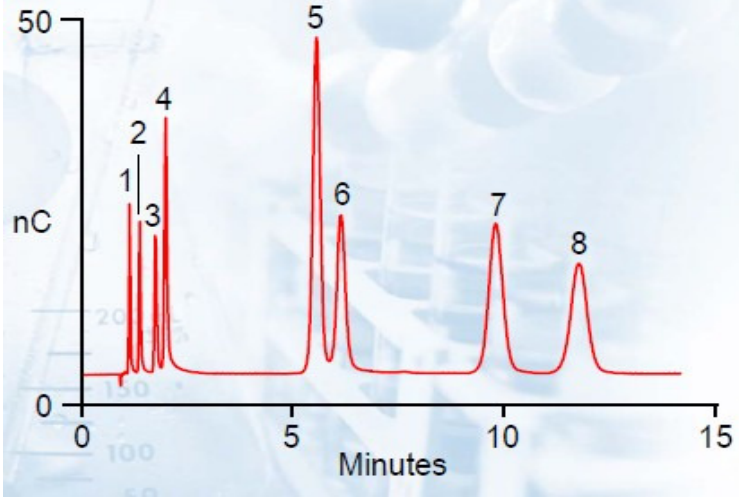
Dionex (now part of Thermo Scientific) Technical Note 21 Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electochemical Detector. Sunnyvale, CA, 1998.

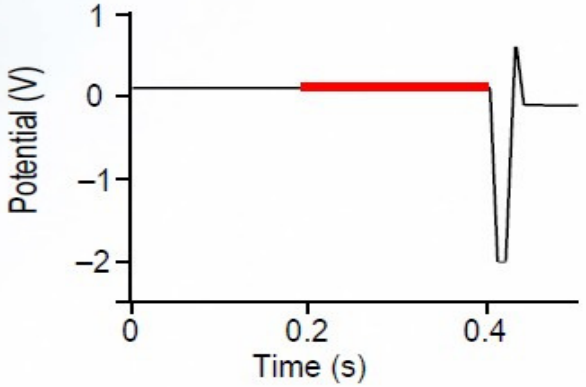
Chromatogram : Example

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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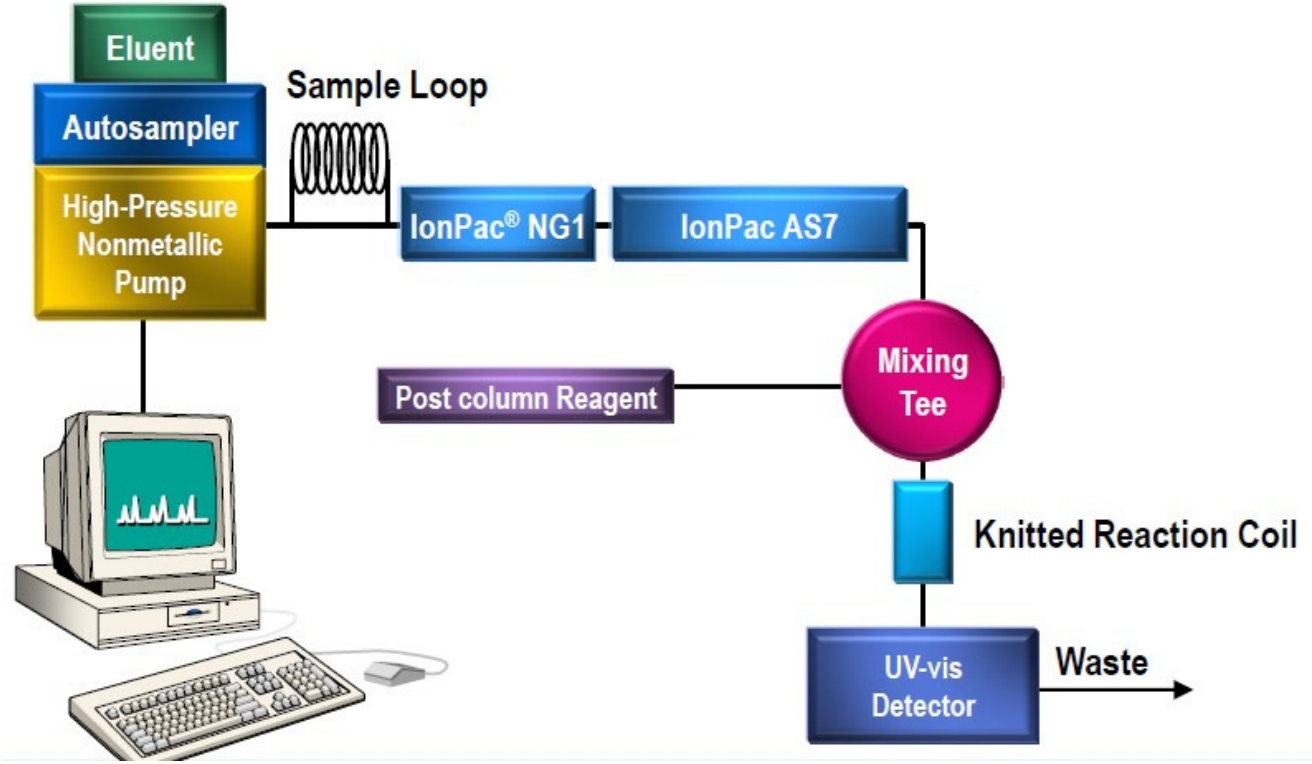
UV-VIS DETECTOR

Flow Chart for PCR + UV-Vis analysis – Transition Metals and Chromium



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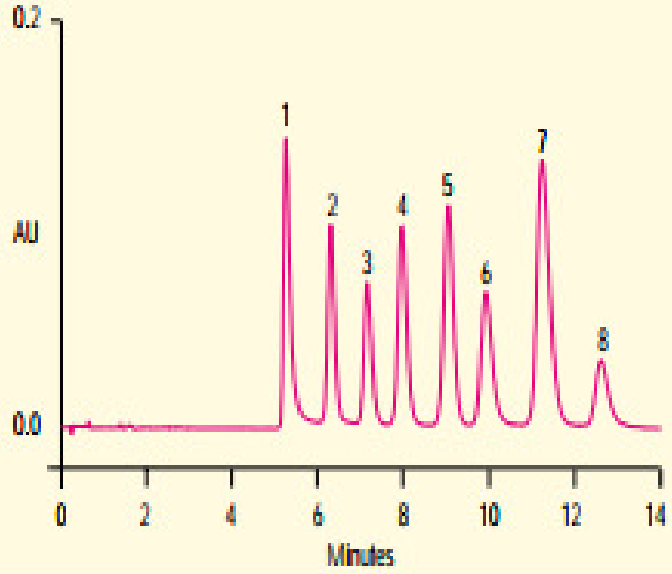
Principle of Separation on Ion Chromatography (Transition metals)

Metals (with net Positive charge)
Eluent Contains Chelating agents like carboxylic acid (Pyridine Dicarboxylic acid-PDCA) or Organic acid (Oxalic acid) acid with pH modifiers
The column CS 5 A will be having both Anion as well as cation exchangers
The metal ions will form Complexes with Chelating agents and these complexes will bear particular charge (either positive or Negative)
With Carboxylic acid Eluent the charges will be negative; while with Oxalic acid eluent the charges will be both positive and Negative.
Metal complexes has different charges and Stability Constants (Log k) on which elution order depends.
This eluate comes in contact with PAR indicator in PCR; where these metal complexes again reacts with PAR indicator to form chromophore groups which further get detected on visible wavelength (530 nm)

Principle of Separation on Ion Chromatography (Transition metals)



Column:	IonPac CS5A, CG5A
Eluent:	MetPac PDCA eluent
Flow Rate:	1.2 mL/min
Inj. Volume:	50 µL
Detection:	Absorbance, 530 nm with PAR in MetPac Postcolumn Reagent Diluent

