



# Increasing Importance of Ion Chromatography for Pharmaceutical Analysis

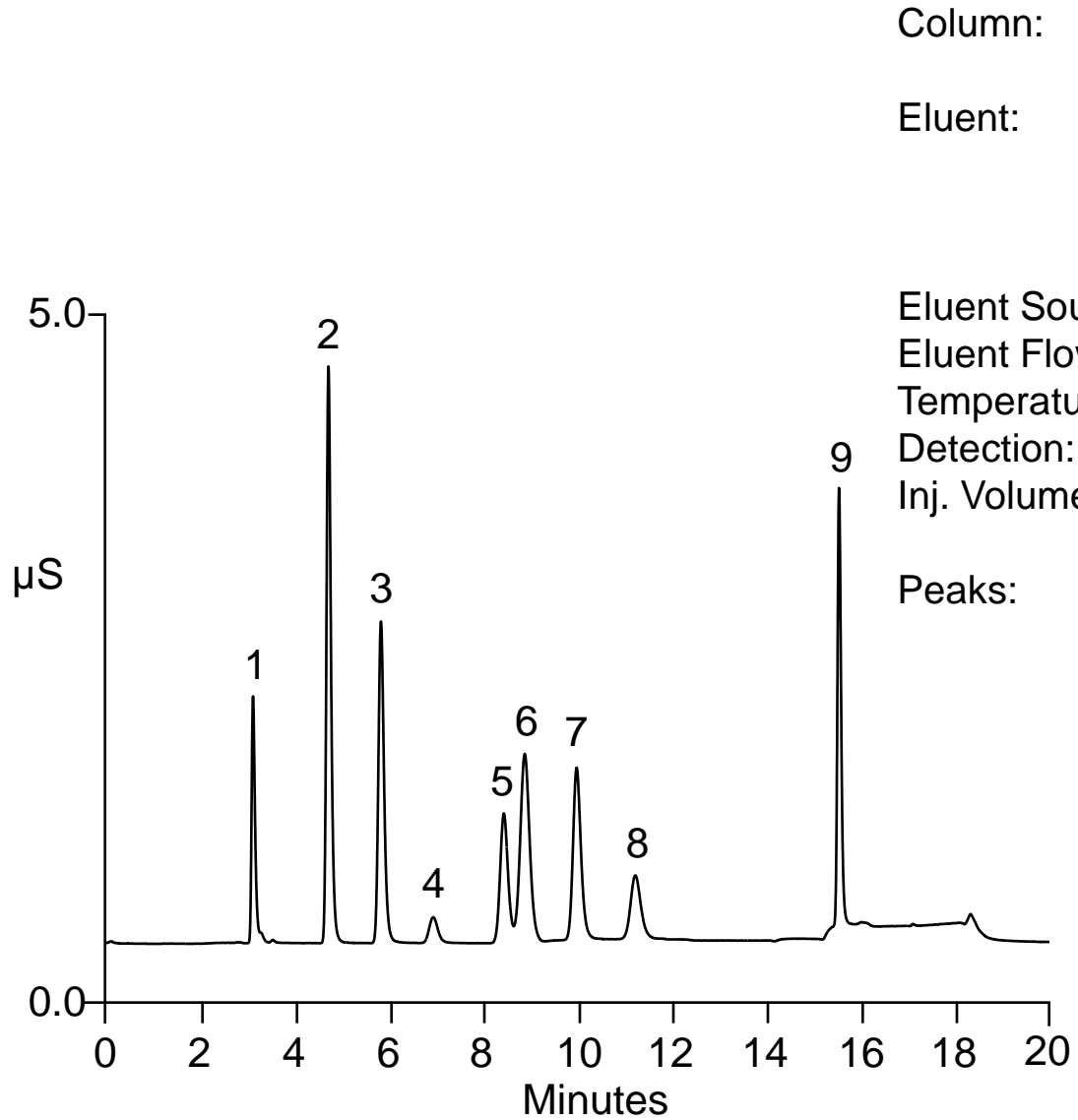
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ISC 2016, Cork, Ireland  
August 31th, 2016

- Introduction to Ion Chromatography
- What Ion Chromatography Offers for Pharmaceutical Analysis
- Review of the Three IC Applications for Pharmaceutical Analysis

At the most basic level....

