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The Past, Present and Future of Ion Chromatography

Joachim Weiss, Ph.D.
Technical Director for Dionex™ Products

Outline

- Introduction
- Areas of progress over the past 44 years
  - Hardware
  - Stationary phases for small-molecular weight ions
    - Anion-exchange chromatography
    - Cation-exchange chromatography
  - Detection
- Current trends
- Conclusions

INTRODUCTION

Ion Analysis Prior to the Introduction of IC

- Photometry
- Titration
- Gravimetry
- Ion-selective electrodes
- Turbidimetry
- Nephelometry
- Atomic absorption
- Polarography
- ...

Some of these methods are:

- Laborious
- Time-consuming
- Prone to interferences

The Legendary Article from Small et al. in 1975

Novel Ion Exchange Chromatographic Method Using Conductimetric Detection

Hamish Small

Central Research, The Dow Chemical Company, Midland, MI 48640

Timothy S. Stevens

Michigan Division Analytical Laboratories, The Dow Chemical Company, Midland, MI, 48640

William C. Bauman

Inorganic Process Research, Texas Division, The Dow Chemical Company, Freeport, TX 77541

Ion exchange resins have a well known ability to provide excellent separation of ions, but the automated analysis of the eluted species is often frustrated by the presence of the background electrolyte used for elution. By using a novel combination of resins, we have succeeded in neutralizing or suppressing this background without significantly affecting the species being analyzed which in turn permits the use of a conductivity cell as a universal and very sensitive monitor of all ionic species either cationic or anionic. Using this technique, automated analytical schemes have been devised for Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, Ca^{2+}, Mg^{2+}, F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, SO_4^{2-}, SO_3^{2-}, PO_4^{3-} and many amines, quaternary ammonium compounds, and organic acids. Elution time can take as little as 1.0 min/ion and is typically 3 min/ion. Ions have been determined in a diversity of backgrounds, e.g., waste streams, various local surface waters, blood serum, urine, and fruit juices.

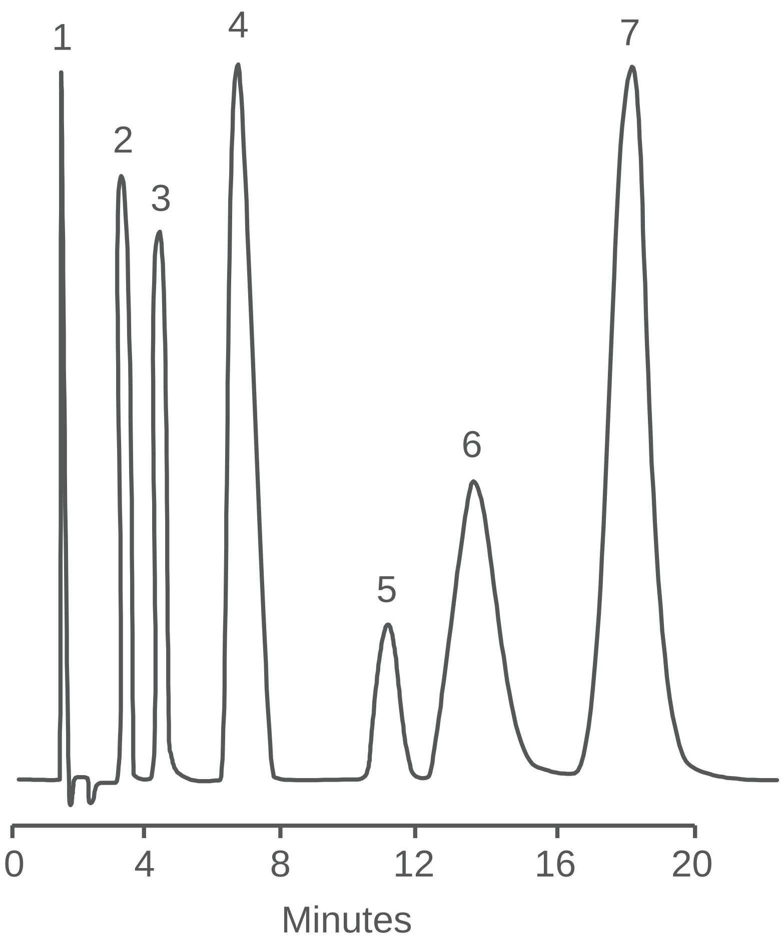
The demand for the determination of ionic species in a variety of aqueous environments is increasing rapidly and, as a result, there is an expanding need for automated or semiautomated analysis of chemical plant streams, environmentally important waters such as waste streams, rivers, and lakes, and fluids of biological interest such as blood, urine, etc. There are many examples where there is a continual need for routine analysis of common species such

IC with Suppressed Conductivity Detection 1975

- **Anion Analysis**
  - Glass separator column (500 mm × 3 mm i.d.)
  - Glass suppressor column (250 mm × 6 mm i.d.)
  - Injection volume: 100 µL
  - Flow rate: 3 mL/min
  - Eluent: carbonate/bicarbonate
  - Analysis time: 20+ min
  - Detection limits: 100 µg/L sulfate
  - Gradient elution: **impossible**

- **Cation Analysis**
  - Glass separator column (250 mm × 6 mm i.d.)
  - Glass suppressor column (250 mm × 10 mm i.d.)
  - Injection volume: 100 µL
  - Flow rate: 3 mL/min
  - Eluent: HCl or HCl with *m*-phenylenediamine
  - Analysis time: 10-20 min (mono- or divalent cations)
  - Detection limits: 100 µg/L sodium
  - Gradient elution: **impossible**

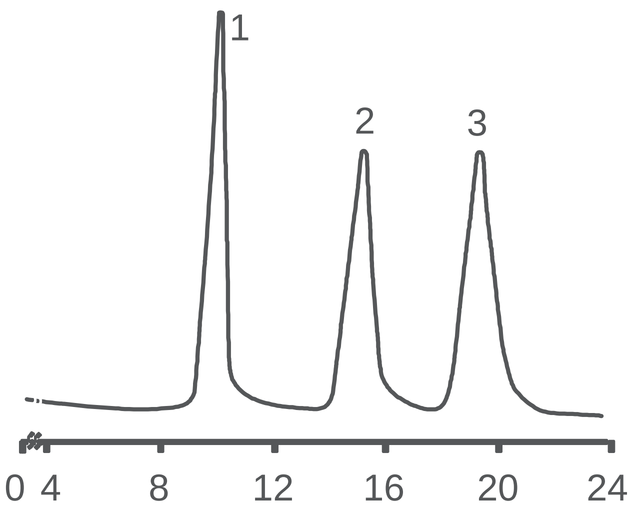
Separation of Standard Anions on the "Anion Column" in 1975



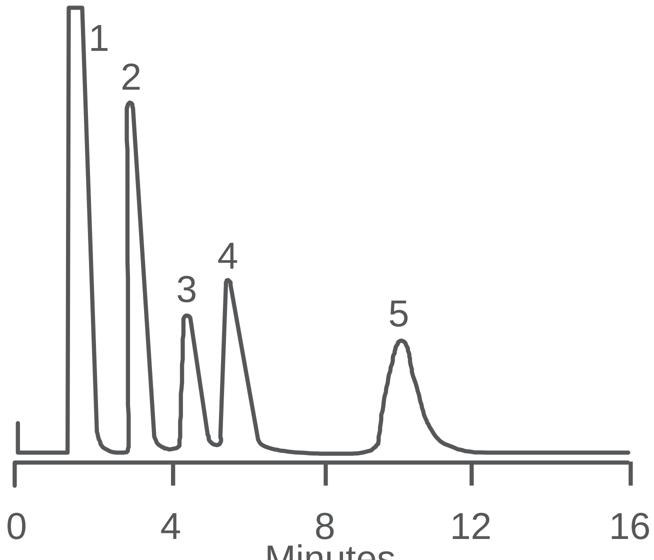
Column:	Dionex IonPac AS1
Eluent:	2.8 mmol/L $NaHCO_3$
	2.2 mmol/L Na_2CO_3
Flow rate:	3 mL/min
Detection:	Suppressed conductivity

Peaks:	1. Fluoride	3	mg/L
	2. Chloride	4	
	3. Nitrite	10	
	4. Orthophosphate	10	
	5. Bromide	10	
	6. Nitrate	20	
	7. Sulfate	25	

Separation of Inorganic Cations in 1975



Column:	150 mm × 3 mm i.d. guard column 250 mm × 6 mm i.d. separator column
Eluent:	5 mmol/L nitric acid
Flow rate:	3.1 mL/min
Inj. volume:	100 µL
Detection:	Suppressed conductivity
Peaks:	1. Sodium 5 mg/L 2. Ammonium 5 3. Potassium 10



Column:	150 mm × 3 mm i.d. guard column 250 mm × 6 mm i.d. separator column
Eluent:	2.5 mmol/L *m*-phenylenediamine·2HCl 2.5 mmol/L nitric acid
Flow rate:	3.1 mL/min
Inj. volume:	100 µL
Detection:	Suppressed conductivity
Peaks:	1. Monovalents - 2. Magnesium 3 mg/L 3. Calcium 3 4. Strontium 10 5. Barium 25

Competing Methods for Ion Analysis Today

- ICP (-OES/-MS)
  - Determination of total metals content
- Atomic absorption
  - Graphite furnace: ultra-trace analysis of metals
  - Hydride generation: determination of antimony, arsenic, selenium, and tellurium
- Polarography
  - Ultra-trace analysis of selected metals
- Titration
  - Determination of selected ions at percent levels
- Ion-selective electrodes
  - Prone to interferences

Competing Methods for Ion Analysis Today

- Capillary Electrophoresis
  - Less expensive stationary phases (fused silica capillaries)
  - Flow rate accuracy and precision in chromatographic systems higher than in CE (stability of electro-osmotic flow)
  - Lack of commercially available detection systems (suppressed conductivity, IPAD, post-column derivatizations)
  - Problems with electrostacking in trace analysis
- Flow Injection Analysis (FIA)/Segmented Flow Analysis (SFA)
  - Perfect choice for high-throughput analysis (> 100)
  - High degree of automation (Autoanalyzer)
  - Complex if many analytes have to be analyzed
- Photometry
  - Still used today for analytes such as nitrite and orthophosphate at very low concentrations

AREAS OF PROGRESS

Areas of Progress During the Past 44 Years

- Improved hardware
- Stationary phase design
- Eluent preparation techniques (RFIC)
- Suppression technology
- Detection limits
- New detection methods
- Analysis times
- Miniaturization
- Applicability
- Ease-of-use
- ...

Thermo Scientific Dionex Ion Chromatography (IC) Systems Today

RFIC

HPIC

Thermo Scientific
Dionex™ Aquion™
Ion Chromatography
(IC)System

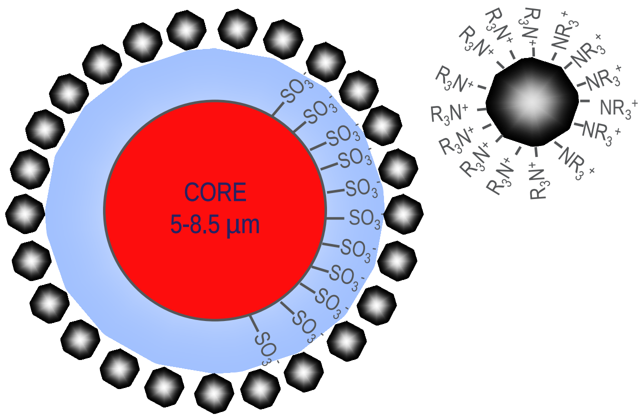
Thermo Scientific™
Dionex™ Integrion™
HPIC™ System

Thermo Scientific™
Dionex™ ICS-4000
Integrated Capillary
HPIC™ System

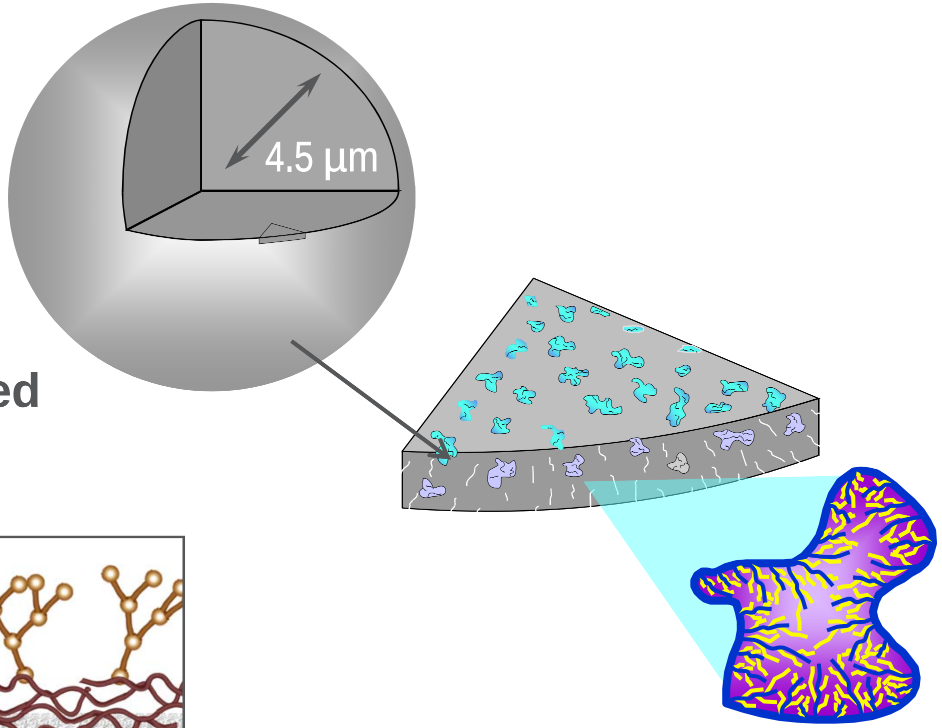
Thermo Scientific™
Dionex™ ICS-6000
Standard Bore
and Microbore HPIC™ Systems

Developments in the Design of Anion Exchangers

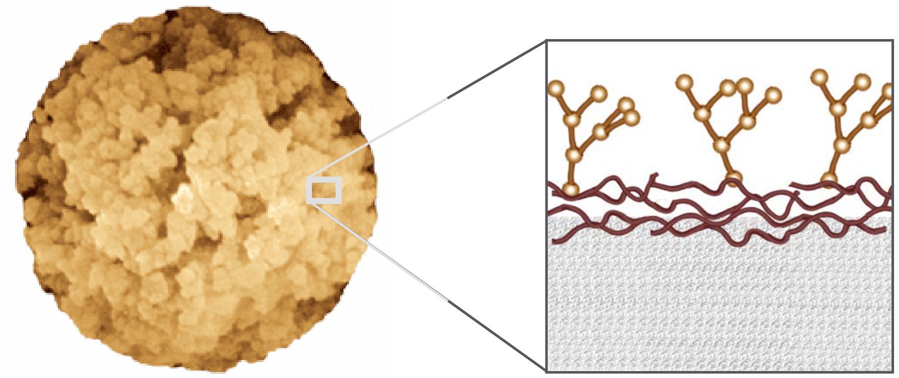
From nanobead-agglomerated …



… to grafted surface-aminated …

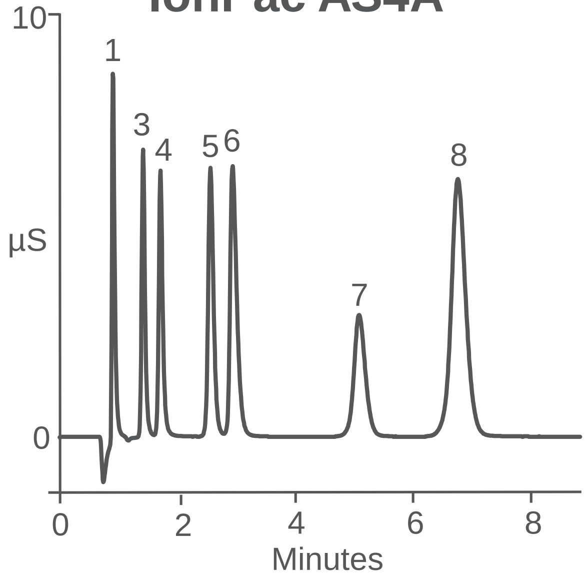


… to hyperbranched anion exchangers.



Chromatographic Results

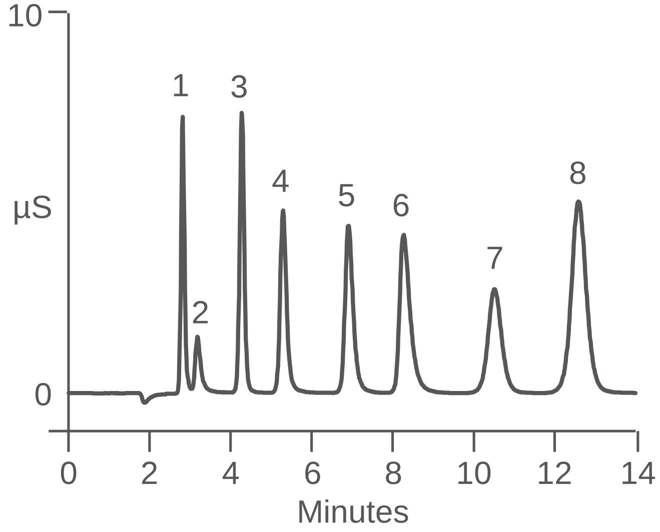
IonPac AS4A



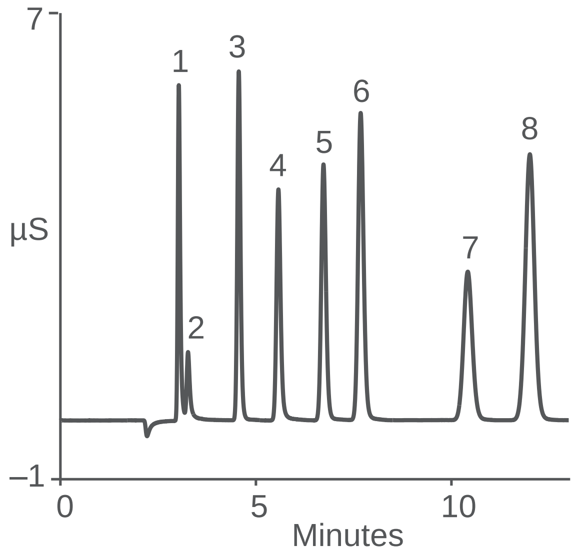
Eluent: Carbonate/bicarbonate
Flow rate: 1.2 –2.0 mL/min
Detection: Suppressed conductivity

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

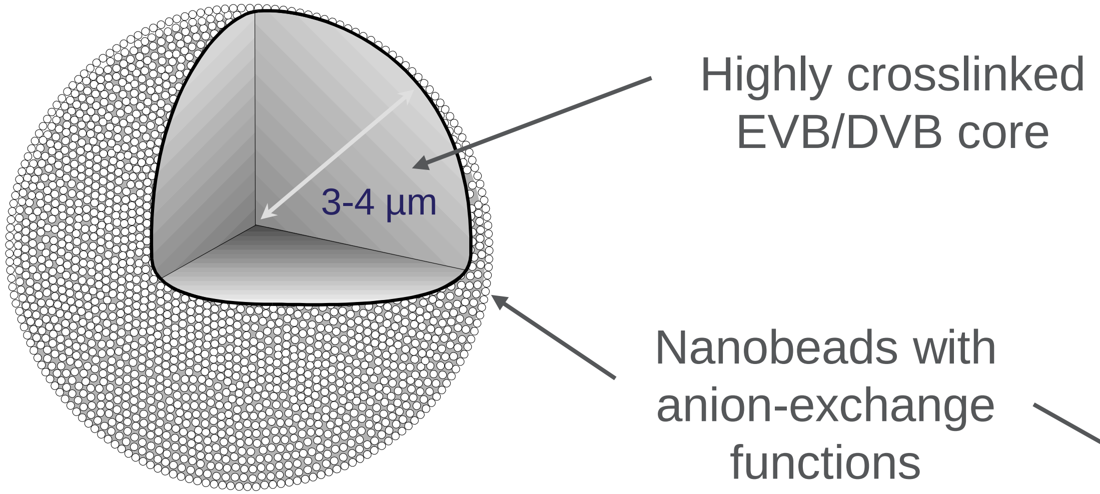
IonPac AS14



IonPac AS22

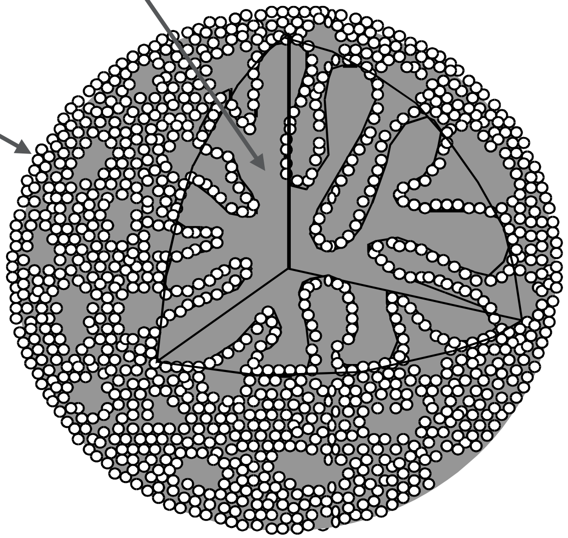


The Importance of High-Capacity Resins



From low-capacity microporous …

- Separation of equally charged ions with large concentration differences
- Large volume injections to facilitate trace-level analysis



… to high-capacity macroporous resins.