Peaks:		
1.	Iron (III)	1.3 mg/L
2.	Copper	1.3
3.	Nickel	2.6
4.	Zinc	1.3
5.	Cobalt	1.3
6.	Cadmium	6.0
7.	Manganese	2.6
8.	Iron (II)	1.3

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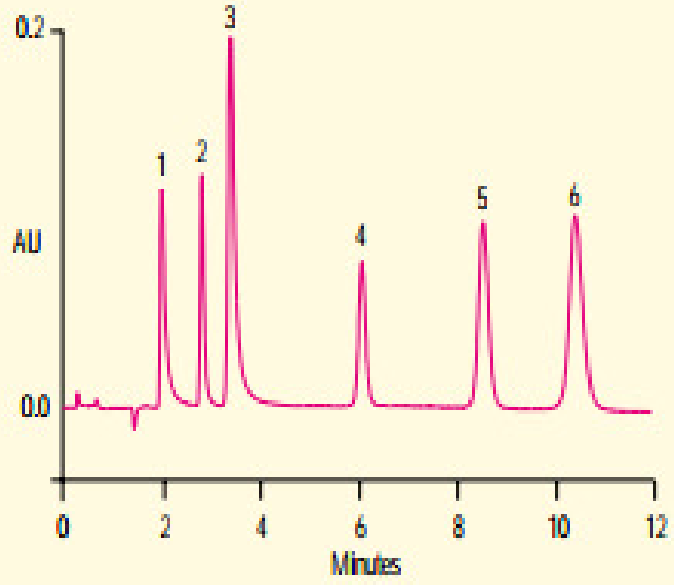
Principle of Separation on Ion Chromatography (Transition metals)



Column:	IonPac CS5A, CG5A, 4 mm
Eluent:	MetPac Oxalic Acid Eluent
Flow Rate:	1.2 mL/min
Inj. Volume:	50 µL
Detection:	Absorbance, 530 nm with PAR in MetPac Postcolumn Reagent Diluent

Peaks:		
1.	Lead	5.0 mg/L (ppm)
2.	Copper	0.7
3.	Cadmium	3.3
4.	Cobalt	0.7
5.	Zinc	1.3
6.	Nickel	2.0

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Species Detected by Ion Chromatography

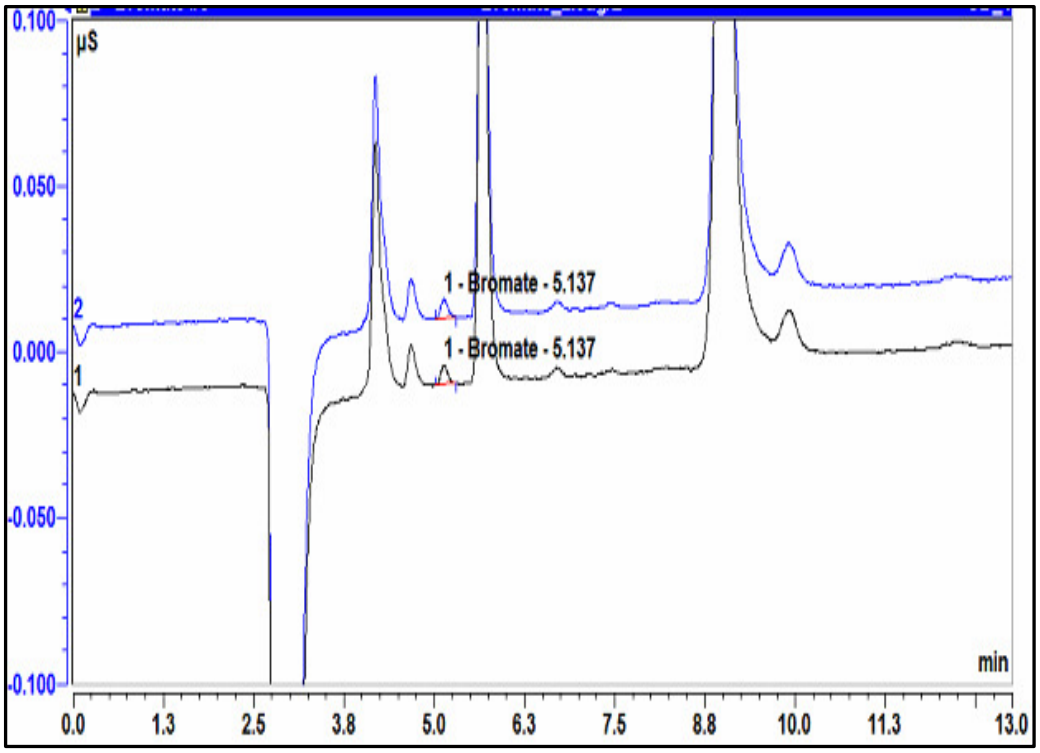
Conductivity	DC Amperometry	Integrated Amperometry	UV-Vis
Inorganic anions	Catecholamines	Carbohydrates	Silicate
Inorganic Cations	Phenols	Aliphatic amines 1^0, 2^0, 3^0	Bromate
Carboxylic acids	Aromatic amines	Amino acids	Chromate
Sulfonic acids	Thiols	Alcohols	Transition metals
Phosphonic acids	Cyanide	Aldehydes	Lanthanides
Amines, 1^0, 2^0, 3^0	Sulfide	S species (except S[VI])	Nitrite, Nitrate
	Iodide	Sulfite	
	Sulfite	Iodide	

APPLICATIONS

Water: Bromate in Drinking water

In Disinfection process of water, Ozonation causes formation of Bromate in water which has been proven to be carcinogenic.

Column	Ion Pac AS 19 (250 X 4mm) + Guard
Eluent	20 mM NaOH
Flow	1.0 ml/Min
Injection Vol	250 µl
detection	Conductivity with Anion Suppressor (Current 50 mA)
LOD	1 µg/L

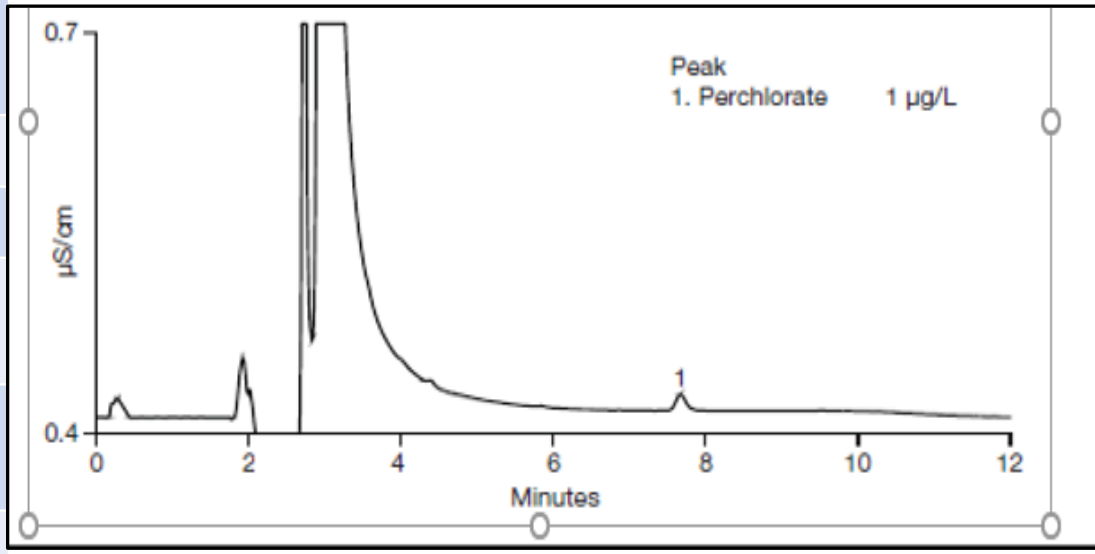


ThermoFisher SCIENTIFIC

Water: Perchlorate in Drinking water

Perchlorate analysis:
Commonly used Oxidizing agent in Solid Propellants, Rockets, fireworks etc. Adverse Health effect because of its presence in ground water:

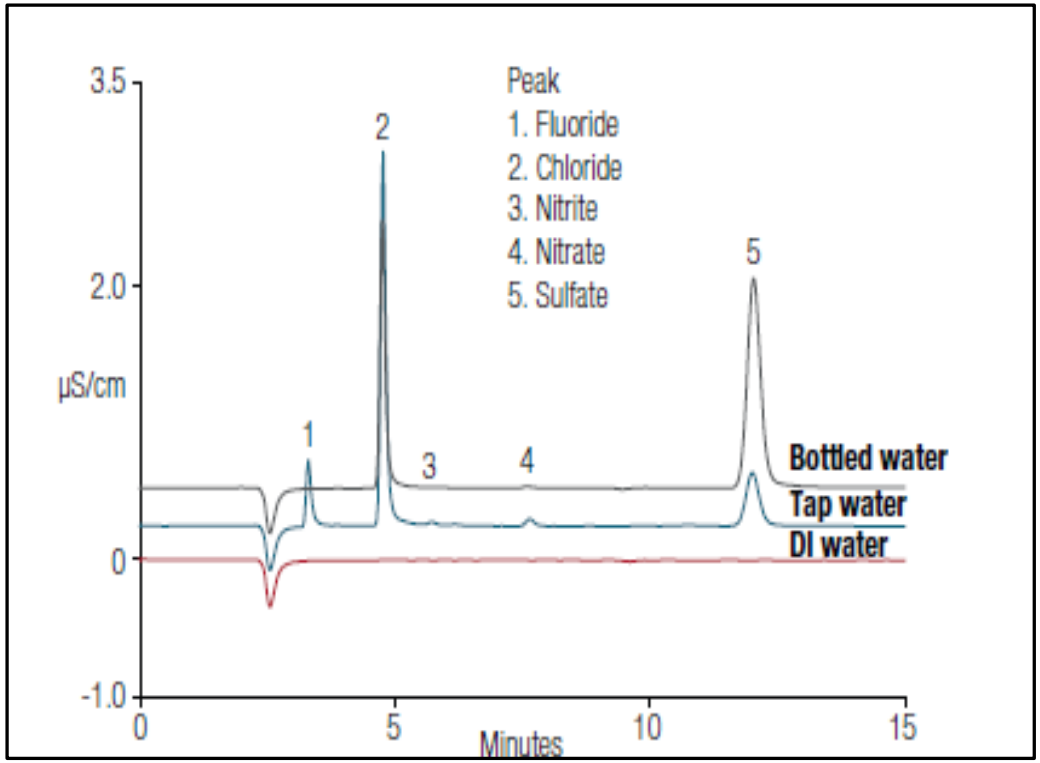
Column	Ion Pac AS 16 (250 X 2mm) 4 µ + Guard
Eluent	65 mM NaOH
Flow	0.38 ml/Min
Injection Vol	250 µl
detection	Conductivity with Anion Suppressor (Current 62 mA)
LOQ	1 µg/L



ThermoFisher SCIENTIFIC

Water: Inorganic anions in Drinking water (EPA Method 300 and 300.1)

Column	Ion Pac AS 22 (250 X 4mm) Guard
Eluent	4.5 mM Carbonate+1.4 mM Bicarbonate
Flow	1.2 ml/Min
Injection Vol	10 µl
detection	Conductivity with Anion Suppressor (Current 31 mA)

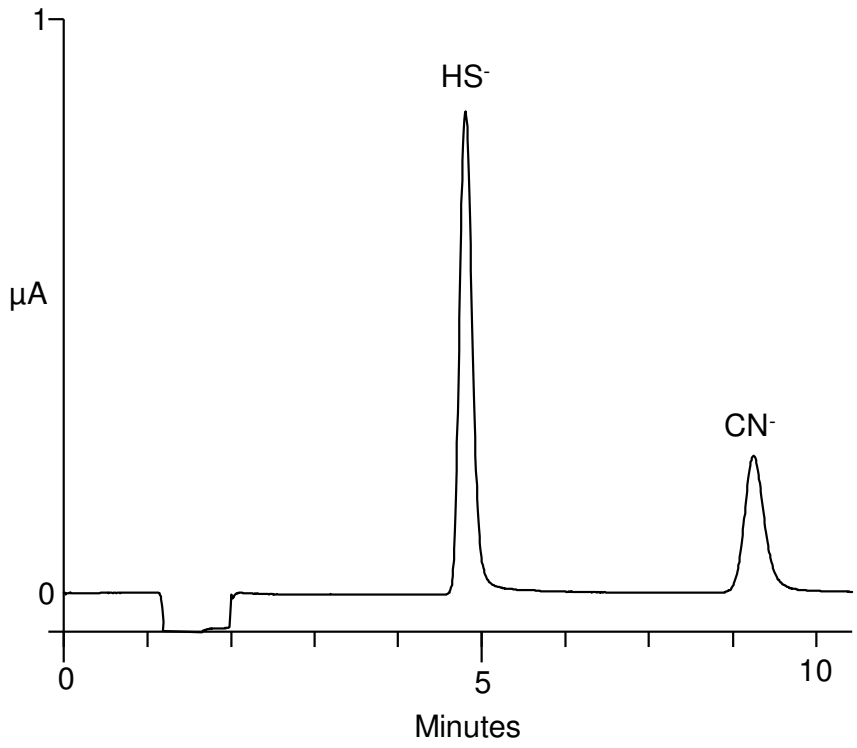


ThermoFisher SCIENTIFIC

Water: Cyanide in Ground water and Drinking water

Cyanide in ground water may be the source. Cyanide is toxic even at low concentrations

Column	Ion Pac AS 7 (250 X 4mm) + Guard
Eluent	0.1 mol/L NaOH+0.5 mol/L NaOAC+0.5 % v/v Ethylenediamine
Flow	1.0 ml/Min
Injection Vol	50 µl
detection	DC Amperometry (Gold working electrode) OR Cyanide Waveform
LOQ	1 µg/L

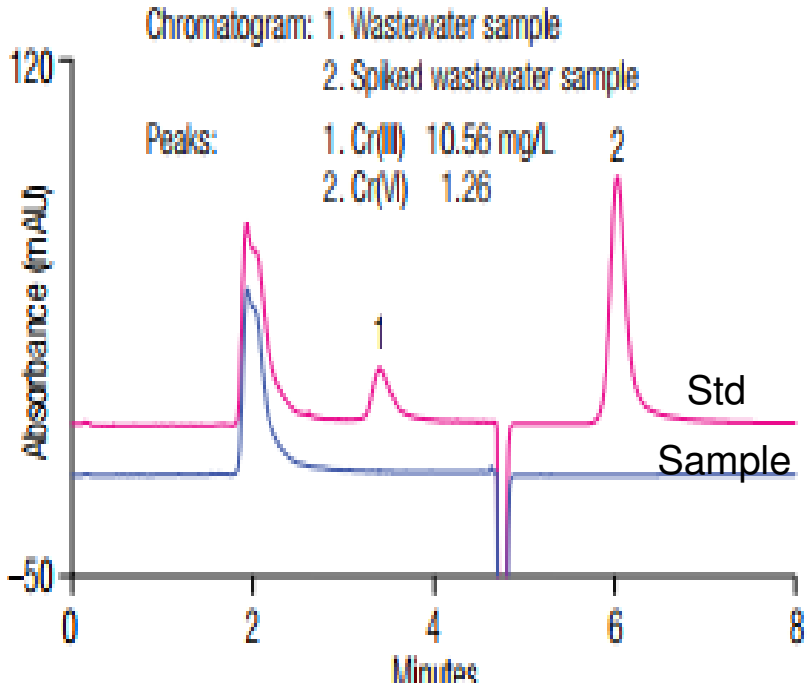


ThermoFisher SCIENTIFIC

Water: Total and Hexavalent Chromium in Drinking water

Hexavalent Chromium can enter in ground water through paints, dyes, wood preservatives etc.