Ion Chromatography =  
Ion-Exchange + Chemically  
Suppressed Conductivity

# Separation of Common Anions and TFA

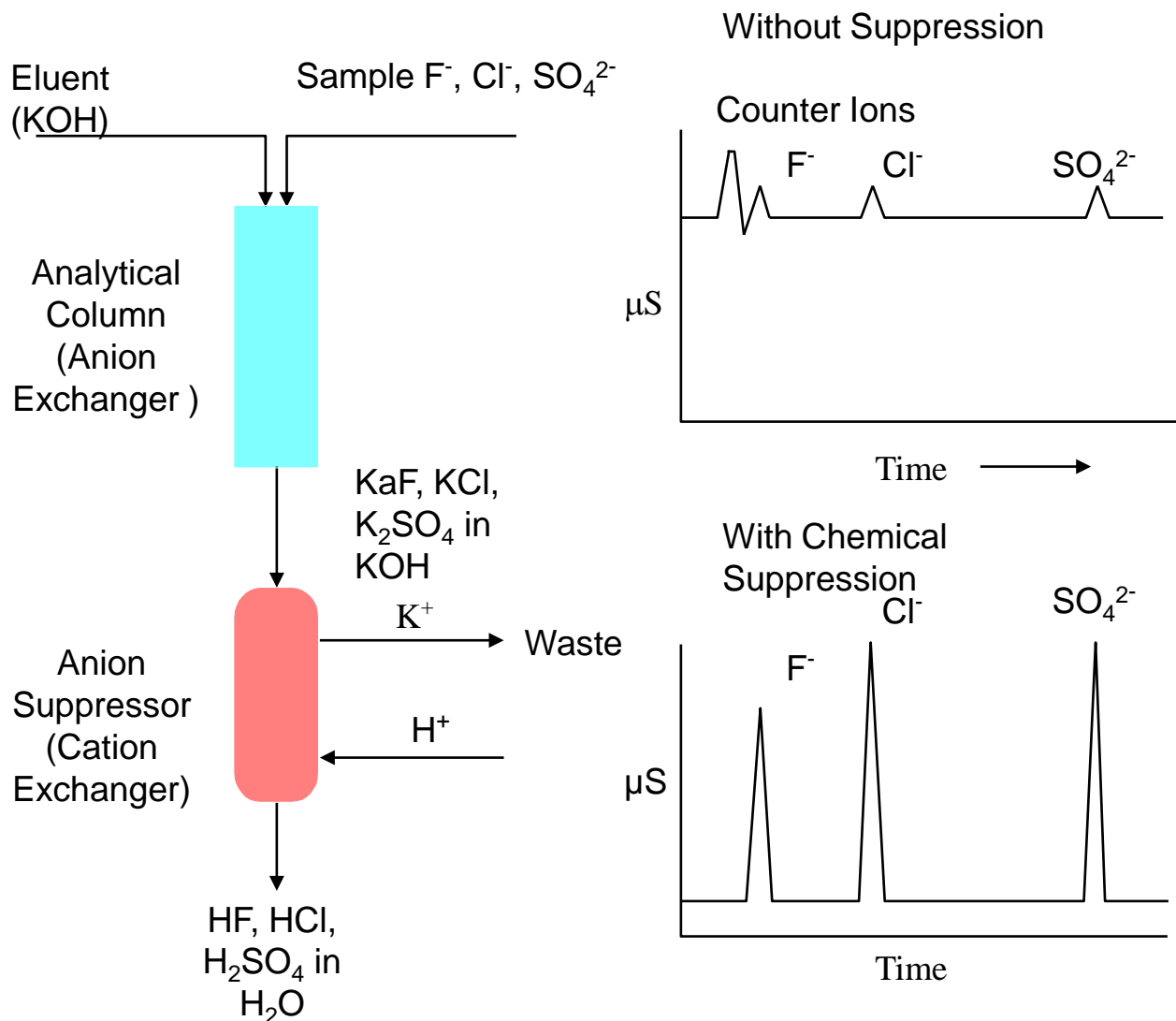


Column: Thermo Scientific™ Dionex™ IonPac™ AG18 and AS18  
Eluent: 22 mM KOH for 0–6 min, 28 mM KOH from 6–12 min, 50 mM KOH for 12–15 min, 22 mM KOH from 15–20 min  
Eluent Source: EG50  
Eluent Flow Rate: 1.0 mL/min  
Temperature: 30 ° C  
Detection: Suppressed conductivity  
Inj. Volume: 5  $\mu\text{L}$