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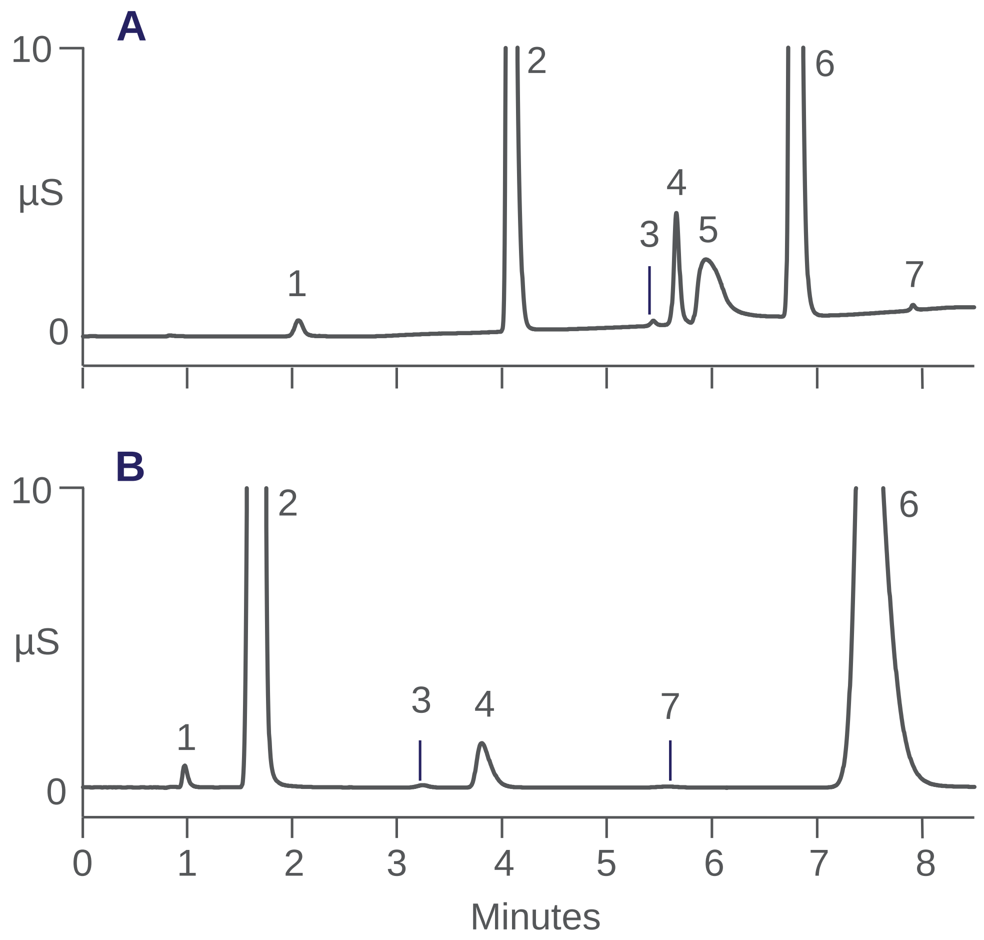
Carbonate-Based or Hydroxide Eluents in Anion-Exchange Chromatography?



Column:	A. IonPac AS17 with guard B. IonPac AS4A-SC with guard		
Eluent:	A. 1–40 mmol/L KOH gradient (EG) B. 1.7 mmol/L $NaHCO_3$ + 1.8 mmol/L Na_2CO_3		
Flow rate:	2 mL/min		
Inj. volume:	A. 25 µL B. 50 µL		
Sample:	Unprocessed water		
Peaks: [mg/L]	1. Fluoride	0.25	0.17
	2. Chloride	76.1	74.4
	3. Bromide	0.16	0.13
	4. Nitrate	3.22	2.93
	5. Carbonate	—	—
	6. Sulfate	51.3	51.2
	7. Orthophosphate	0.20	0.34

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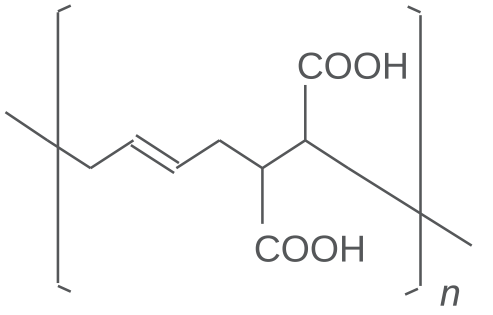
Conventional Anion-Exchange Chromatography Today

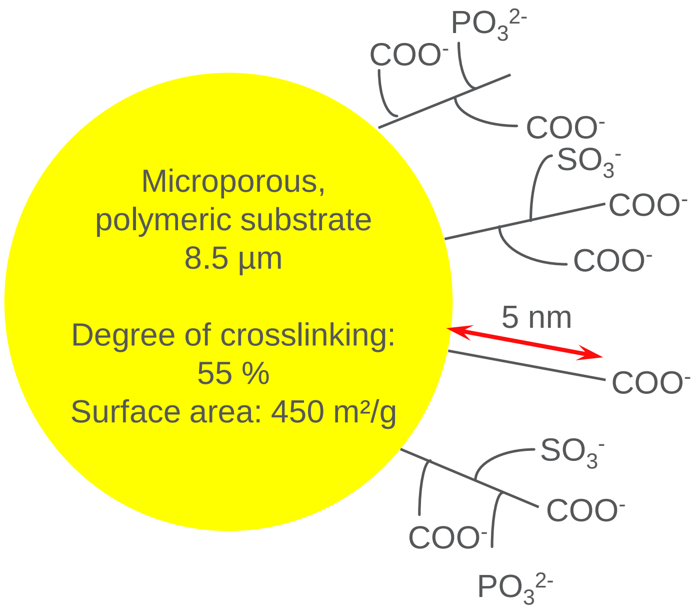
- PEEK separator and guard columns
(various particle diameters and dimensions)
- Auto Suppression (recycle or external water mode)
- Injection volumes: 0.4 – 25 µL
- Flow rates: 10 µL – 1.5 mL/min
- Eluents: carbonate/bicarbonate or hydroxide
- Analysis times: 5 – 12 min
- Detection limits: 5 – 10 µg/L (for 25 µL injection volume)
- Gradient elution: **possible** (with hydroxide eluents)

From polymer-coated silica …



Poly(butadiene-maleic acid), PBDMA

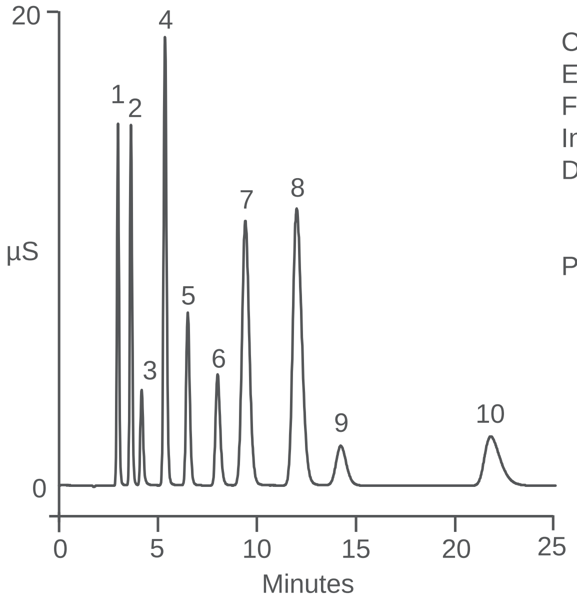
P. Kolla, J. Kohler and G. Schomburg (1987) *Chromatographia,* **23,** 465



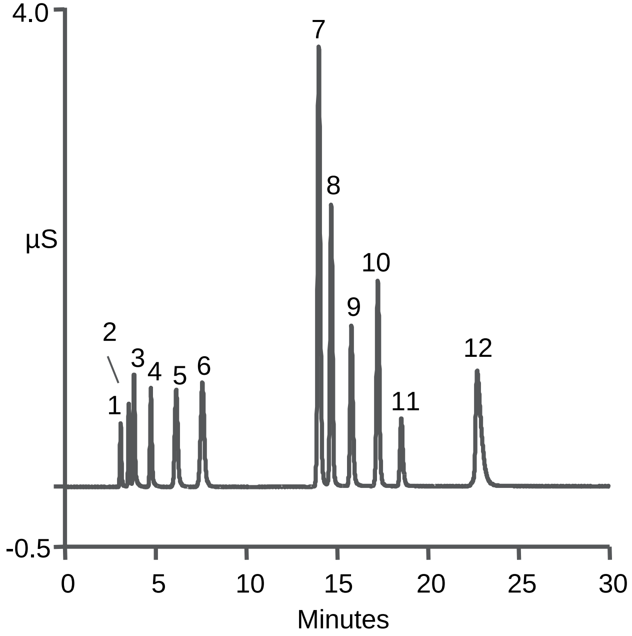
… to polymeric weak/strong acid cation exchangers.

Chromatographic Results

… for inorganic cations …



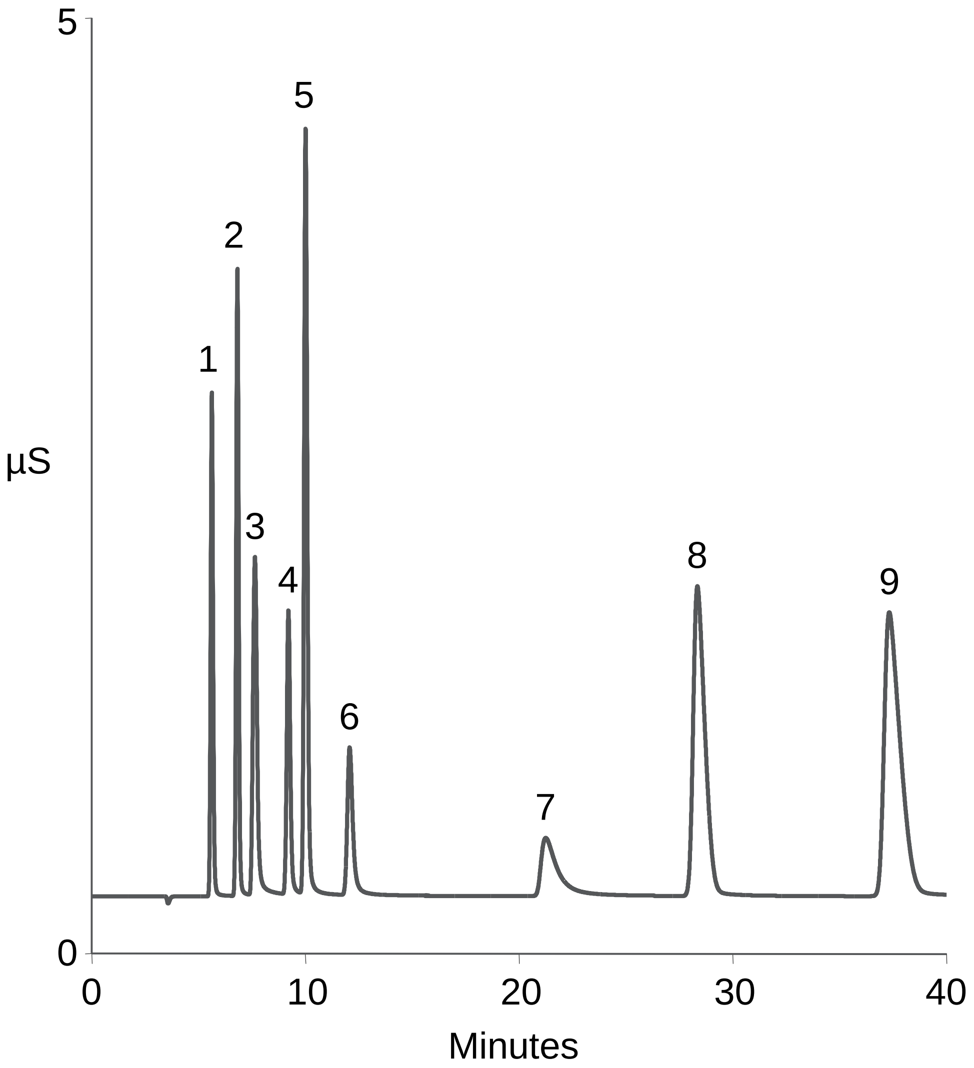
Column:	IonPac CS12A	
Eluent:	18 mmol/L MSA	
Flow rate:	1 mL/min	
Inj. volume:	25 µL	
Detection:	Suppressed conductivity, AutoSuppression, recycle mode	
Peaks:	1. Lithium	1 mg/L
	2. Sodium	4
	3. Ammonium	5
	4. Potassium	10
	5. Rubidium	10
	6. Cesium	10
	7. Magnesium	5
	8. Calcium	10
	9. Strontium	10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



Column:	IonPac CS19 (250 mm × 2 mm i.d.)			
Eluent:	MSA (EG)			
Gradient:	10 mmol/L for 7 min, 10–40 mmol/L over 6 min, 40–60 mmol/L over 7 min			
Flow rate:	0.3 mL/min			
Inj. volume:	0.4 µL			
Temperature:	30 °C			
Detection:	Suppressed conductivity			
Peaks:	1. Lithium	0.1	7. Putrescine	15
[mg/L]	2. Sodium	0.1	8. Cadaverine	9
	3. Ammonium	0.1	9. Histamine	13
	4. Potassium	0.1	10. Agmatine	10
	5. Magnesium	0.1	11. Spermidine	3
	6. Calcium	0.1	12. Spermine	6

… or biogenic amines.

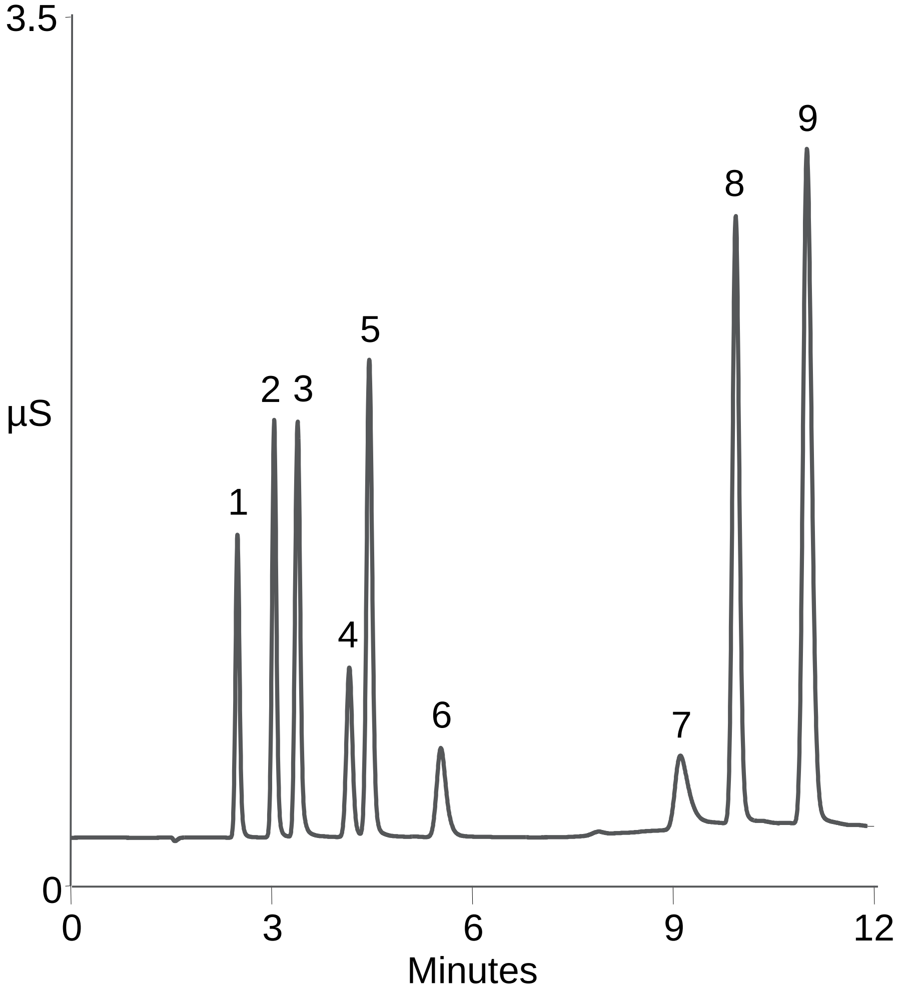
Isocratic Elution of Common Cations and Ethylamines



Column:	Dionex IonPac CS19-4µm with guard
Dimensions:	250 mm × 0.4 mm i.d.
Temperature:	30 ºC
Eluent:	4 mmol/L MSA (EG)
Flow rate:	10 µL/min
Inj. volume:	0.4 µL
Detection:	Suppressed conductivity, AutoSuppression, recycle mode

Peaks:		
1. Lithium	0.125	mg/L
2. Sodium	0.5	
3. Ammonium	0.62	
4. Monoethylamine	0.7	
5. Potassium	1.25	
6. Diethylamine	1.0	
7. Triethylamine	2.0	
8. Magnesium	0.62	
9. Calcium	1.25	

Gradient Elution of Common Cations and Ethylamines



Column:	Dionex IonPac CS19-4µm with guard	
Dimensions:	250 mm × 0.4 mm i.d.	
Temperature:	30 °C	
Eluent:	MSA (EG)	
Gradient:	Isocratic 4 mmol/L to 5 min, gradient to 8 mmol/L at 7.5 min, isocratic to 10 min	
Flow rate:	20 µL/min	
Inj. volume:	0.4 µL	
Detection:	Suppressed conductivity, AutoSuppression, recycle mode	
Peaks:	1. Lithium	0.125 mg/L
	2. Sodium	0.5
	3. Ammonium	0.62
	4. Monoethylamine	0.7
	5. Potassium	1.25
	6. Diethylamine	1.0
	7. Triethylamine	2.0
	8. Magnesium	0.62
	9. Calcium	1.25