Column	Ion Pac CS 5A (250 X 4mm) + Guard
Eluent	PDCA Eluent
Flow	1.0 ml/Min
Injection Vol	50 µl
detection	UV Detector at 365 nm
LOQ	50 µg/L

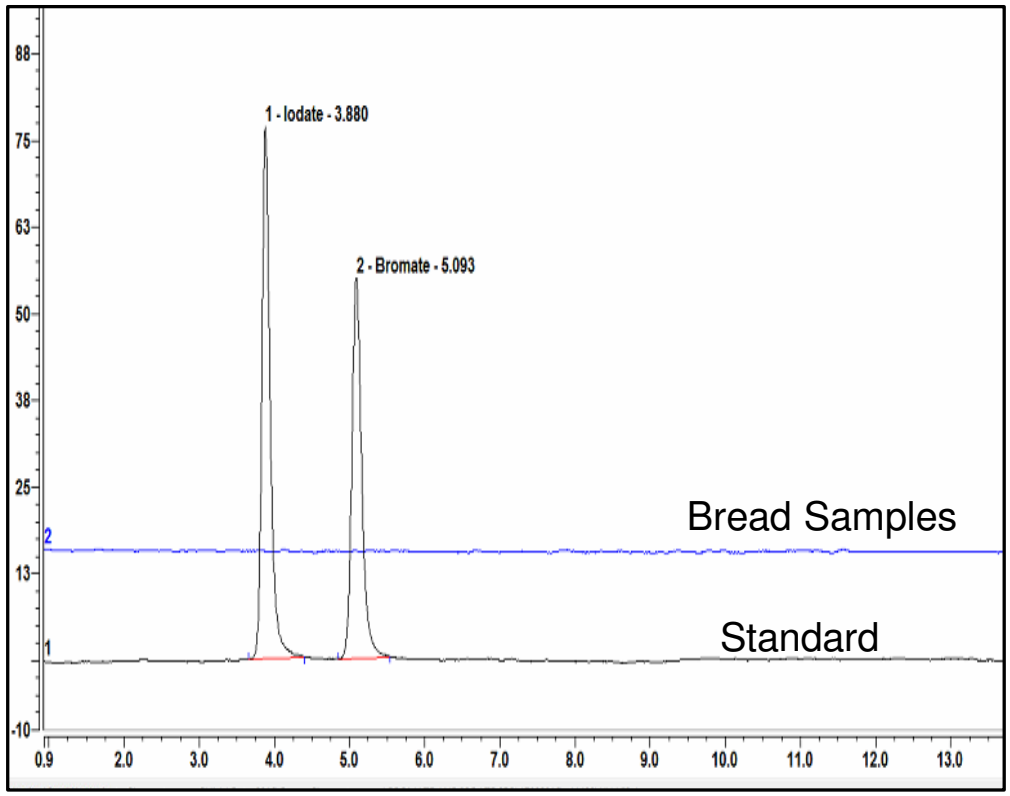


ThermoFisher SCIENTIFIC

Food: Bromate and iodate in Bread and its floor.

Potassium Bromate and Iodate are added in Bread to make it spongier and improve its size. But Bromate is Carcinogenic.

Column	Ion Pac AS 19 (250 X 4mm) + Guard
Eluent	20 mM NaOH
PCR	0.3 % Promethazine in 5M HCl (0.5 ml/min)
Eluent Flow	1.0 ml/Min
Injection Vol	25 µl
detection	UV detector 515 nm
LOD	0.03 mg/L



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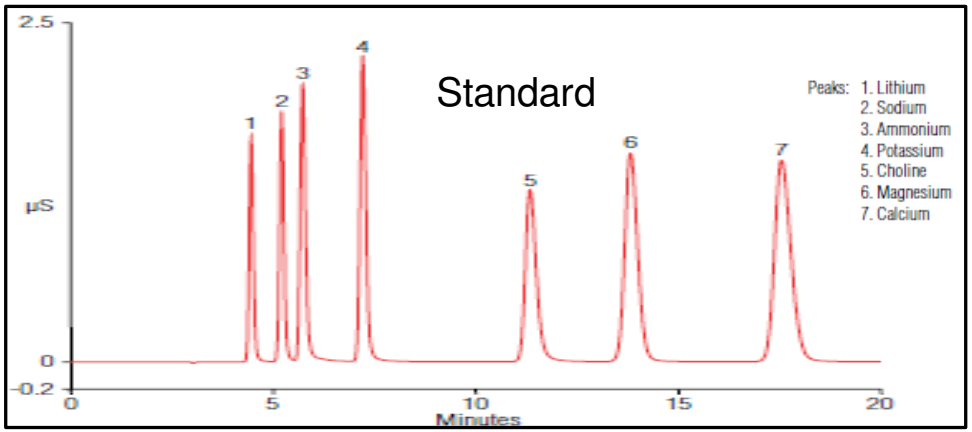
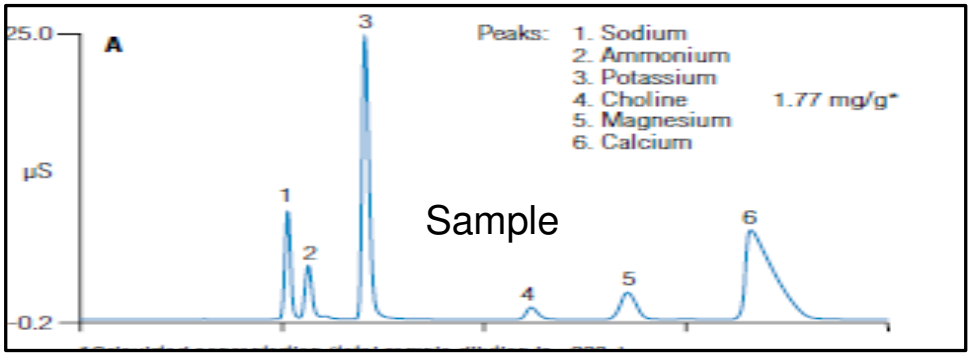
Food: Choline in Infant Milk

Choline is required additive in infant formulas and hence need to be evaluated

Column	Ion Pac CS 19 (250 X 2mm) + Guard
Eluent	6.5 mM MSA
Eluent Flow	0.25 ml/Min
Injection Vol	5 µl
detection	Conductivity Detector with cation suppressor (Current 4 mA)





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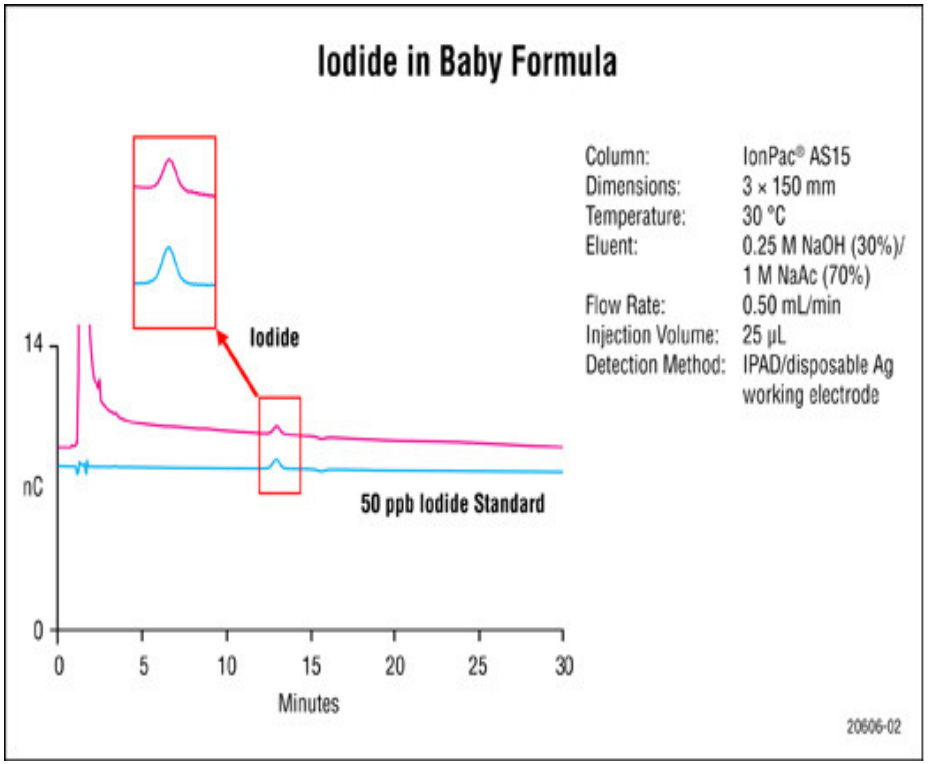
Food: Iodide in Infant Milk