Conventional Cation-Exchange Chromatography Today

- PEEK separator and guard columns
  (various particle sizes and dimensions)
- AutoSuppression (recycle or external water mode)
- Injection volumes: 0.4 – 25 µL
- Flow rates: 10 µL/min – 1 mL/min
- Eluent: MSA
- Analysis times: >5 – 12 min (mono- **and** divalent cations)
- Detection limits: 1 – 2 µg/L (for 25 µL injection volume)
- Gradient elution: **possible** (with MSA)

Progress in Eluent Preparation Techniques

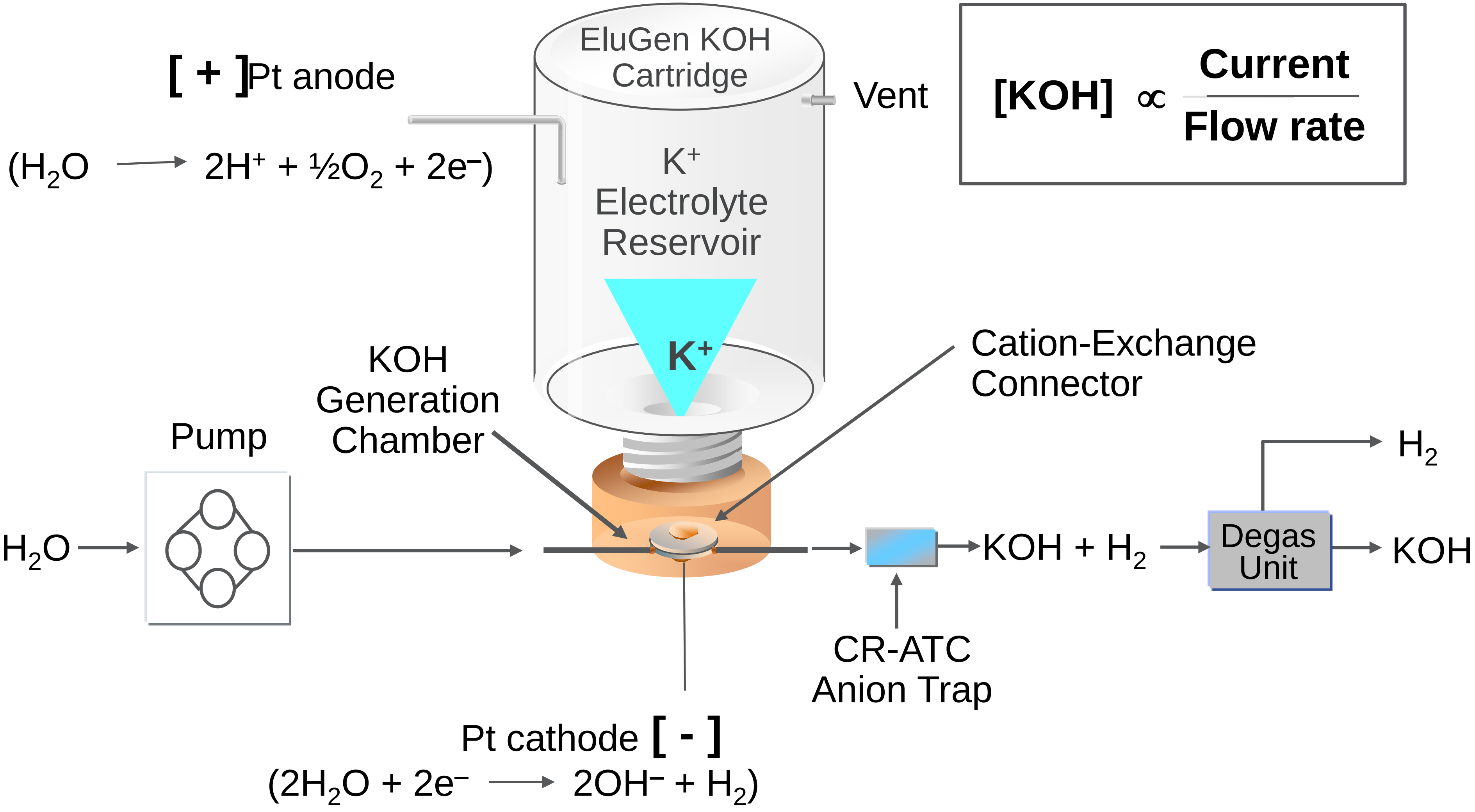
Conventional IC

- Manual preparation of eluents and regenerants
  - A source of labor and uncertainty
- Regenerant flow through the suppressor has to be monitored
  - Another source of labor and uncertainty
- Gradient elution in anion exchange
  - Not practical with carbonate-based eluents

Electrolytic Eluent Preparation (RFIC-EG)

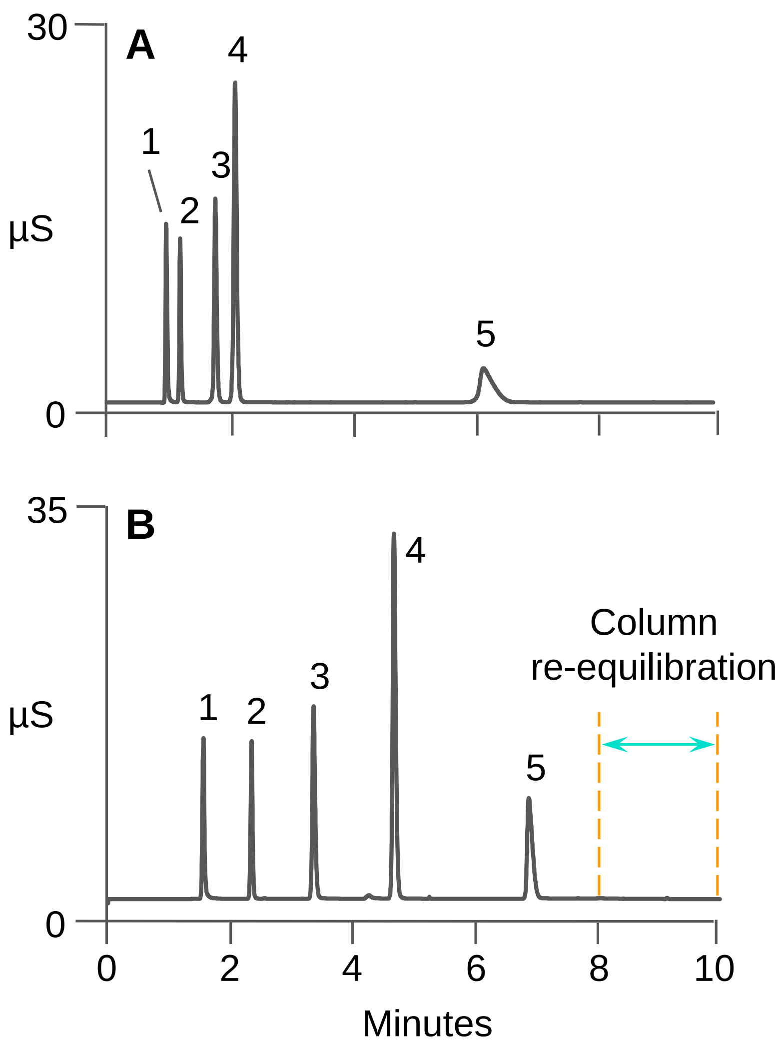
- Only **deionized water** is used as the carrier
- Generation of **high-purity**, contaminant-free acid and base eluents online
- **Electrical** control of eluent concentration with only 15 µL delay volume
- Capable of generating acids or bases at concentrations up to 100 mmol/L at 1 mL/min or 200 mmol/L at 10 µL/min
- Control via Chromeleon 6.8 and 7.x

Hydroxide Eluent Generation for Anion Analysis



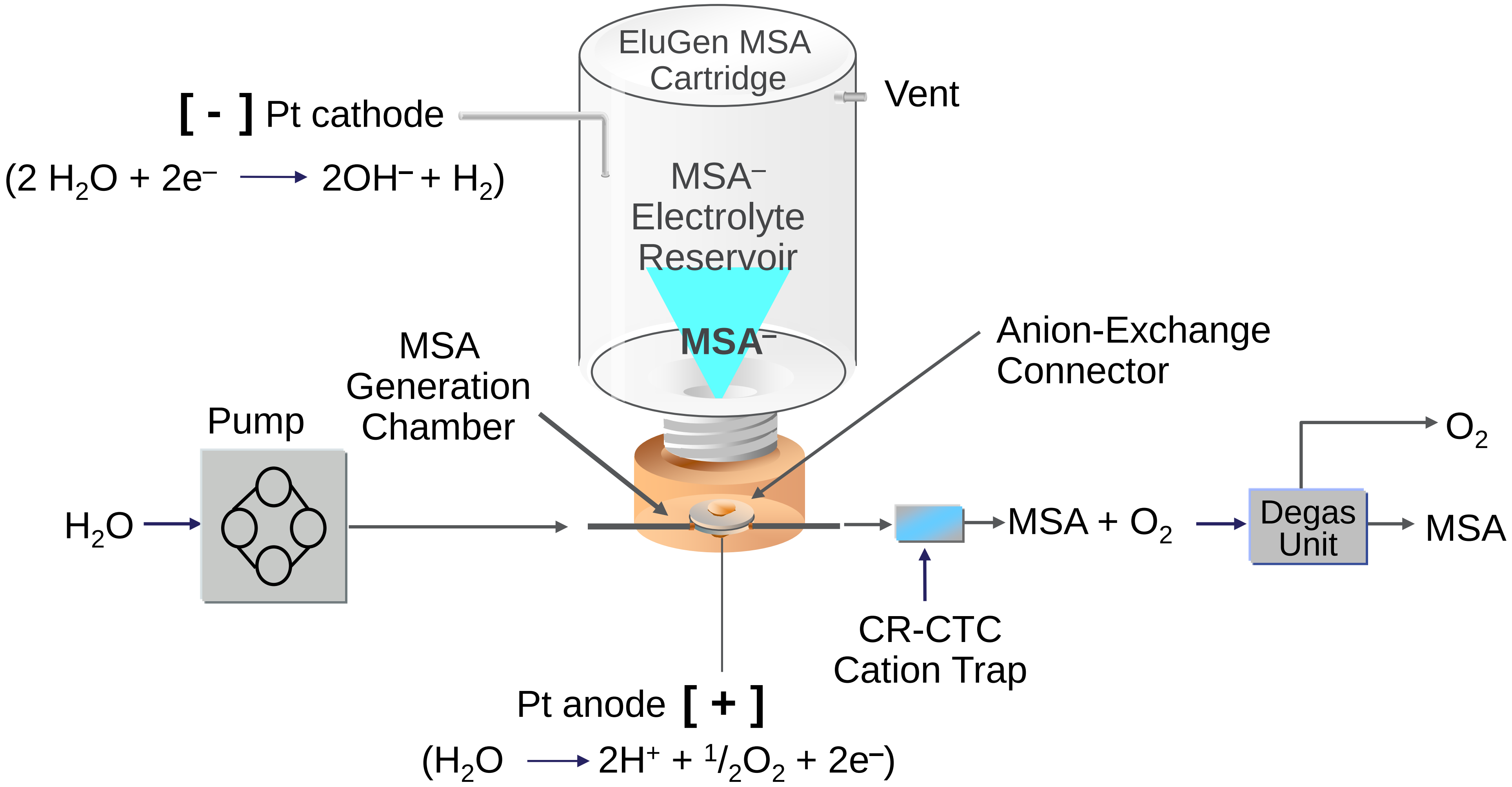
$$[KOH] \propto \frac{\text{Current}}{\text{Flow rate}}$$

Isocratic vs. Gradient Elution of Standard Anions Using Electrolytically Generated KOH



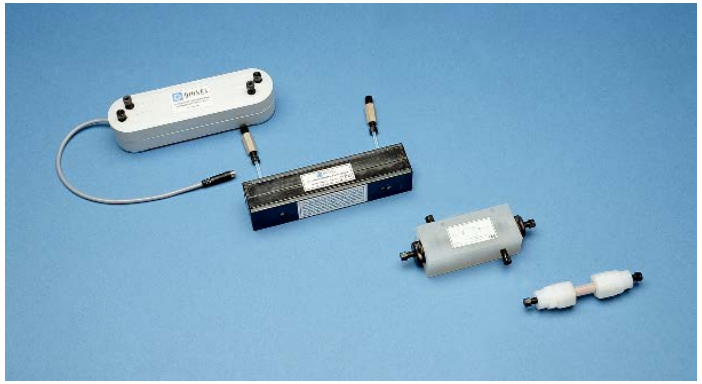
Column:	Dionex IonPac AS11 with guard	
Dimensions:	250 mm × 4 mm i.d.	
Eluent:	A: 15.5 mmol/L KOH	
	B: 0.5–25 mmol/L KOH in 8 min	
Flow rate:	2 mL/min	
Inj. volume:	25 µL	
Detection:	Suppressed conductivity	
	AutoSuppression, recycle mode	
Peaks:	1. Fluoride	2 mg/L
	2. Chloride	3
	3. Nitrate	10
	4. Sulfate	15
	5. Phosphate	15

MSA Eluent Generation for Cation Analysis



Progress in Suppression Technology

From periodically regenerated packed bed suppressors …



… to continuously regenerated membrane or capillary devices.

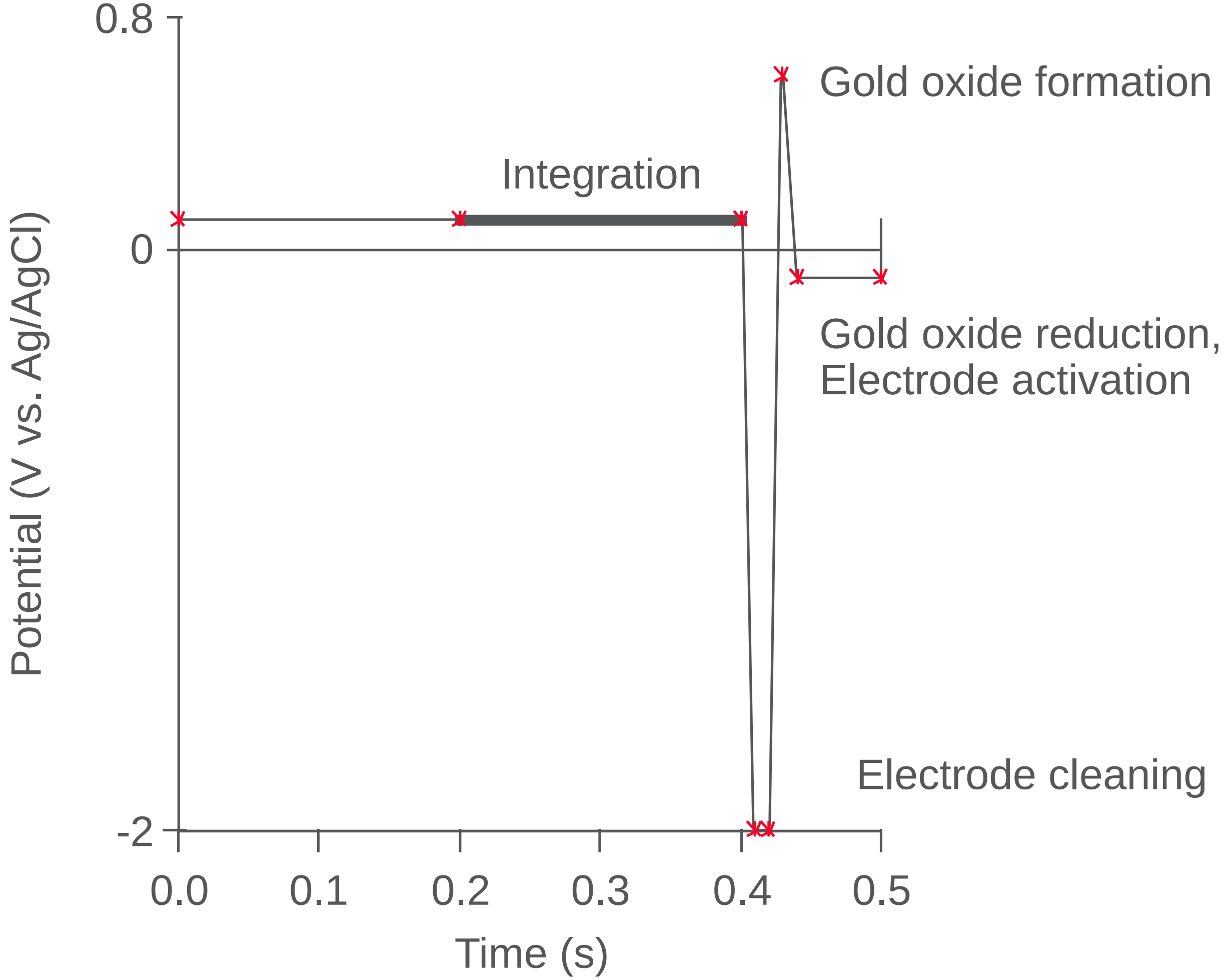
The Purpose of Suppressors in Ion Chromatography

- Reduction of background conductance caused by the eluent resulting in lower baseline noise
- Conversion of the analyte ions into a more strongly conductive form
- Conversion of a bulk property conductivity detector into a solute-specific one
- Sensitivity improvement
- Use of high-capacity separator columns with higher ionic strength eluents
- Use of gradient elution techniques in combination with conductivity detection

Major Improvements in the Field of Detection

- Electrochemical detection
  - Pulsed amperometry for carbohydrate analysis
  - Integrated pulsed amperometry for the analysis of amino acids, amines, and divalent sulfur components
  - Multicyclic waveforms for the analysis of divalent sulfur components
  - Disposable electrodes
  - 3D amperometry
- Spectrometric detection
  - Postcolumn derivatization in combination with UV/Vis or fluorescence detection for a wide variety of compound classes
  - Hyphenation with ICP–MS or ESI–MS

Four-Potential Waveform for Carbohydrate Analysis



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	

High-Performance Anion-Exchange Chromatography for Carbohydrate Analysis

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

Anion-Exchange Columns for Carbohydrate Analysis

Column	Application
CarboPac MA1	Monosaccaride alditols, mixtures of mono- and di-saccharides, and mono- and di-saccharide alditols
CarboPac PA1	General purpose, wood sugars, Rhm/GalN
CarboPac SA10	Fast analysis of mono- and di-saccharides, biofuel monosaccharides
CarboPac PA10 CarboPac PA20	Monosaccharides, incl. low-level analyses
CarboPac PA100 CarboPac PA200	Oligosaccharides

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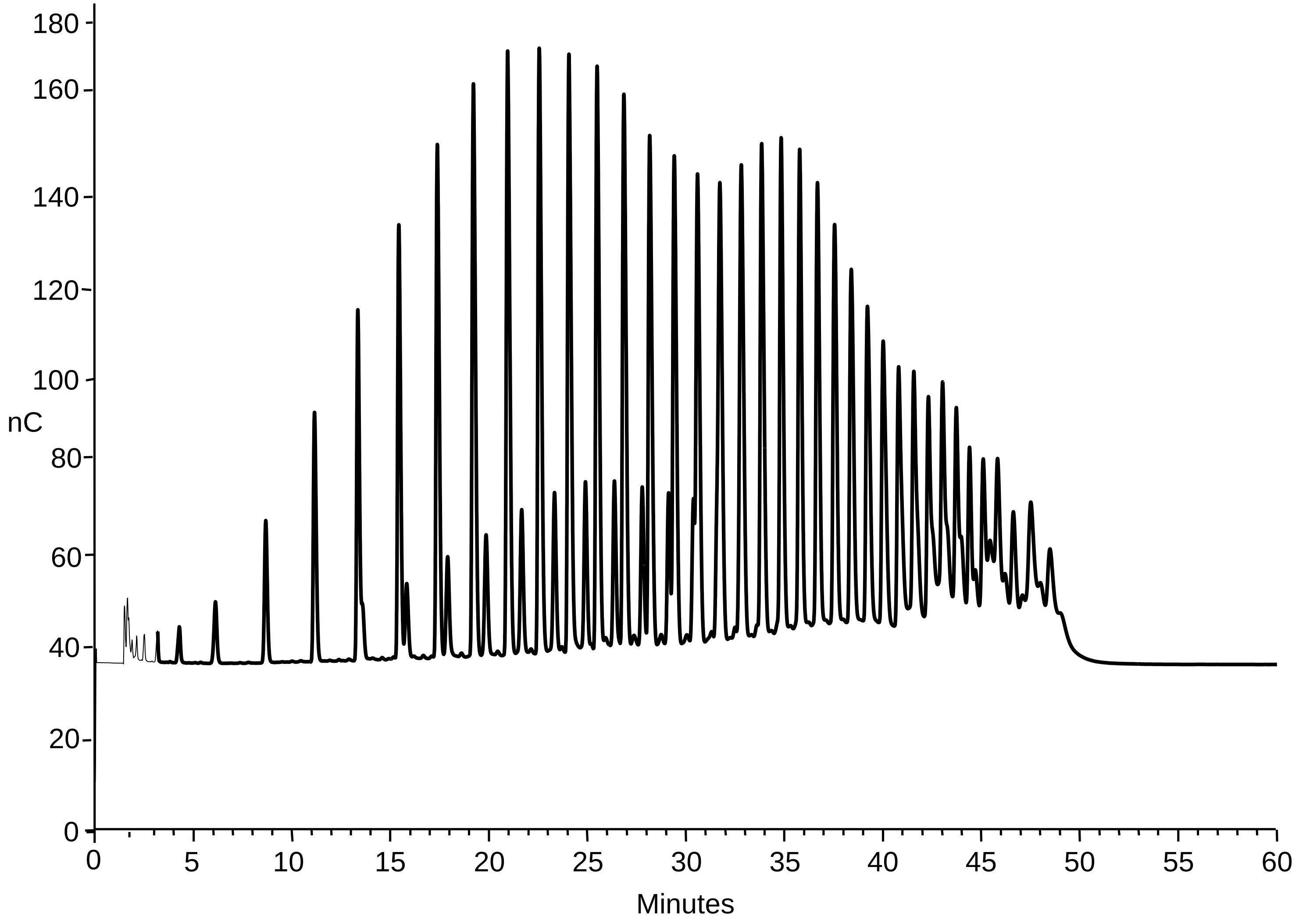
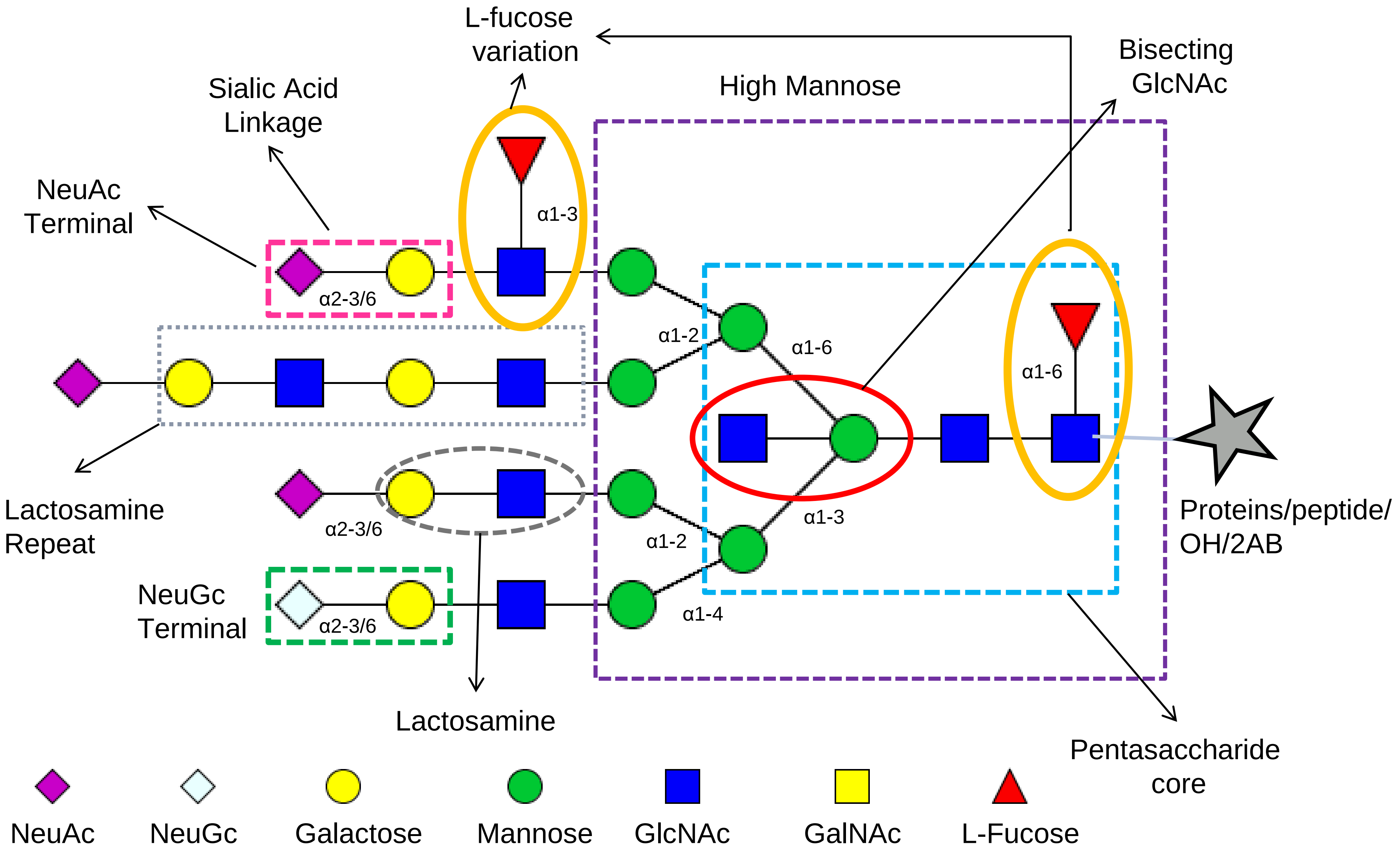
Analysis of Inulin



Column: Dionex CarboPac PA200
Gradient: 120–320 mmol/L NaOAc in 100 mmol/L NaOH in 40 min
Flow rate: 0.5 mL/min
Detection: Integrated pulsed amperometry, Au electrode

Heterogeneity and Diversity of Glycans Derived from Glycoproteins



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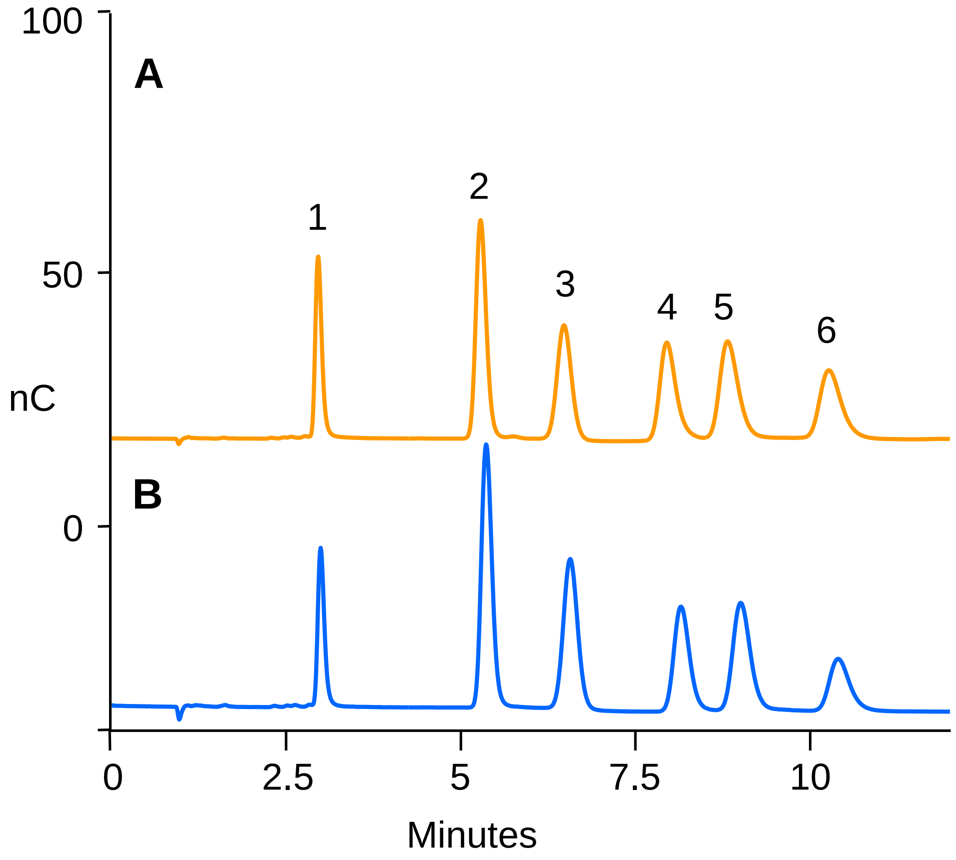
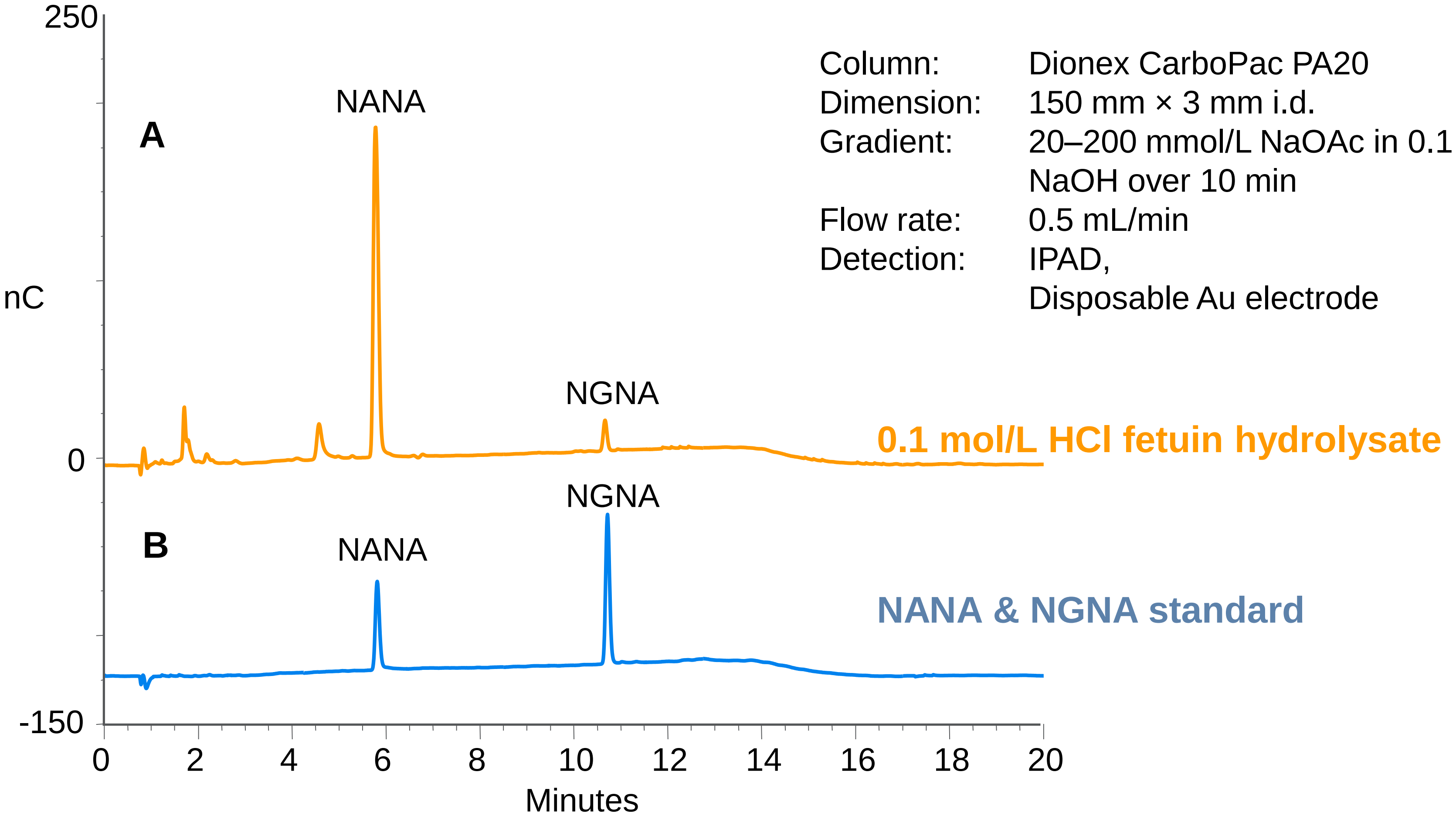
Analysis of Monosaccharide Derived from Glycoproteins



Column:	Dionex CarboPac PA20 with guard
Temperature:	30 ºC
Eluent:	10 mmol/L NaOH
Flow rate:	0.5 mL/min
Inj. volume:	10 µL
Detection:	IPAD, Au electrode
	A) Conventional WE
	B) Disposable WE

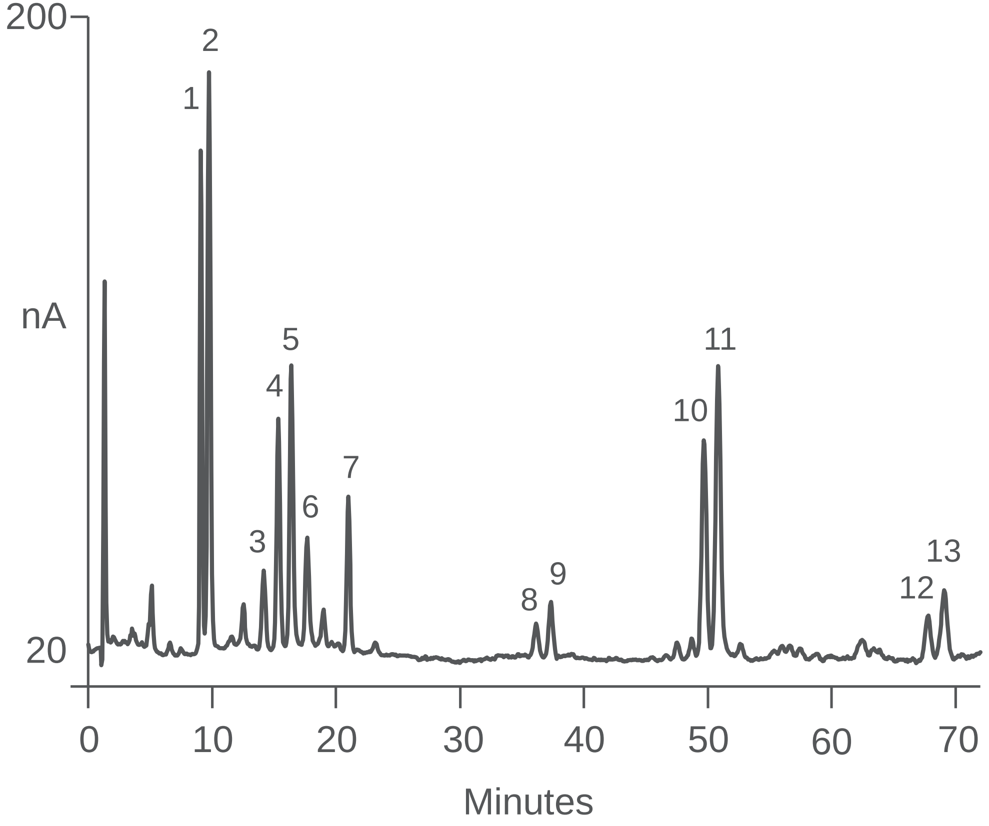
Peaks:	1. Fucose	100 pmol
	2. Galactosamine	100
	3. Glucosamine	100
	4. Galactose	100
	5. Glucose	100
	6. Mannose	100

Sialic Acid Analysis of Bovine Fetuin



Column:	Dionex CarboPac PA20
Dimension:	150 mm × 3 mm i.d.
Gradient:	20–200 mmol/L NaOAc in 0.1 mol/L NaOH over 10 min
Flow rate:	0.5 mL/min
Detection:	IPAD, Disposable Au electrode

Separation of Neutral and Sialylated Oligosaccharides



Column: Dionex CarboPac PA100
Eluent: 0–250 mmol/L NaOAc in 100 mmol/L NaOH over 110 min
Flow rate: 1 mL/min
Detector: PAD, Au electrode

Peaks:
1. $Man_3GlcNAc_2$, Fucosylated
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 bi (reduced)
10, 11. Trisialylated tri (reduced)
12, 13. Tetrasialylated tri (reduced)

Thermo Scientific Dionex Carbonate, Methanesulfonic Acid, and Hydroxide EGC Eluent Generator Cartridges

Main Components of Dual EGC - For Analytical & Cap Formats

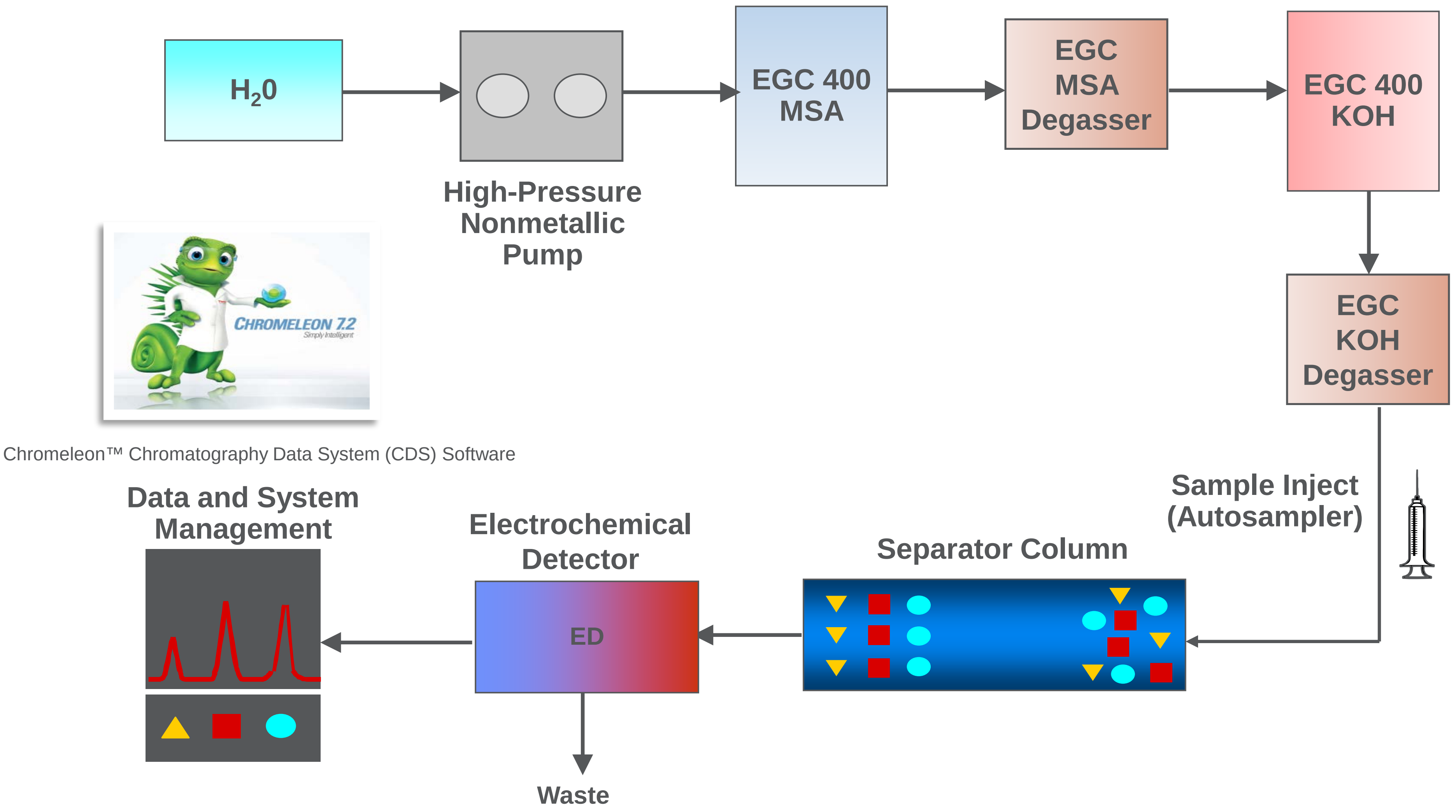
Thermo Scientific™ Dionex™ Carbonate, Methanesulfonic Acid, and Hydroxide EGC Eluent Generator Cartridges