Excess Iodide may lead to Thyroid disorders in babies

Column	Ion Pac AS 15 (150 X 3 mm) + Guard
Eluent	0.25 M NaOH+1 M NaOAC
Eluent Flow	0.5 ml/Min
Injection Vol	25 µl
detection	Pulsed Amperometry with Silver working electrode

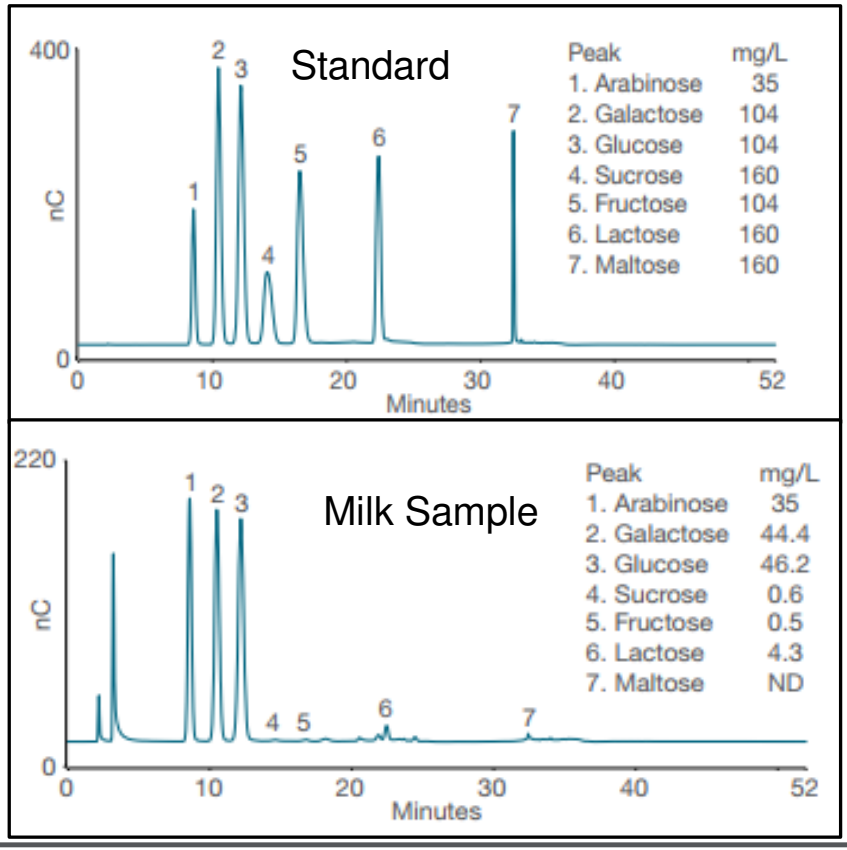


ThermoFisher SCIENTIFIC

Food: Sugars in Dairy Products

Sugar contents need to be Specified on Nutritional fact labels of dairy products.

Column	CarboPac PA 1 Analytical + Guard
Eluent	Hydroxide Gradient
Eluent Flow	0.25 ml/Min
PCR	0.3 M NaOH at 0.3 ml/min Flow rate
Injection Vol	5 µl
Detection	Pulsed Amperometry with Gold working electrode and Ag/AgCl Reference electrode.



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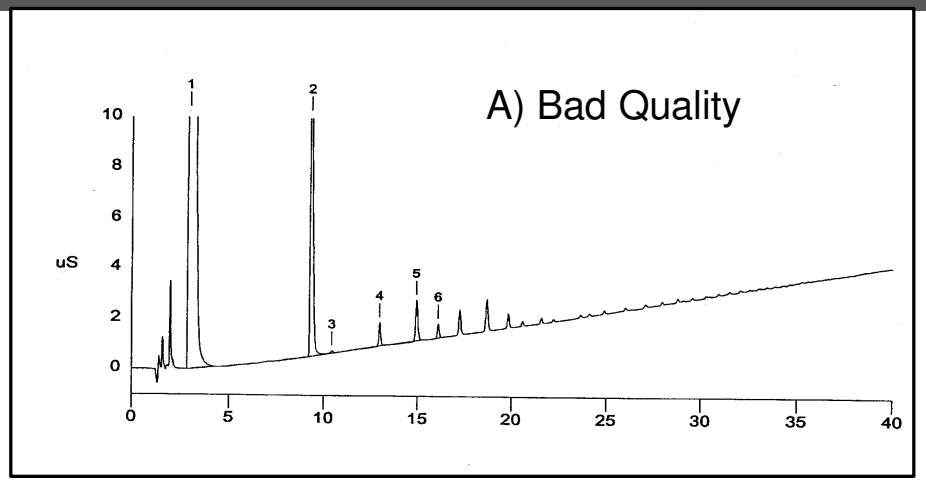
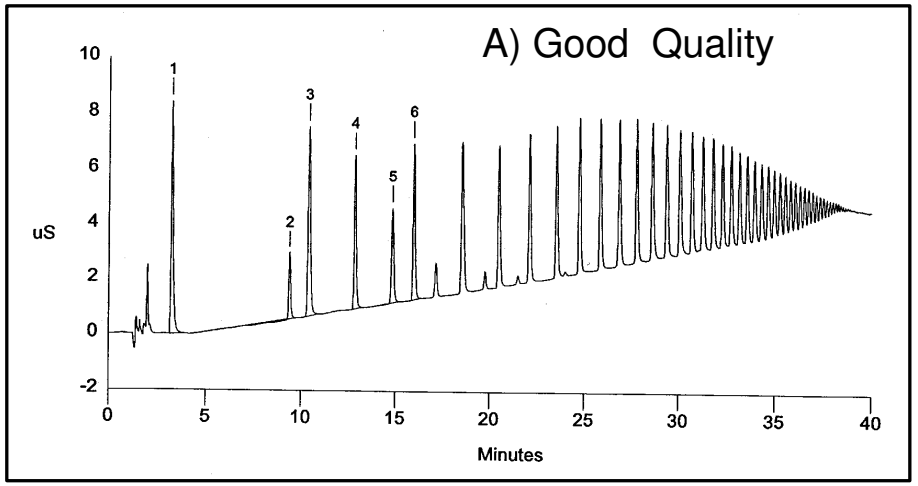
Food: Polyphosphates in Cheese samples

Column	AS 11 (250 X 2 mm) + AG 11 (50 X 2 mm)
Eluent	Hydroxide Gradient
Eluent Flow	0.3 ml/Min
Injection Vol	10 µl
detection	Suppressed Conductivity (Anion Suppressor)

Peaks:

1. PO_4
2. P_2O_7
3. P_3O_9
4. P_3O_{10}
5. P_4O_{12}
6. P_4O_{13}





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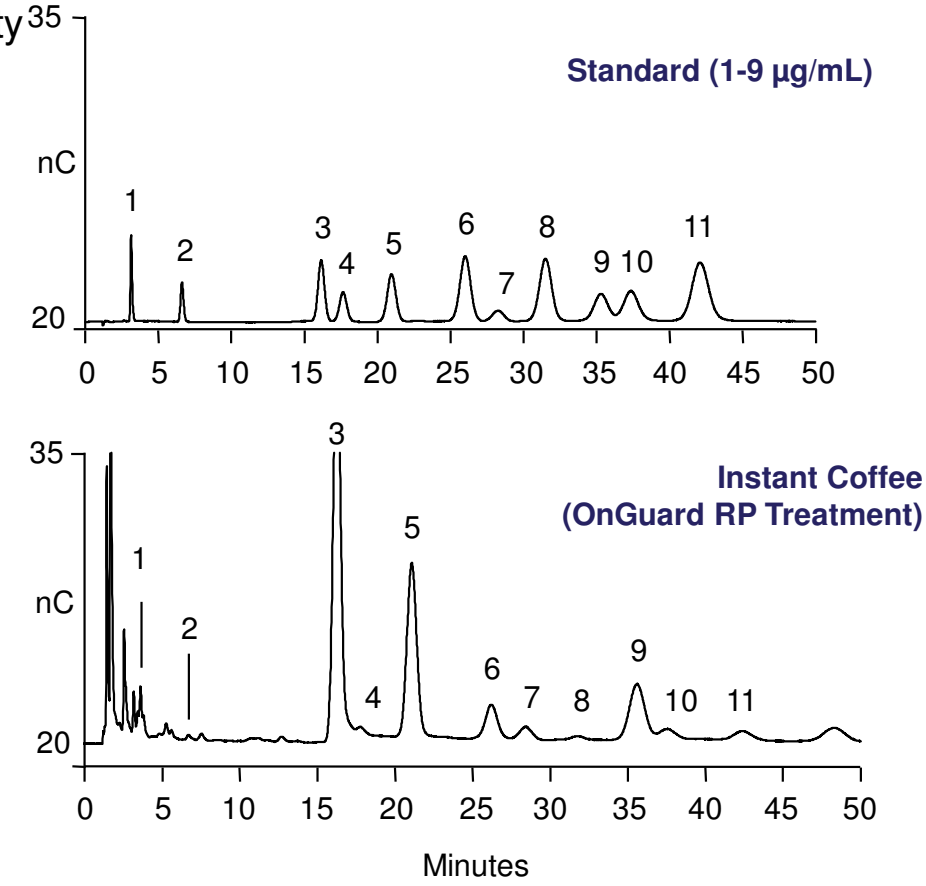
Monosaccharides in Soluble Coffee

Profile of Monosaccharides in coffee sample evaluates its quality

Column	CarboPac PA 10 with Guard
Eluent	1 mM KOH
Eluent Flow	1.0 ml/min (Post Column Addition of 0.3 M NaOH)
Injection Vol	5 µl
detection	Pulsed Amperometry with Gold working electrode

Peaks :

1. Mannitol
2. Fucose
3. Arabinose
4. Rhamnose
5. Galactose
6. Glucose
7. Sucrose
8. Xylose
9. Mannose
10. Fructose
11. Ribose



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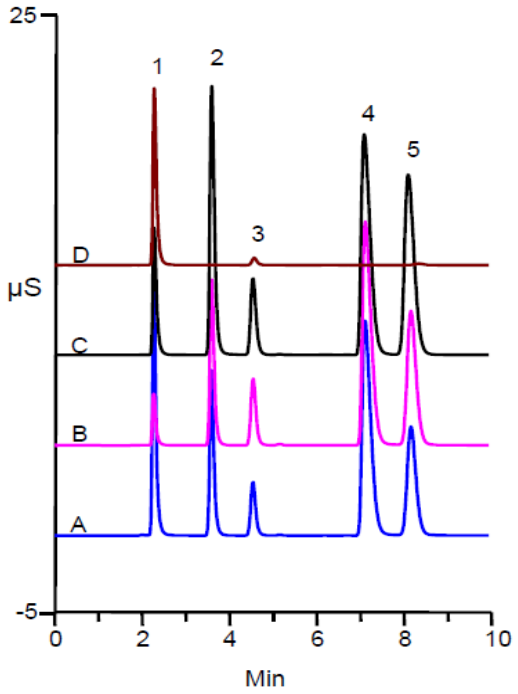
Food: Fluoride in Tea

Fluoride may cause dental fluorosis, Bone fractures etc. better Alternative for current Ion selective electrode method NY/T 838-2004

Column	Ion Pac AS 18 (150 X 4 mm) + Guard
Eluent	20 mM KOH
Eluent Flow	1.0 ml/min
Injection Vol	100 µl
detection	Suppressed Conductivity with Anion Suppressor



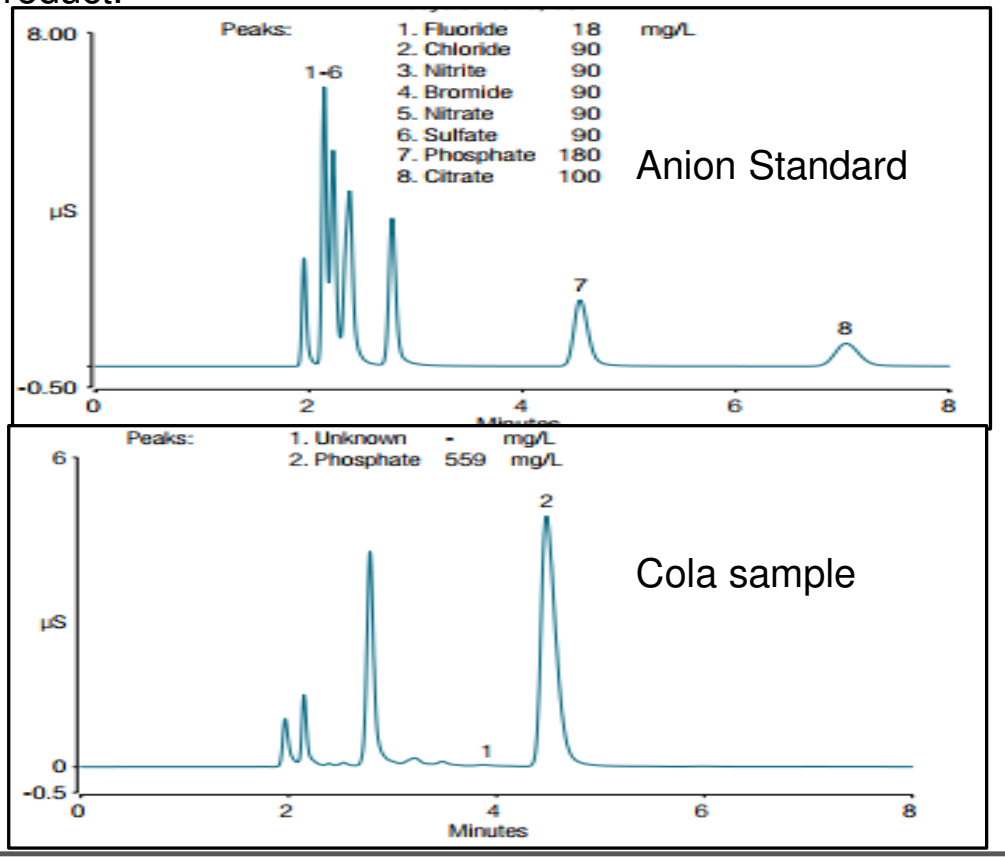
Sample :	Fluoride (mg/Kg)
A Tea 1	279
B Tea 2	55
C Tea 3	129
D 200 ppm F^-(NH_4F)	200

Peaks:
1. Fluoride
2. Chloride
3. Nitrite
4. Sulfite
5. Sulfate

Beverages: Citrate and Phosphate in Soft Drinks

Phosphoric acid and Citric acid used in Cola Syrup as a Stabilizer and Taste maker. Their concentrations are monitored in Manufacturing of Syrup and bottling of Cola Product.