Thermo Scientific™ Dionex™ IonPac™ columns

Thermo Scientific™ Chromeleon™ CDS Software

- ✓ Leverages RFIC capabilities to electrolytically generate potassium hydroxide/potassium methanesulfonate (KOH/KMSA) eluents
- ✓ Can replace manual NaOH/NaOAc gradients required for analyzing complex carbohydrates
- ✓ Will now allow our Cap IC systems to perform the complex carbohydrate analysis
- ✓ Available formats – capillary (0.4 mm) and micro (1.0 mm)

System Workflow Under Dual EGC Mode



Chromeleon™ Chromatography Data System (CDS) Software

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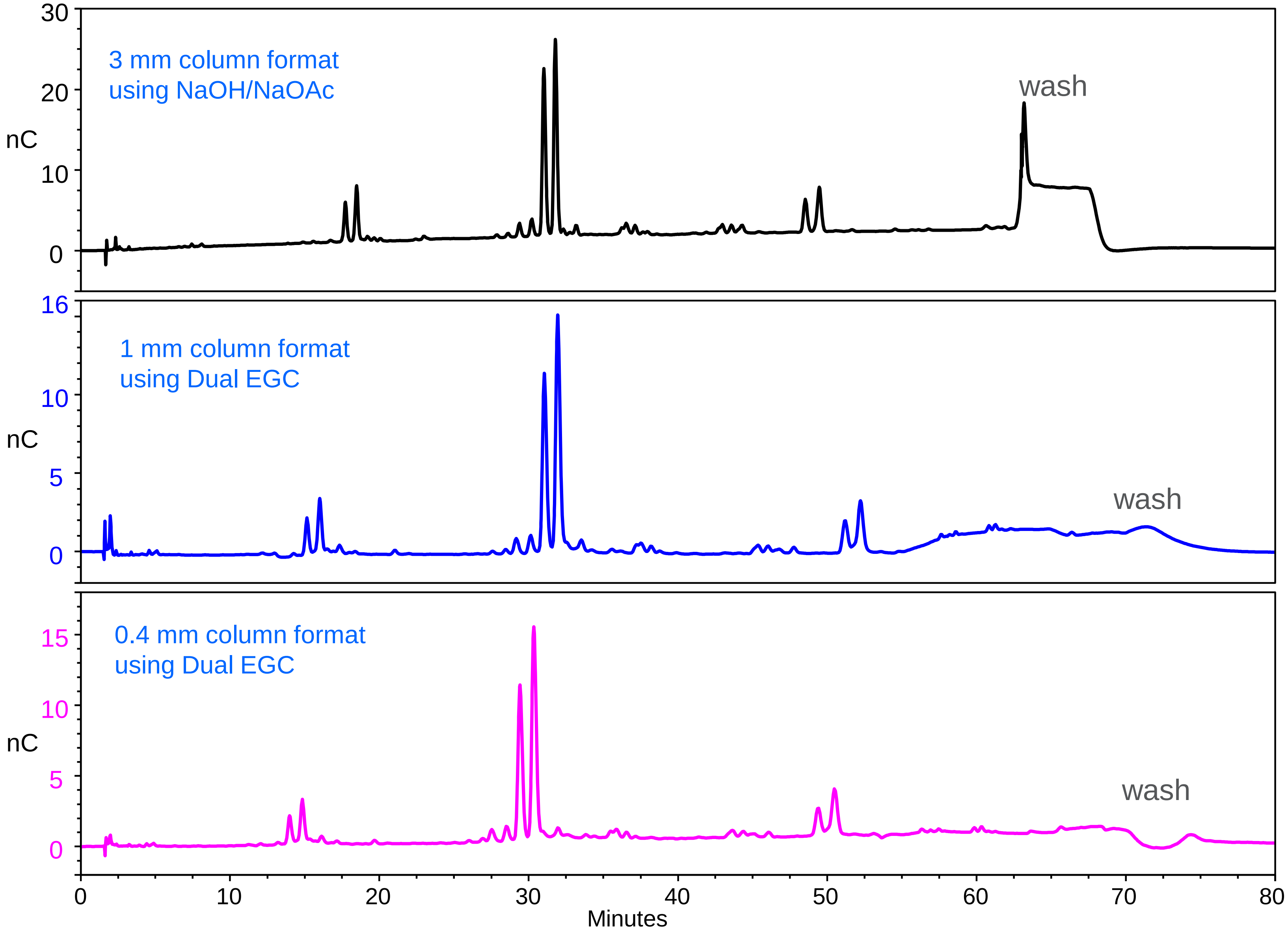
Fetuin Oligosaccharide Alditol Analysis Using Dual EGC



Columns: Dionex CarboPac PA200, 3 mm (guard + separator)
Dionex CarboPac PA200 1 mm (guard + separator)
Dionex CarboPac PA200 0.4 mm (guard + separator)

Gradients: Dionex CarboPac PA200, 250 mm × 3 mm i.d.
0 to 60 min: 20–150 mmol/L NaOAc in 100 mmol/L NaOH, 60 to 65 min: 500 mmol/L NaOAc in 100 mmol/L NaOH, 65 to 80 min: 20 mmol/L NaOAc in 100 mmol/L NaOH

Dionex CarboPac PA200, 250 mm × 1 mm i.d.
0 to 50 min: 15–64 mmol/L KMSA in 136 mmol/L KOH
50 to 60 min: 80 mmol/L KMSA in 90 mmol/L KOH
60 to 65 min: 100 mmol/L KMSA in 100 mmol/L KOH
65 to 80 min: 15 mmol/L KMSA in 136 mmol/L KOH

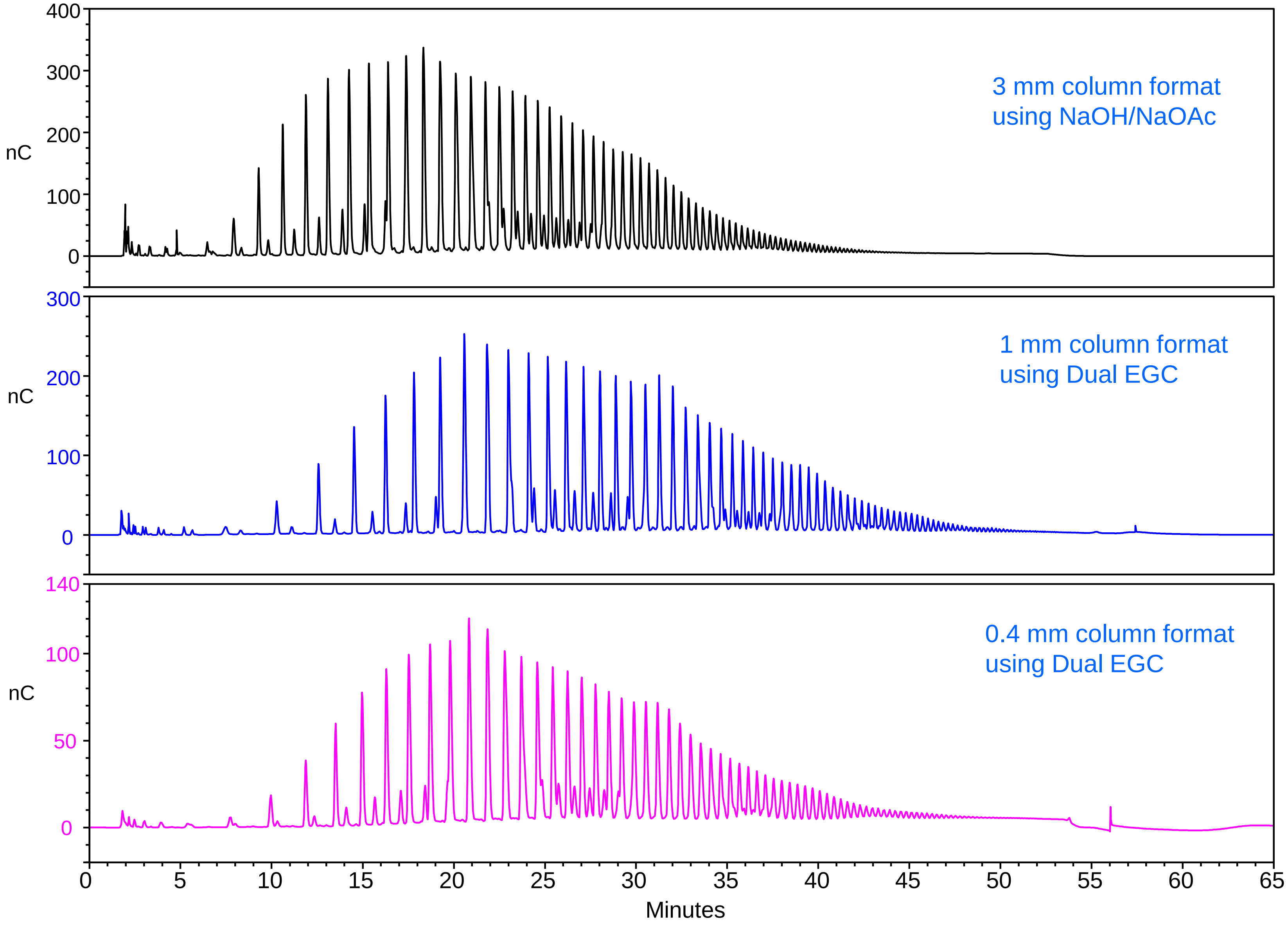
Dionex CarboPac PA200, 250 mm × 0.4 mm i.d.
0 to 50 min: 15–64 mmol/L KMSA in 136 mmol/L KOH
50 to 60 min: 80 mmol/L KMSA in 90 mmol/L KOH
60 to 65 min: 100 mmol/L KMSA in 100 mmol/L KOH
65 to 80 min: 15 mmol/L KMSA in 136 mmol/L KOH

Flow rate: CarboPac PA200, 250 mm × 3 mm i.d.: 0.5 mL/min
CarboPac PA200, 250 mm × 1 mm i.d.: 0.063 mL/min
CarboPac PA200, 250 mm × 0.4 mm i.d.: 0.010 mL/min

Detection: Au on PTFE, Ag/AgCl reference
CarboPac PA200, 250 mm × 3 mm i.d.: 2 mil gasket
CarboPac PA200, 250 mm × 1 mm i.d.: 1 mil gasket
CarboPac PA200, 250 mm × 0.4 mm i.d.: 1 mil gasket

Samples: 50 µmol/L fetuin oligosaccharide alditol standard

Inulin Analysis Using Dual EGC



Columns: Dionex CarboPac PA200, 3 mm (guard + separator)
Dionex CarboPac PA200, 1 mm (guard + separator)
Dionex CarboPac PA200, 0.4 mm (guard + separator)

Gradient: Dionex CarboPac PA200, 250 mm × 3 mm i.d.
0 to 45 min: 100–430 mmol/L NaOAc in 100 mmol/L NaOH
45 to 50 min: 430 mmol/L NaOAc in 100 mmol/L NaOH
50 to 65 min: 100 mmol/L NaOAc in 100 mmol/L NaOH

Dionex CarboPac PA200, 250 mm × 1 mm i.d.
0 to 45 min: 40 mmol/L KMSA/60 mmol/L KOH to 156 mmol/L KMSA/22 mmol/L KOH
45 to 50 min: 156 mmol/L KMSA/22 mmol/L KOH
50 to 65 min: 40 mmol/L KMSA/60 mmol/L KOH

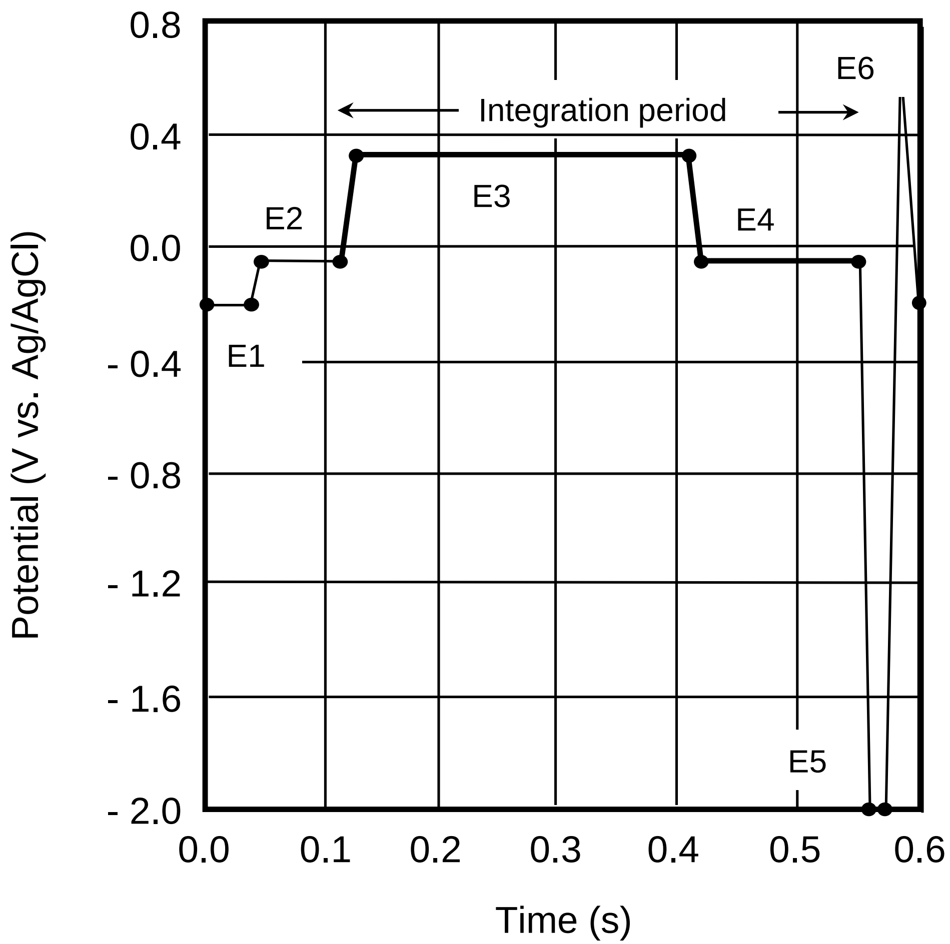
Dionex CarboPac PA200, 250 mm × 0.4 mm i.d.
0 to 45 min: 40 mmol/L KMSA/70 mmol/L KOH to 190 mmol/L KMSA/10 mmol/L KOH
45 to 50 min: 190 mmol/L KMSA/10 mmol/L KOH
50 to 65 min: 40 mmol/L KMSA/70 mmol/L KOH

Flow rate: CarboPac PA200, 250 mm × 3 mm i.d.: 0.5 mL/min
CarboPac PA200, 250 mm × 1 mm i.d.: 0.063 mL/min
CarboPac PA200, 250 mm × 0.4 mm i.d.: 0.010 mL/min

Detection: Au on PTFE, Ag/AgCl reference
CarboPac PA200, 250 mm × 3 mm i.d.: 2 mil gasket
CarboPac PA200, 250 mm × 1 mm i.d.: 1 mil gasket
CarboPac PA200, 250 mm × 0.4 mm i.d.: 1 mil gasket

Samples: 5 mg/mL inulin (from chicory)

Waveform for Amino Acid Analysis

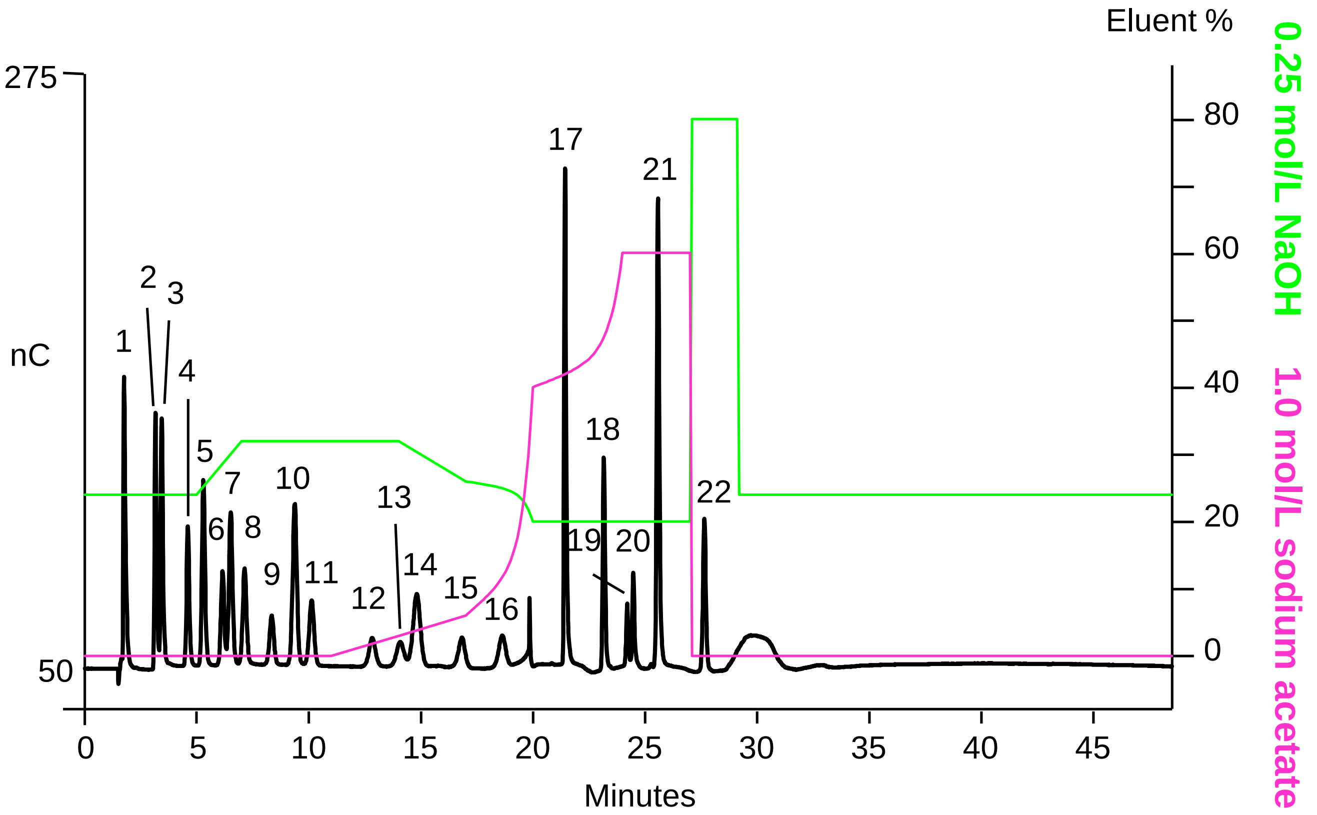


Time [s]	Pot. [V]	Integ.
0.00	– 0.20	
0.04	– 0.20	
0.05	– 0.05	
0.11	– 0.05	Begin
0.12	+ 0.28	
0.41	+ 0.28	
0.42	– 0.05	
0.56	– 0.05	End
0.57	– 2.00	
0.58	– 2.00	
0.59	+ 0.60	
0.60	– 0.20	