Column	Ion Pac Anion III A (150 X 3 mm) + Guard
Eluent	22 mM KOH
Eluent Flow	0.5 ml/Min
Injection Vol	25 µl
detection	Suppressed Conductivity



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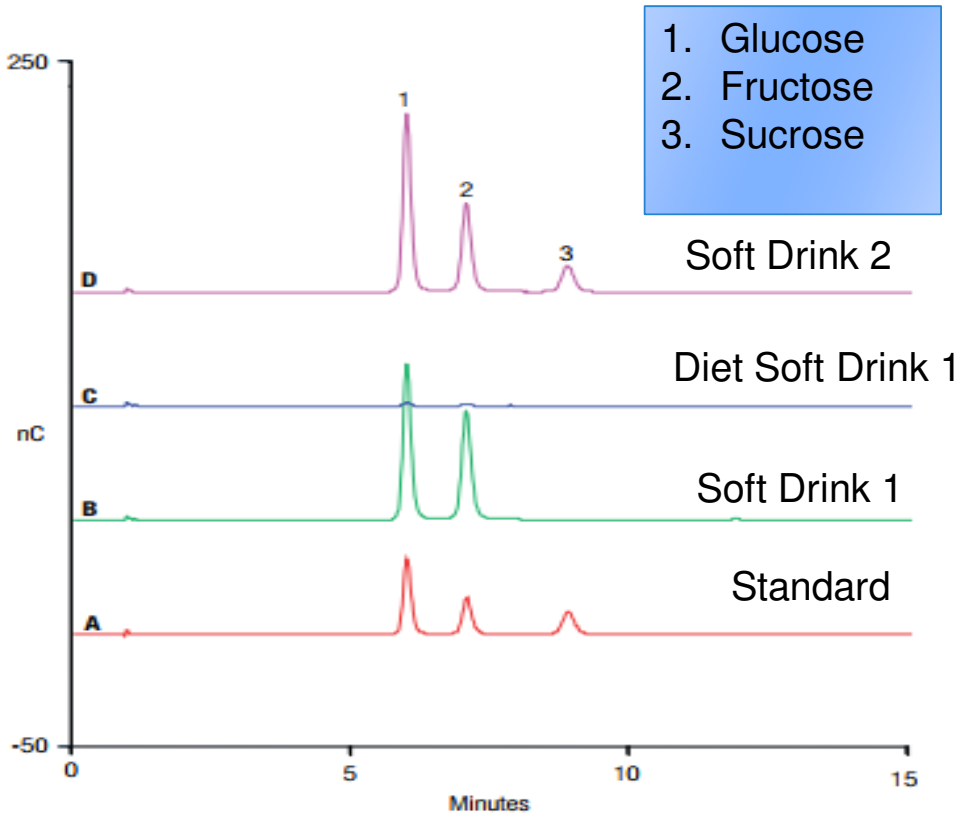
Beverages: Sugars in Soft Drinks

Sugars should be monitored in Soft drinks for quality purpose.

Column	Carbopac PA 20 Analytical + Guard
Eluent	33 mM KOH
Eluent Flow	0.5 ml/Min
Injection Vol	10 µl
Detection	Pulsed Amperometry with Ag/AgCl Ref. electrode Quadrapole Waveform



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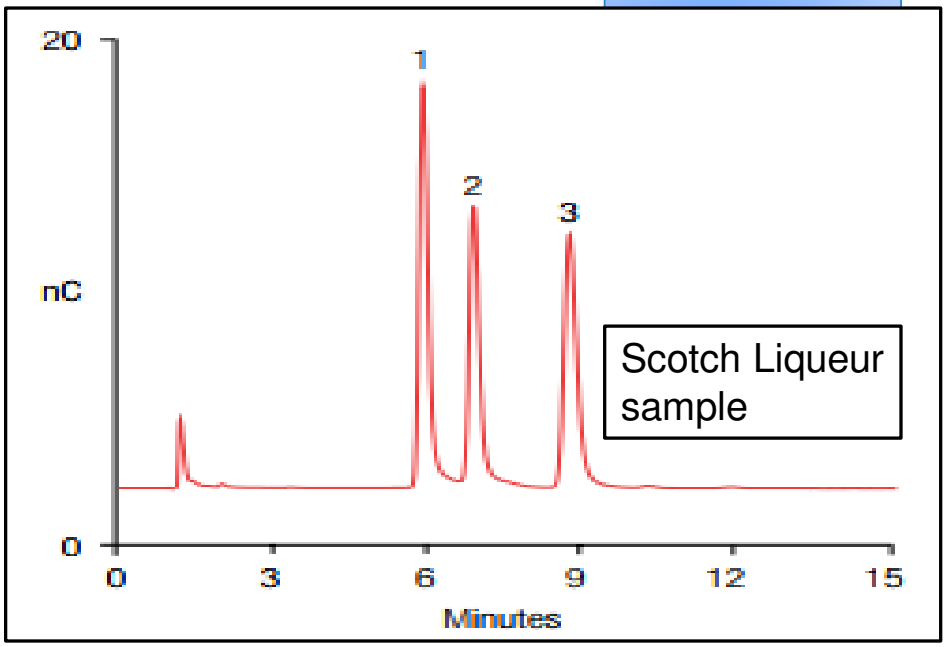
Beverages: High Conc Sugars in Scotch Liqueur Sample

In Scotch Liqueur samples Sugars are monitored for Quality testing purpose

Column	Carbopac PA 20 Analytical + Guard
Eluent	35 mM KOH with 100 mM KOH wash
Eluent Flow	0.5 ml/Min
Injection Vol	0.4 µl
Detection	Pulsed Amperometry with Ag/AgCl Ref. electrode Quadrapole Waveform

1. Glucose
2. Fructose
3. Sucrose



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Beverages: Organic acid Content in Beer samples

Organic acids are end products of yeast fermentation critical to the flavor of beer, but are also products of bacterial fermentation that introduce a sour flavor, either purposely or unintentionally due to spoilage.

Column	Ion Pac AS 11 HC Column l + Guard
Eluent	Hydroxide gradient
Eluent Flow	0.38 ml/Min
Injection Vol	2.5 µl
Detection	Suppressed Conductivity

Peaks:	mg/L		mg/L
1. Quinate	10	21. Nitrate	10
2. Fluoride	3	22. Citramalate	15
3. Lactate	10	23. Malate	15
4. Acetate	10	24. Carbonate	15
5. 2-Hydroxybutyrate	10	25. Malonate	15
6. Propionate	10	26. Citraconitate	15
7. Formate	10	27. Maleate	15
8. Butyrate	10	28. Sulfate	15
9. Methanesulfonate	10	29. α-Ketoglutarate	15
10. Pyruvate	10	30. Oxalate	15
11. Isovalerate	10	31. Fumarate	15
12. Valerate	10	32. Tungstate	20
13. Monochloroacetate	10	33. Phosphate	20
14. Bromate	10	34. Phthalate	20
15. Chloride	5	35. Arsenate	20
16. 2-Oxovalerate	10	36. Citrate	20
17. Nitrite	10	37. Chromate	20
18. Ethylphosphate	10	38. Isocitrate	20
19. Trifluoroacetate	10	39. *cis*-Aconitate	–
20. Bromide	10	40. *trans*-Aconitate	20

Beverages: Organic acid Content in Beer samples

Peaks:	mg/L		mg/L
1. Quinate	10	21. Nitrate	10
2. Fluoride	3	22. Citramalate	15
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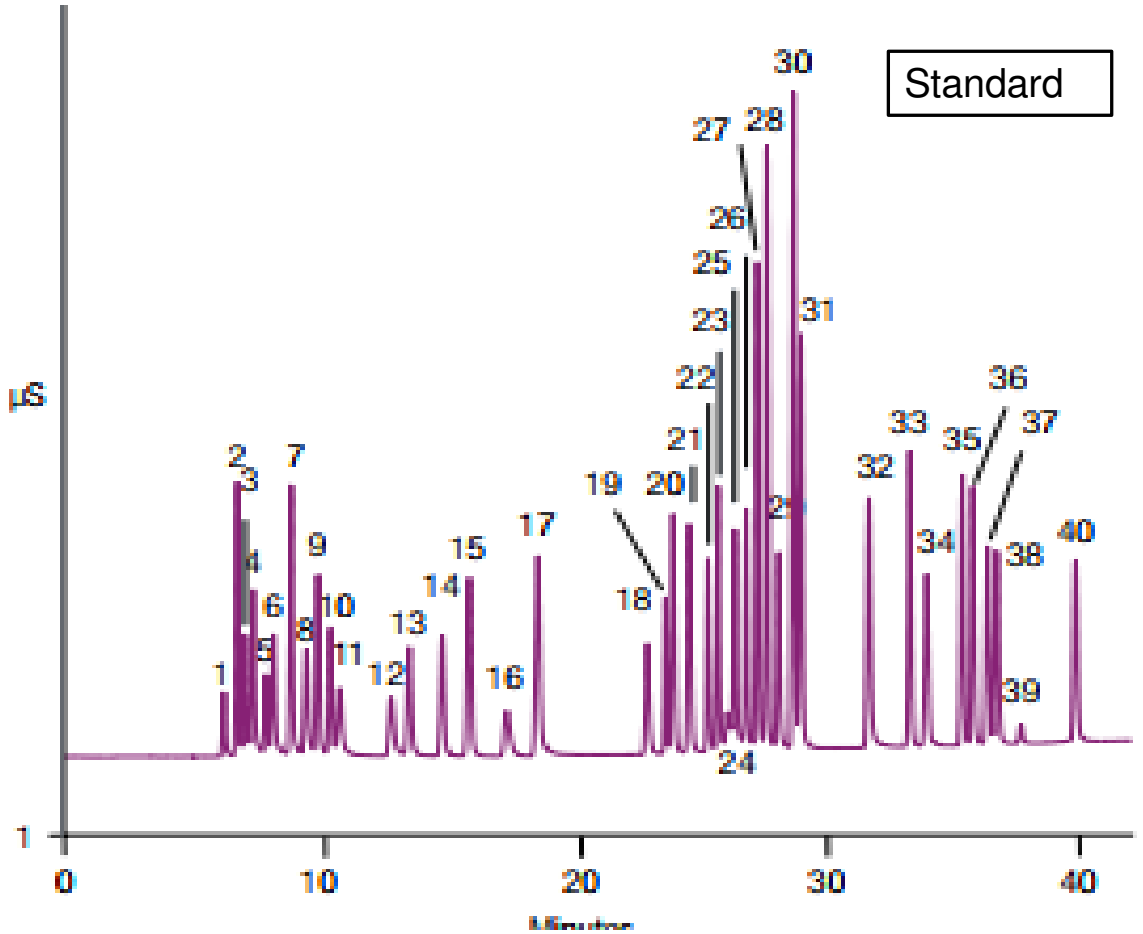
20. Bromide	10	40. *trans*-Aconitate	20



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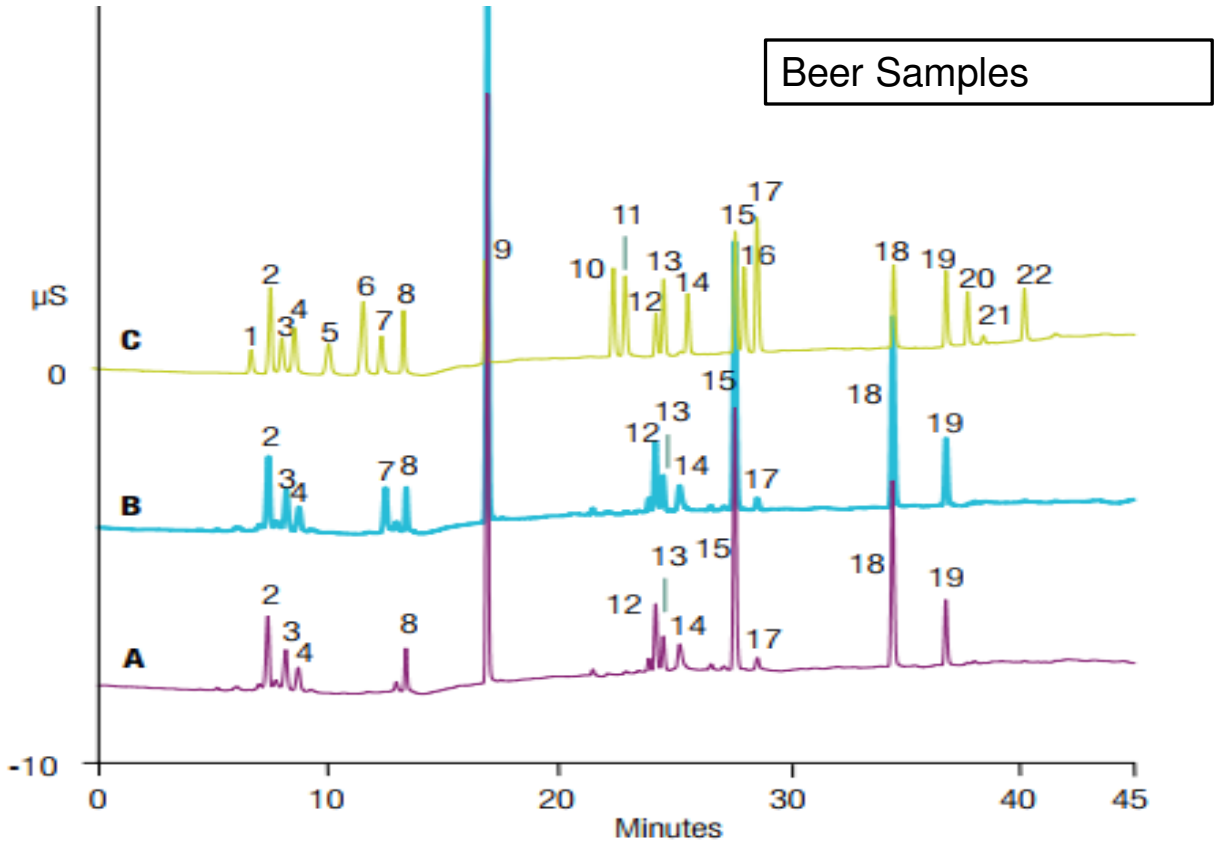
Beverages: Organic acid Content in Beer samples

Peaks:	mg/L		mg/L
1. Quinate	10	21. Nitrate	10
2. Fluoride	3	22. Citramalate	15
3. Lactate	10	23. Malate	15
4. Acetate	10	24. Carbonate	15
5. 2-Hydroxybutyrate	10	25. Malonate	15
6. Propionate	10	26. Citraconitate	15
7. Formate	10	27. Maleate	15
8. Butyrate	10	28. Sulfate	15
9. Methanesulfonate	10	29. α-Ketoglutarate	15
10. Pyruvate	10	30. Oxalate	15
11. Isovalerate	10	31. Fumarate	15
12. Valerate	10	32. Tungstate	20
13. Monochloroacetate	10	33. Phosphate	20
14. Bromate	10	34. Phthalate	20
15. Chloride	5	35. Arsenate	20
16. 2-Oxovalerate	10	36. Citrate	20
17. Nitrite	10	37. Chromate	20
18. Ethylphosphate	10	38. Isocitrate	20
19. Trifluoroacetate	10	39. *cis*-Aconitate	–
20. Bromide	10	40. *trans*-Aconitate	20



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