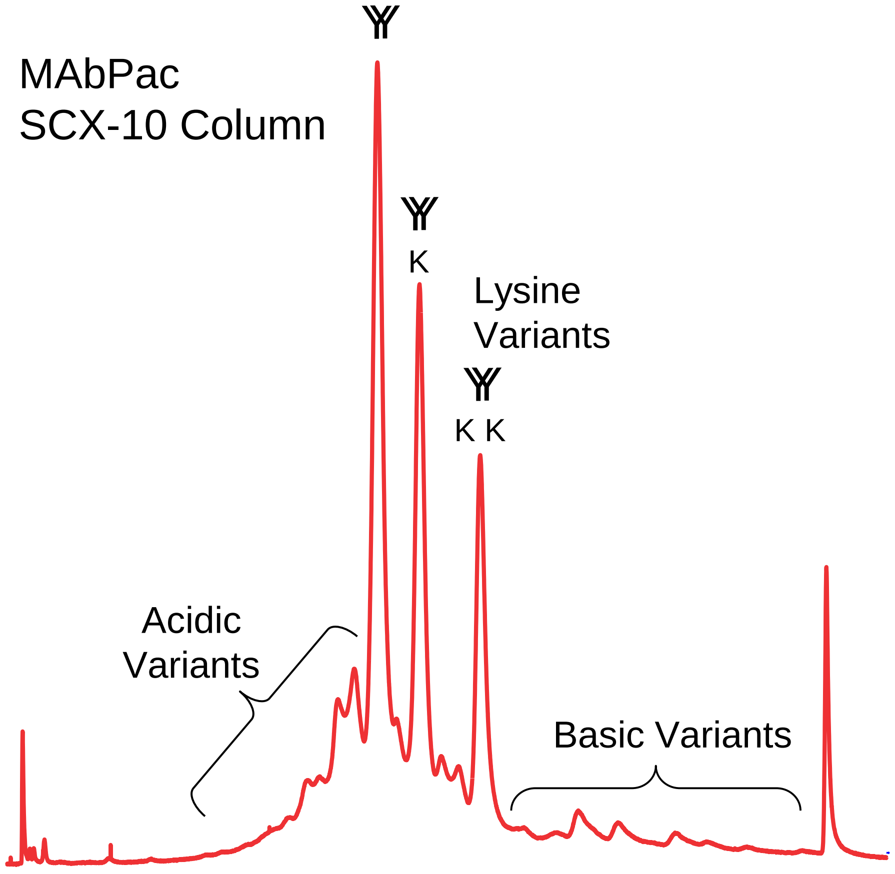
Gradient Elution of Amino Acids on a Nanobead-Agglomerated Anion Exchanger

Column: Dionex AminoPac PA10
Dimensions: 250 mm × 2 mm i.d.
Eluent: NaOH/NaOAc gradient
Flow rate: 0.25 mL/min

Peaks:
1. Arginine
2. Ornithine
3. Lysine
4. Glutamine
5. Asparagine
6. Alanine
7. Threonine
8. Glycine
9. Valine
10. Serine
11. Proline
12. Isoleucine
13. Leucine
14. Methionine
15. Norleucine
16. Taurine
17. Histidine
18. Phenylalanine
19. Glutamate
20. Aspartate
21. Cystine
22. Tyrosine

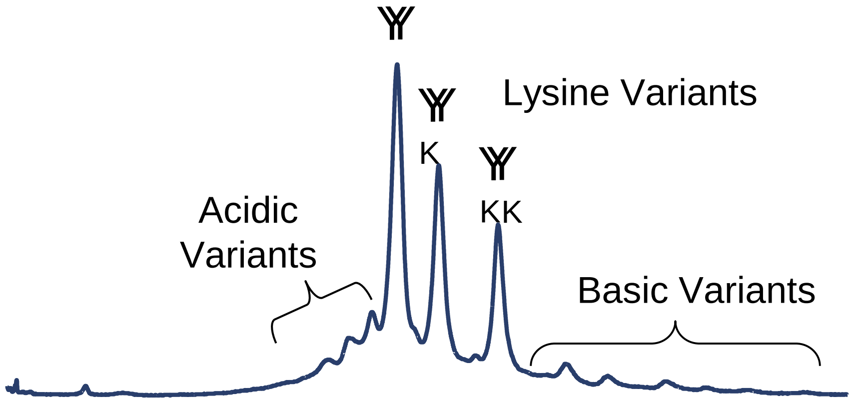


Cation-Exchange Chromatography – The Gold Standard for mAb Analysis

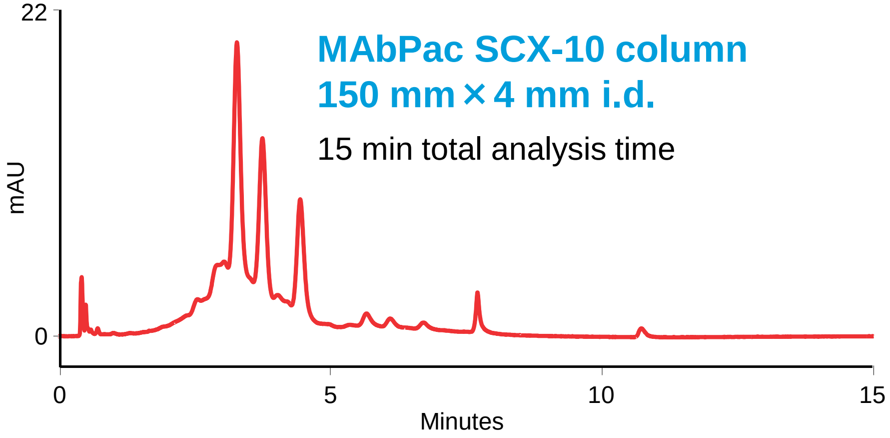


MAbPac SCX-10 column, 250 mm × 4 mm i.d.

60 min total analysis time



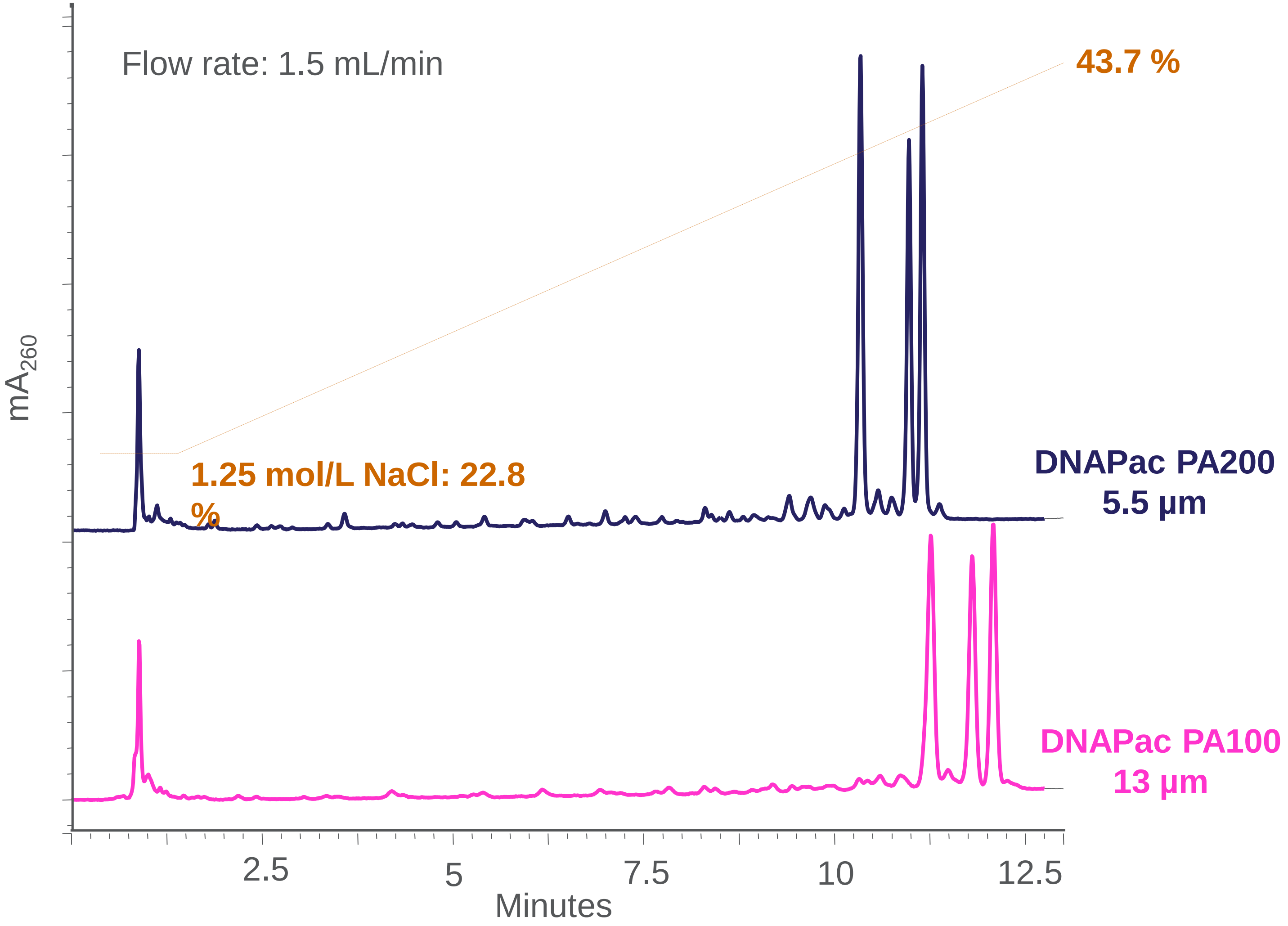
ProPac WCX-10 column, 250 mm × 4 mm i.d.



MAbPac SCX-10 column
150 mm × 4 mm i.d.

15 min total analysis time

Cation-Exchange Chromatography – The Gold Standard for Nucleic Acid Analysis



NaCl, pH 8, 25 ^{o}C, 8 µL d(AC)$_{10-11}$ 20-22mers

CURRENT TRENDS

Current Trends in IC

- System miniaturization into the capillary scale
- Ion chromatography systems with higher pressure tolerance
- Ion exchangers with smaller particle diameter
  - Increase in sample throughput
  - High-resolution IC
- Stationary phases with ion-exchange and reversed-phase properties
- Hyphenation of IC with ICP and ESI–MS *

* Separate presentation

SYSTEM MINIATURIZATION INTO THE CAPILLARY SCALE

The Dimension of Scale – Analytical vs. Capillary IC

Parameter	Analytical IC		Capillary IC
Column diameter	4 mm		0.4 mm
Flow rate	1.0 mL/min		10 µL/min
Injection volume	25 µL		0.4 (0.1) µL
Eluent consumption	43.2 L/month		0.432 L/month
EGC lifetime (@75 mmol/L)	28 days		18 months
Absolute detection limit (with IC x IC)	700 ng		7 ng

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The Most Important Values of Capillary IC

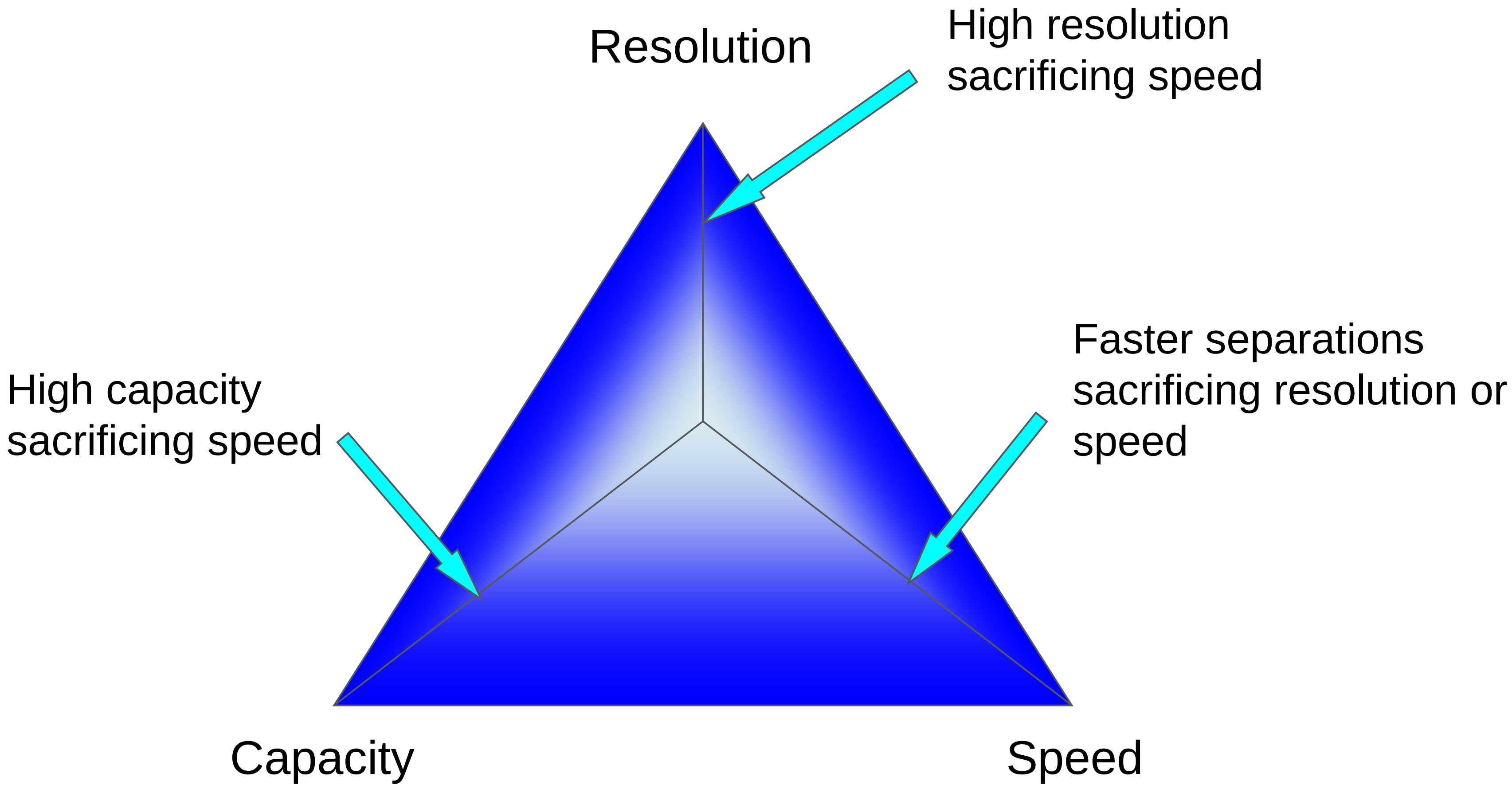
- **“IC on Demand”**
  - Permanent availability of the system
  - Higher laboratory productivity (typical waiting times for equilibration are omitted)
  - Less calibration efforts
  - Isocratic and gradient elution due to RFIC
- **Higher mass sensitivity**
  - High sensitivity with less sample volume
  - 100-fold increase in absolute sensitivity in comparison to 4 mm systems
  - IC × IC (2D IC) – detection limits in the ng/L range with only 1 mL of sample
- **Lower cost of ownership**
  - Lower eluent consumption, less waste
  - 18 months lifetime of the EG cartridges

ION CHROMATOGRAPHY SYSTEMS WITH HIGHER PRESSURE TOLERANCE

High-Pressure IC Started with Capillary IC

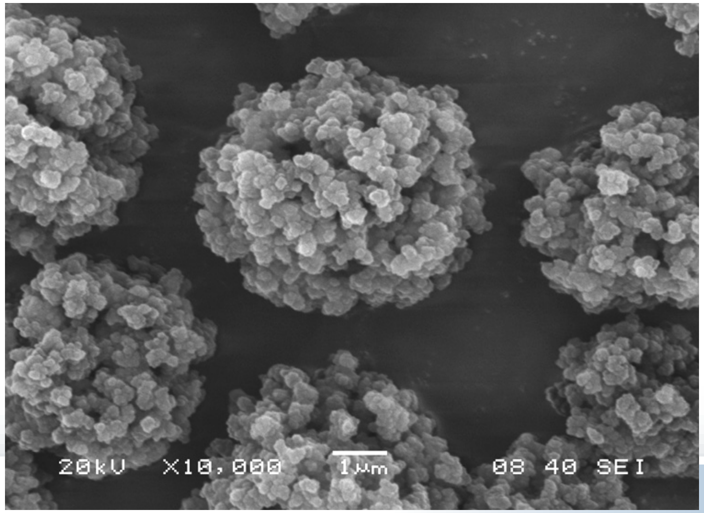
- **What do we understand under the term HPIC?**
  - Analytical and capillary IC systems with a pressure tolerance of 34.5 MPa (5000 psi) under continuous operation
- **Why is it important?**
  - Users are tending to IC/LC systems tolerating a much higher back pressure to be able to use packing materials with smaller particle diameters.
  - Smaller particle diameter = higher chromatographic efficiency
  - Higher chromatographic efficiency = faster separations without sacrificing resolution
    (However, smaller particle diameters result in a much higher back pressure.)
  - **Or:** Higher resolution at standard flow rates and standard column formats!

The Challenge: Speed, Capacity, and Resolution



The Vicious Triangle in IC

Thermo Scientific Dionex IonPac Columns 4 µm



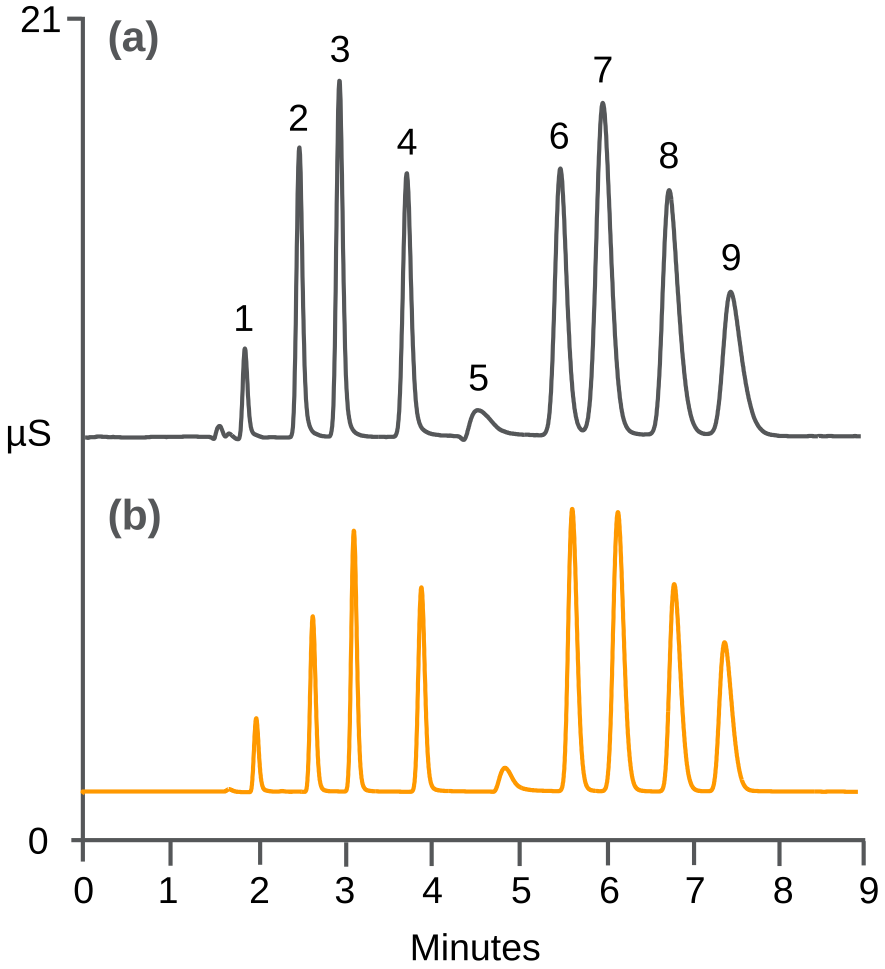
- Smaller resin particles produce more efficient peaks
  - Existing ion exchange materials are based on 5–10 µm particle diameters
- Optimal combination of chromatographic speed and resolution
- Obtain more accurate and more reliable results with easier peak integration
- Increase sample throughput without compromising data quality
- Inorganic anions and cations in diverse sample matrices

Why Do We Need Faster Separations in IC?

- Make laboratories more productive
- Save laboratories time and eluent
- Provide faster answers to analytical questions (e.g., forensic samples)
- Improve LODs and LOQs in some cases

Faster separations are as accurate and precise as conventional methods!

Comparison of IonPac AS18-Fast and AS18-4µm



Column:	(a) Dionex IonPac AS18-Fast, (150 mm × 2 mm i.d.)
	(b) Dionex IonPac AS18-4µm, (150 mm × 2 mm i.d.)
Temperature:	30°C
Eluent:	23 mmol/L KOH (EG)
Flow rate:	0.25 mL/min
Inj. volume:	(a) 5 µL, (b) 2.5 µL
Detection:	Suppressed conductivity, AutoSuppression, recycle mode

Peaks:		
	1. Fluoride	0.5 mg/L
	2. Chlorite	5.0
	3. Chloride	3.0
	4. Nitrite	5.0
	5. Carbonate	20.0
	6. Bromide	10.0
	7. Sulfate	10.0
	8. Nitrate	10.0
	9. Chlorate	10.0

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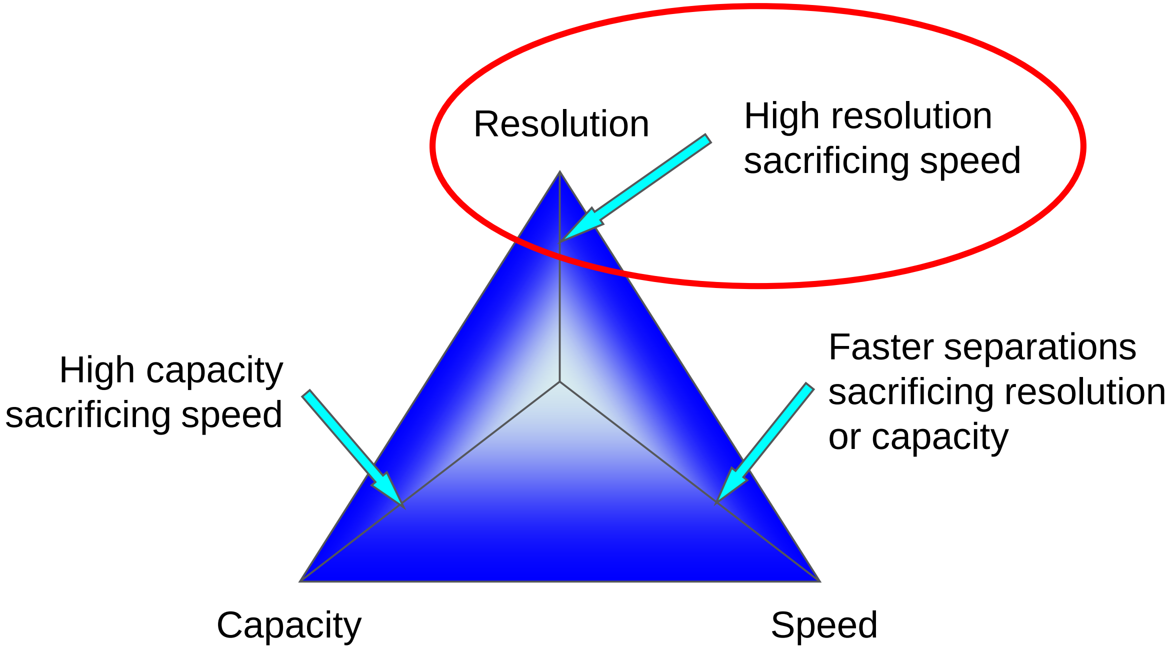
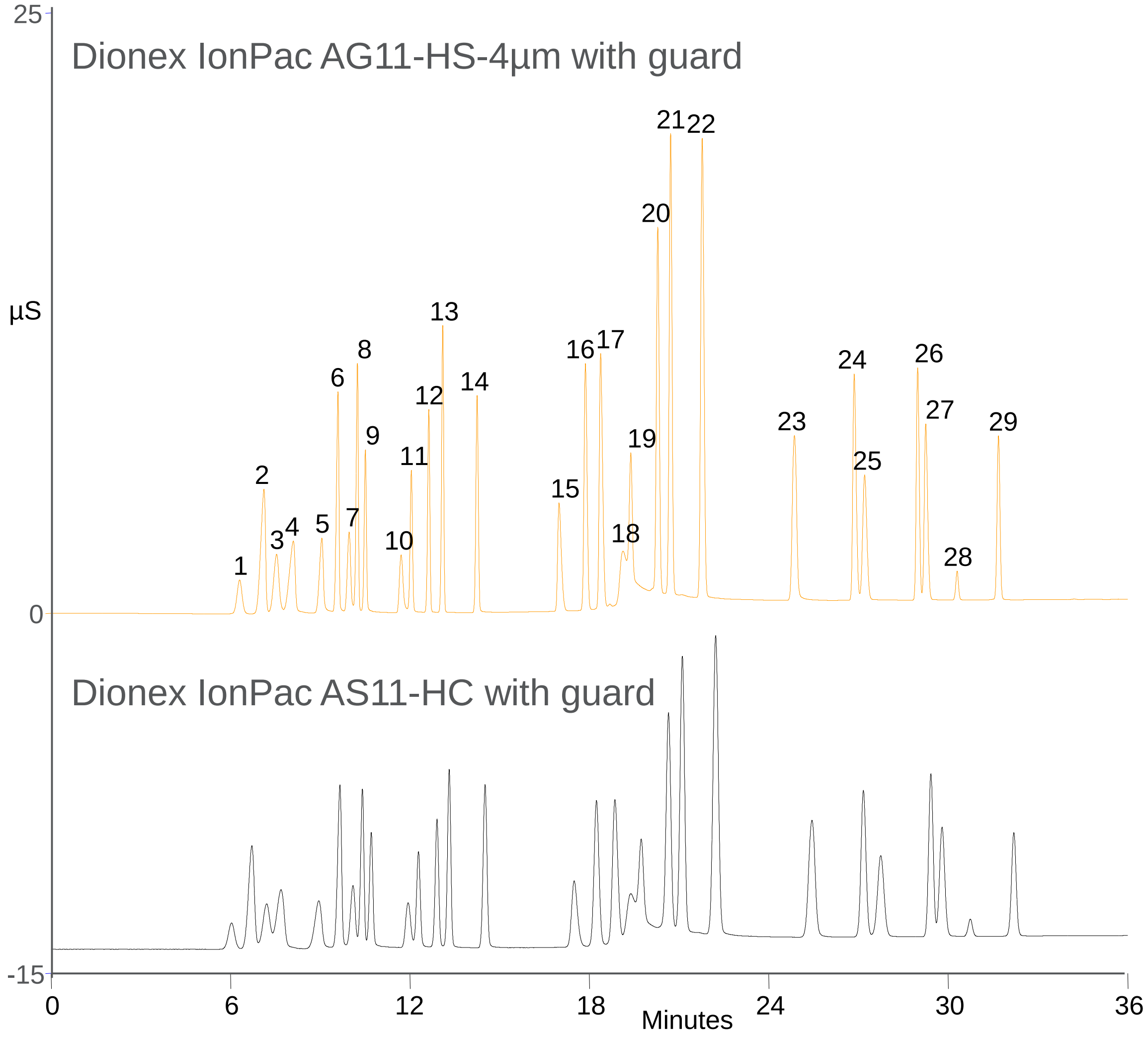
High-Resolution IC

- Instead of shortening columns for faster separations, we can **use 250 mm columns (or two columns in series)**, but we sacrifice speed.
- In addition, it is possible to decrease the particle diameter, but we sacrifice column back pressure.



Comparison of Dionex IonPac AS11-HC and AS11-HC-4µm Capillary Columns



Column:	see chromatogram
Dimensions:	250 mm × 0.4 mm i.d.
Temperature:	30 °C
Eluent :	KOH (EG)
Gradient:	1 mmol/L from 0 to 5 min, 1–15 mmol/L from 5 to 14 min,15–30 mmol/L from 14 to 23 min, 30–60 mmol/L from 23 to 31 min
Flow rate:	15 µL/min
Inj. volume:	0.4 µL
Detection:	Suppressed conductivity, AutoSuppression, recycle mode

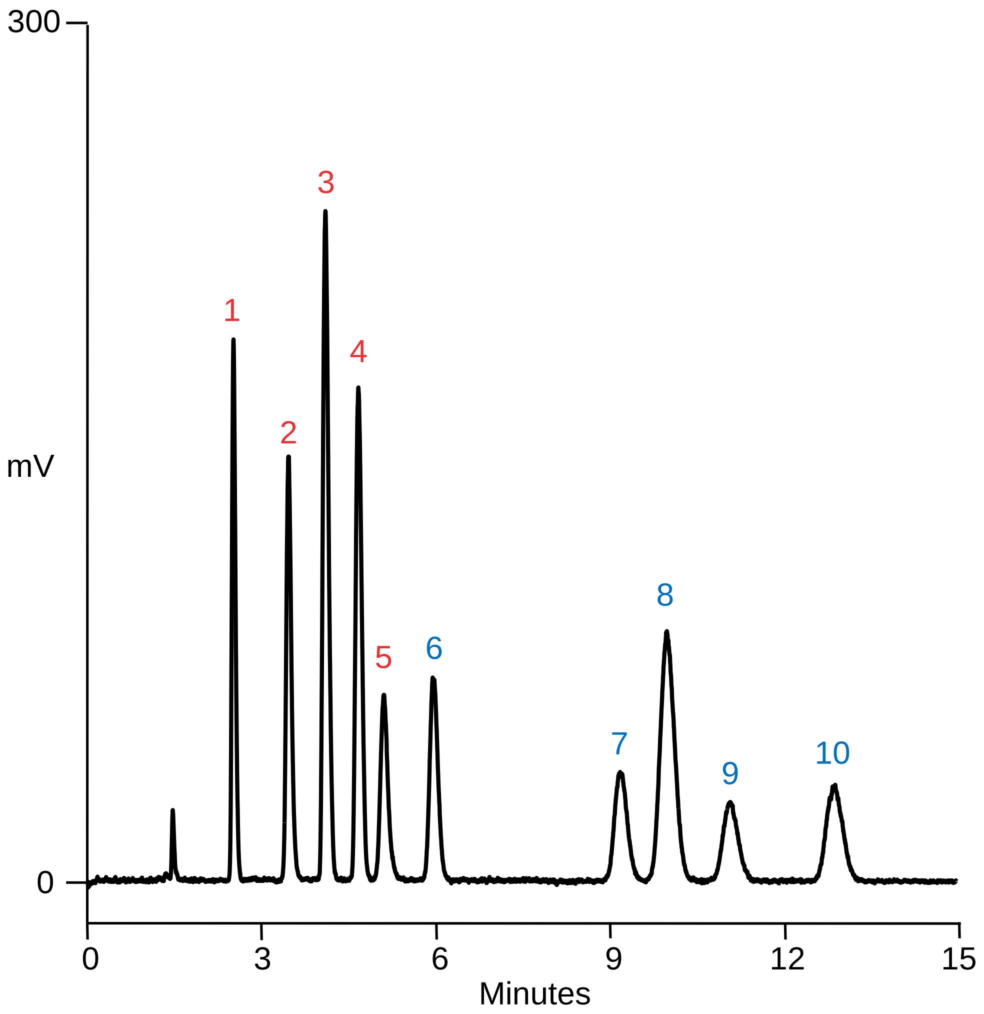
Peaks:	mg/L		mg/L
1. Quinate	5.0	16. Bromide	5.0
2. Fluoride	1.5	17. Nitrate	5.0
3. Lactate	5.0	18. Carbonate	---
4. Acetate	5.0	19. Malonate	7.5
5. Propionate	5.0	20. Maleate	7.5
6. Formate	5.0	21. Sulfate	7.5
7. Butyrate	5.0	22. Oxalate	7.5
8. Methylsulfonate	5.0	23. Tungstate	10.0
9. Pyruvate	5.0	24. Orthophosphate	10.0
10. Valerate	5.0	25. Pthalate	10.0
11. Monochloroacetate	5.0	26. Citrate	10.0
12. Bromate	5.0	27. Chromate	10.0
13. Chloride	2.5	28. cis-Aconitate	---
14. Nitrite	5.0	29. trans-Aconitate	10.0
15. Triflouroacetate	5.0		

STATIONARY PHASES WITH REVERSED-PHASE AND ION-EXCHANGE PROPERTIES

What Are Mixed-Mode Phases?

- Definition
  - Hydrophobic (or hydrophilic) interactions + ion-exchange interactions
- Benefits
  - Adjustable selectivity for optimal separations
  - Simplified mobile phases (no need of ion-pair reagents)
  - Simultaneous separation of different classes of analytes
- Types
  - Anion-exchange/reversed-phase (AEX/RP)
  - Cation-exchange/reversed-phase (CEX/RP)
  - Anion-exchange/cation-exchange/reversed-phase (AEX/CEX/RP)
  - AEX/HILIC
  - CEX/HILIC
  - AEX/CEX/HILIC

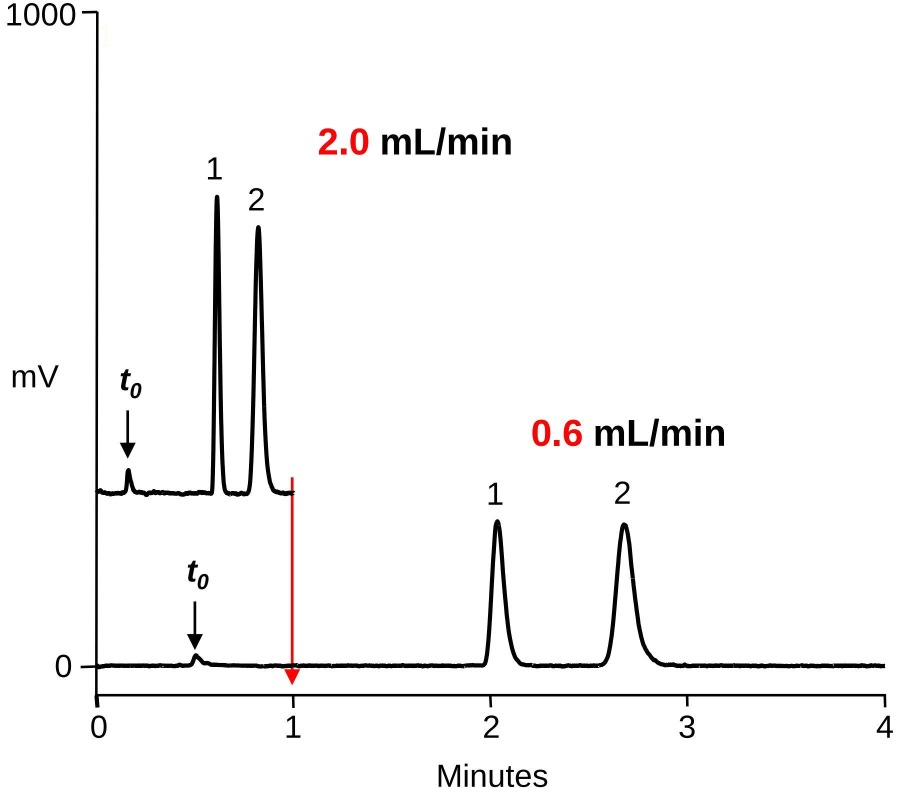
Separation of Pharmaceutically Relevant Ions



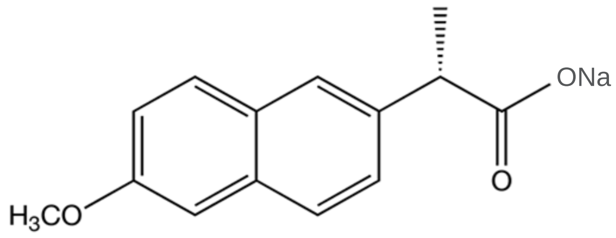
Column:	Dionex Acclaim Trinity P1, 3 µm
Dimensions:	100 mm × 3 mm i.d.
Temperature:	30 °C
Eluent:	60:40 v/v MeCN/20 mmol/L (total) NH_4OAc, pH 5
Flow rate:	0.5 mL/min
Inj. volume:	2 µL
Detection:	CAD (Corona *ultra,* gain: 100 pA; Filter: med; Neb. Temp.: 30°C)
Peaks:	(50–100 mg/L)

1. Choline
2. Tromethamine
3. Sodium
4. Potassium
5. Meglumine
6. Mesylate
7. Nitrate
8. Chloride
9. Bromide
10. Iodide

Separation of Naproxen and Counterion



Column:	**Acclaim Trinity P1**, 3 µm
Dimensions:	50 mm × 3 mm i.d.
Temperature:	30 °C
Eluent:	80:20 v/v MeCN/20 mmol/L (total) NH_4OAc, pH 5
Flow rate:	see chromatograms
Inj. volume:	2.5 µL
Detection:	CAD (Corona *ultra*, Gain: 100 pA; Filter: med; Neb. Temp.: 30°C)
Sample:	Na, Naproxen (0.2 mg/mL in eluent)
Peaks:	1. Sodium 2. Naproxen



HYPHENATION OF IC WITH ICP OR ESI–MS

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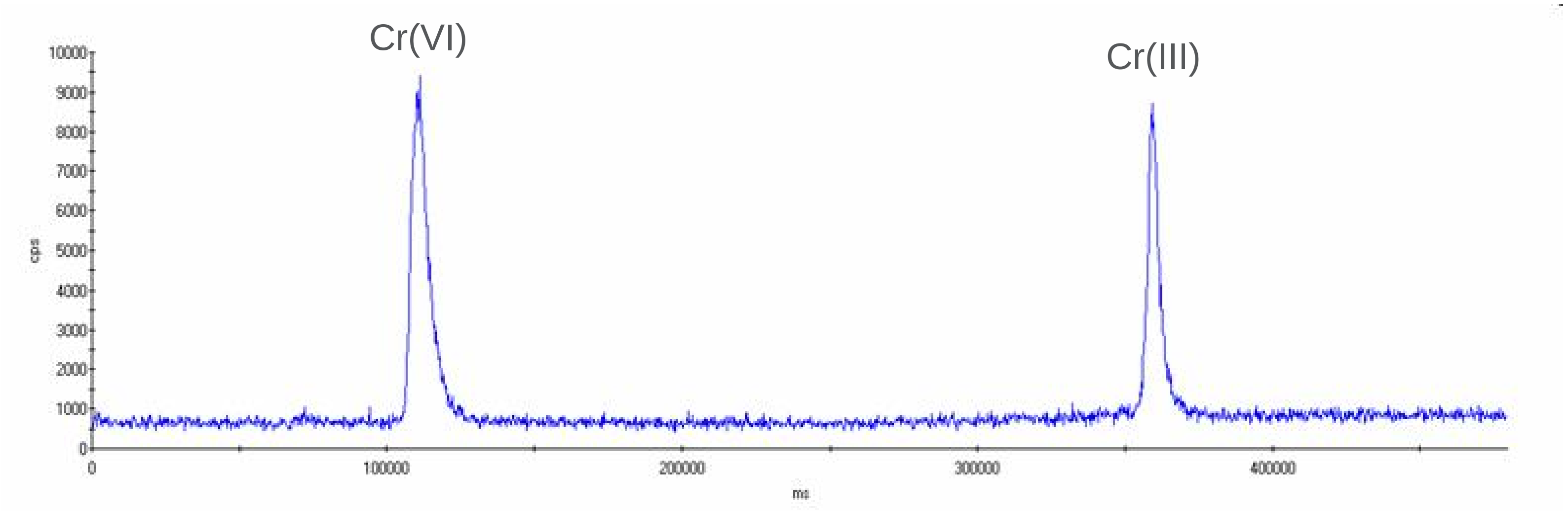
Benefits of Hyphenated Techniques

- Coupling of ion chromatography with ESI–MS or ICP–MS solves complex separation problems
- Provides high sensitivity and specificity
- Provides valuable information for peak verification or peak identification
- Less prone to matrix effects
- Allows to distinguish between different oxidation states of an element (speciation)

Speciation of Chromium Using IC–ICP/MS

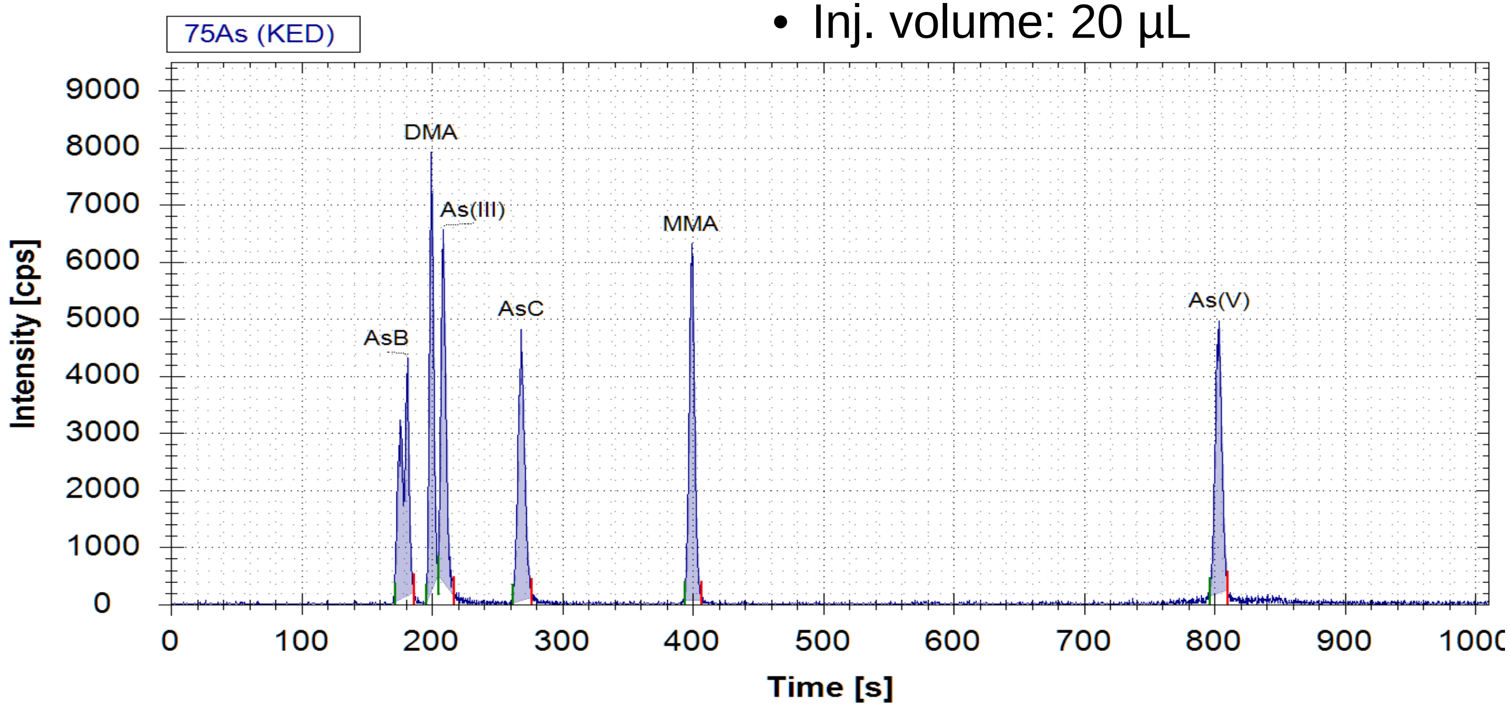
- Commercial standards prepared in deionized water
- 100 µL injection volume, 2 ng/mL standard
- Dionex IonPac CS5A (250 mm × 2 mm i.d.) plus guard column
- Gradient elution (0.3–1 mol/L nitric acid, 0.5 mL/min)
- X Series ICP-MS conditions
- Peltier cooled spray chamber, PlasmaScreen and Xt interface



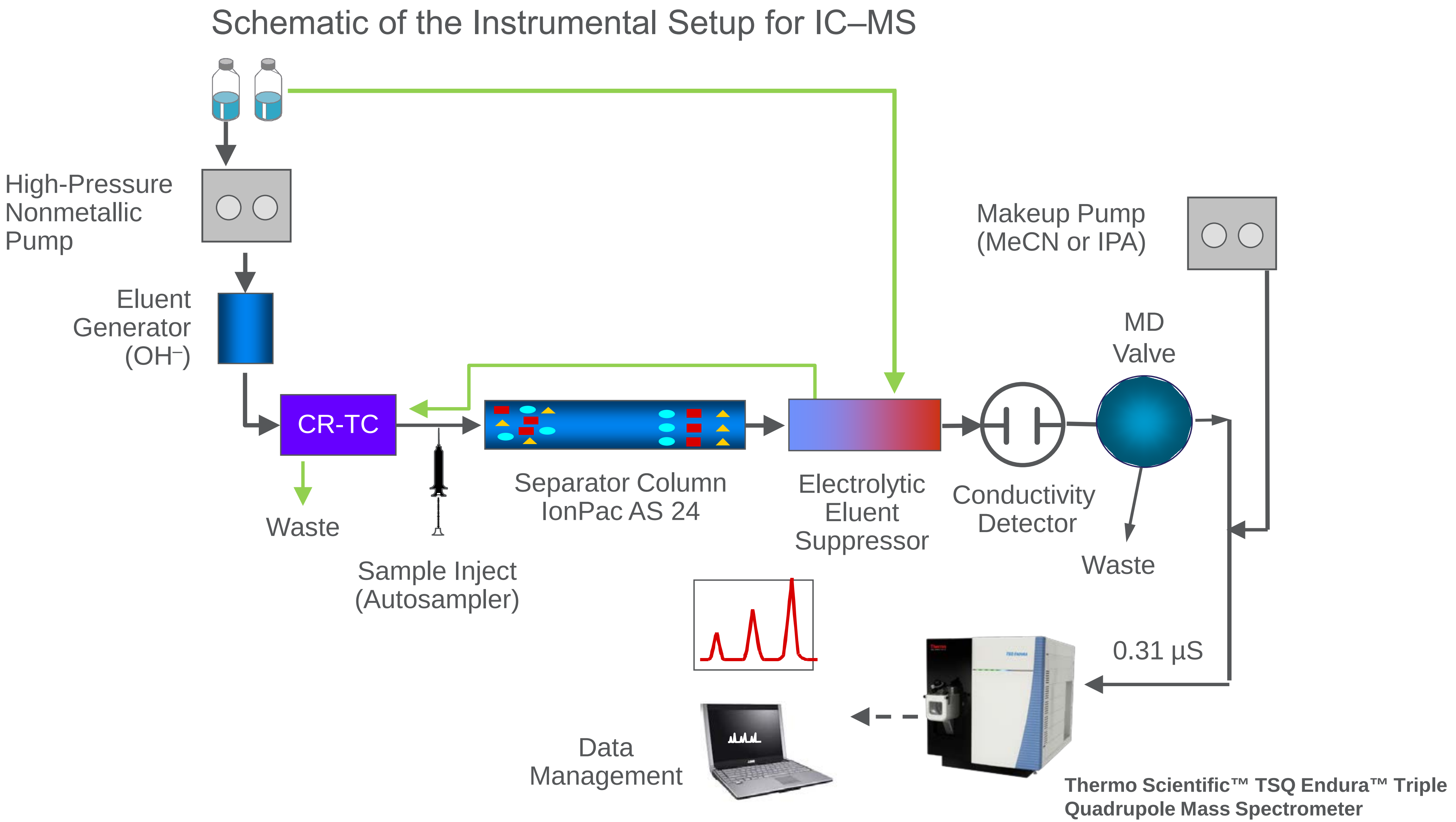
Arsenic Speciation with an iCAP Qc ICP–MS

- 0.45 µg/L of each As standard
- 6 species
- ~8000 cps/µg/L
- ~15 min analysis

- Anion exchange:
  - Dionex IonPac AS7 (250 mm × 2 mm i.d.)
  - Gradient elution with 20–200 mmol/L ammonium carbonate
  - Flow rate: 0.3 mL/min
  - Inj. volume: 20 µL



Thermo Scientific Ion Chromatography Mass Spectrometry (IC-MS)



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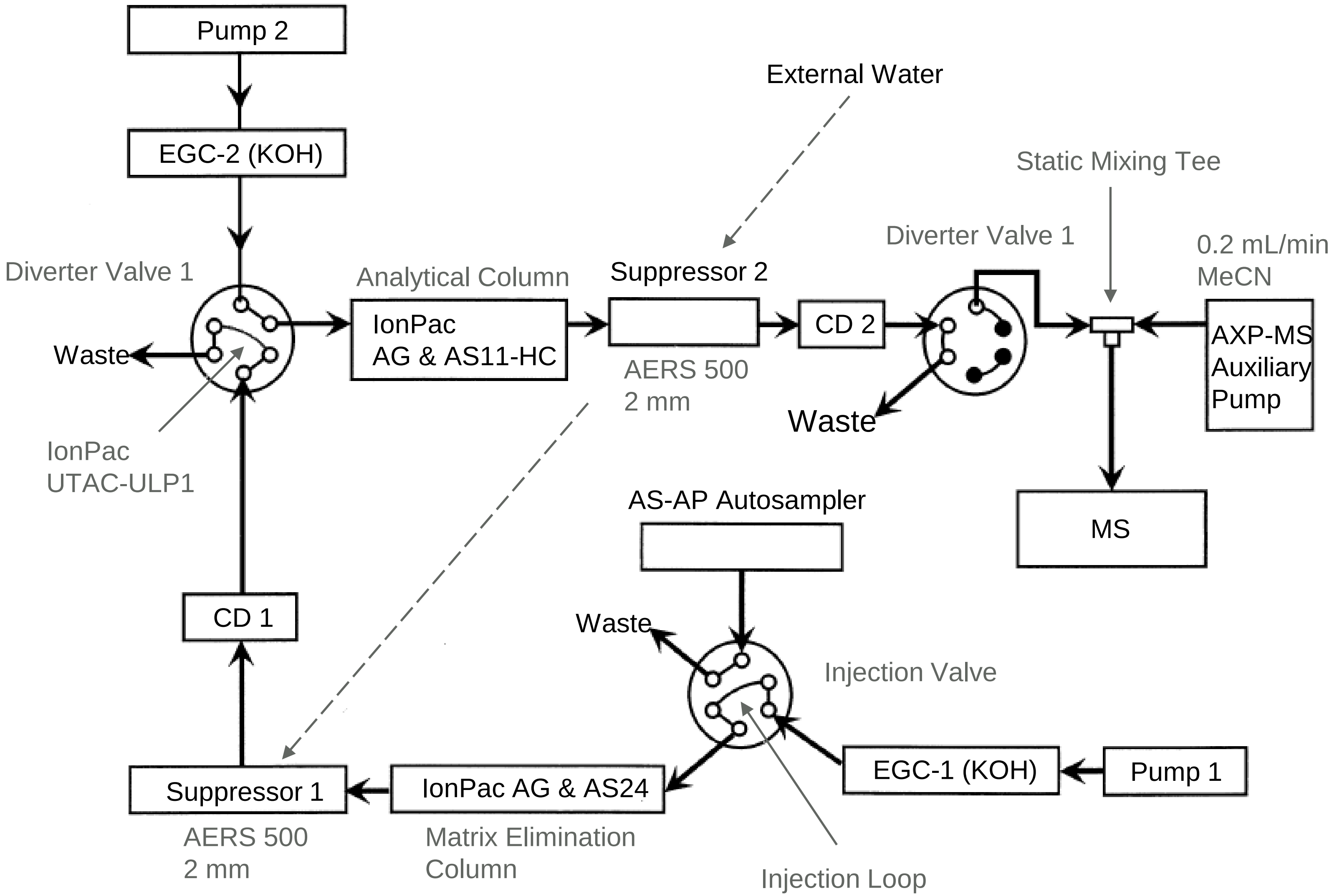
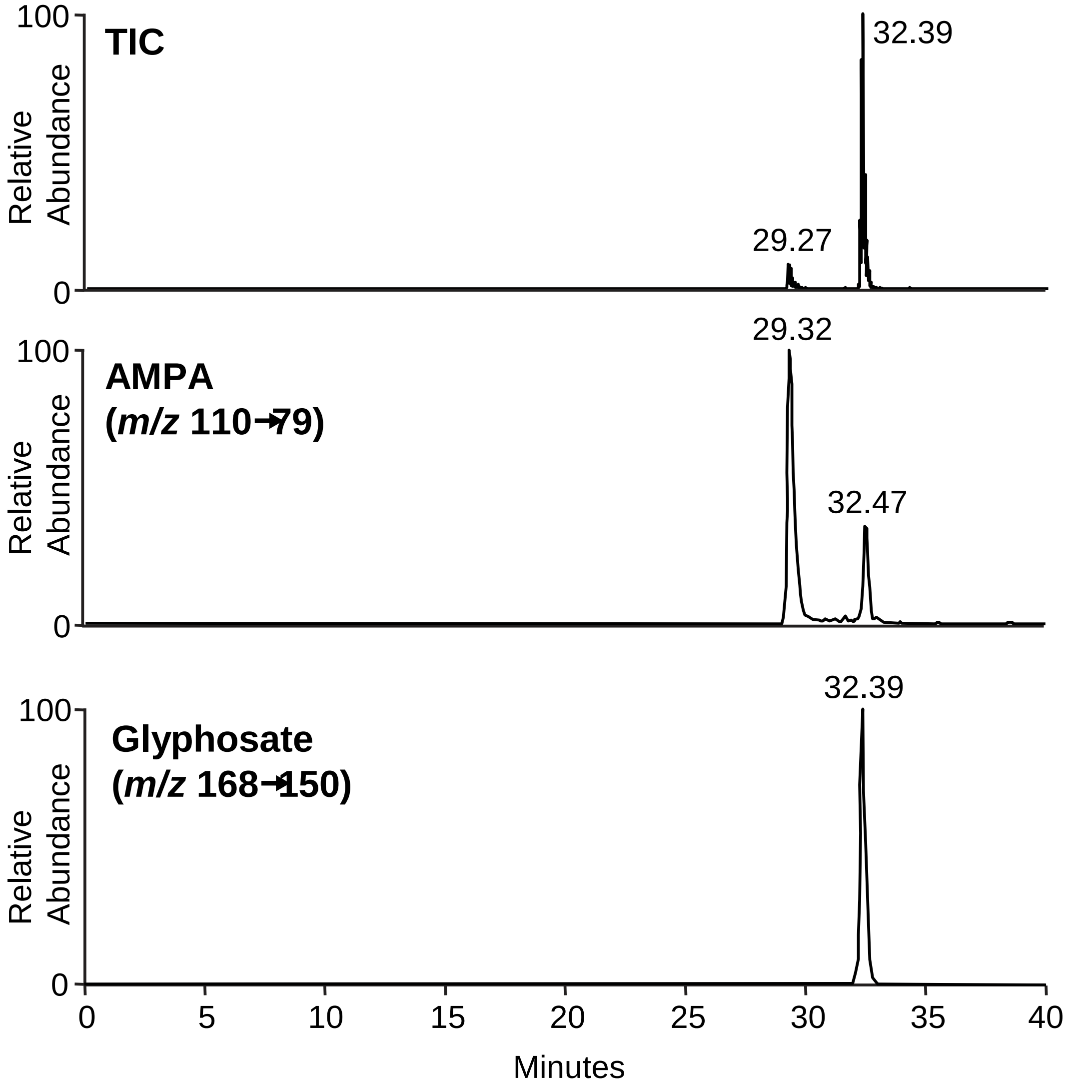
Schematic Diagram of a Reagent-Free IC × IC–MS System



Thermo Scientific Ion Chromatography Mass Spectrometry (IC-MS)

TIC of Glyphosate and AMPA with IC × IC–MS/MS Detection



Channel 1:

Column:	Dionex IonPac AS19;
Dimensions:	250 mm × 2 mm i.d.
Temperature:	30 ºC
Eluent:	KOH (EG)
Gradient:	8 mmol/L for 0-12 min, then 8-40 mmol/L for 12-16 min, then 40 mmol/L for 16-21 min
Flow rate:	300 µL/min
Detection:	Suppressed conductivity
Inj. volume:	200 µL;

Channel 2:

Column:	Dionex IonPac AS21
Dimensions:	250 mm × 2 mm i.d.
Temperature:	35 ºC
Eluent:	KOH (EG)
Gradient:	1 mmol/L for 0-20 min, then 1-40 mmol/L for 20-30 min, then 40 mmol/L for 30-35 min
Flow rate:	300 µL/min
Detection:	MS/MS (TSQ Quantum Access) -ESI, 3 kV, 400ºC
Sample:	5 µg/L glyphosate and AMPA spiked into high ionic strength matrix consisting of 250 mg/L chloride and sulfate, 150 mg/L bicarbonate, and 20 mg/L nitrate.

Applicability

From standard inorganic anions and cations …

… to

- Organic acids
- Amino acids (aliphatic, aromatic)
- Polyvalent anions
(Sequestering agents)
- Polarizable anions
(Oxy metal anions, sulfur species, etc.)
- Anionic surfactants
- Carbohydrates
- Proteins
- Nucleic acids
- …

- Amines
- Transition metals
- Lanthanides
- Phosphonium compounds
- Sulfonium compounds
- Hydrazinium compounds
- Cationic surfactants
- …

Modern Definition of the Term *Ion Chromatography*

Ion Chromatography is an umbrella term for all liquid chromatographic techniques that are suitable to separate and detect ionic and ionizable species.

Conclusions

- Highly reproducible isocratic and gradient separations of analytes with analytical and capillary RFIC utilizing suppressed conductivity detection.
- Analytical and capillary high-pressure IC with longer columns or smaller particles results in higher resolution.
- Acclaim mixed-mode phases support multiple retention mechanisms such as reversed-phase, ion-exchange, and HILIC.
- Adequate retention for ionic and ionizable compounds without adding ion-pair reagents.
- Analysis of APIs and counterions.
- Simultaneous separation of acidic, basic, and neutral compounds.
- The advantage of coupling ICP with IC is the possibility to analyze metals with different oxidation states.

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Acknowledgment

- Hamish Small
- Christopher Pohl and his entire team in R&D
- Prof. Sandy Dasgupta, Prof. Paul Haddad, Prof. Dennis Johnson, Prof. William LaCourse, Prof. Pavel Nesterenko, and many others

Thanks for Your Attention!

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Thank You

Please return our survey to receive a drink ticket for our daily networking event where you can continue discussions with our experts!