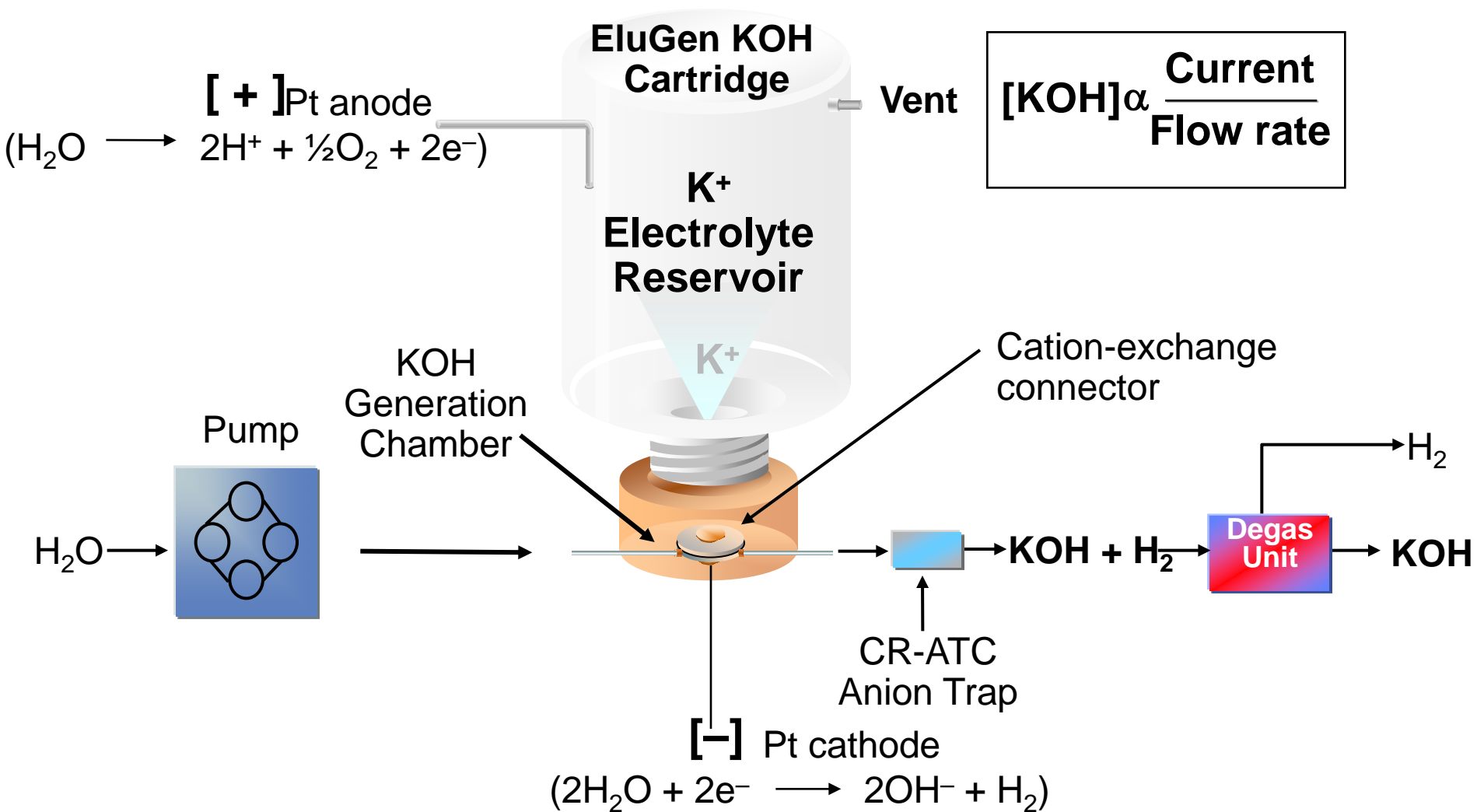
Peaks:

1. Fluoride	2 mg/L
2. Chloride	4
3. Nitrite	10
4. Carbonate	–
5. Bromide	10
6. Sulfate	10
7. Nitrate	10
8. Trifluoroacetate	10
9. Phosphate	20

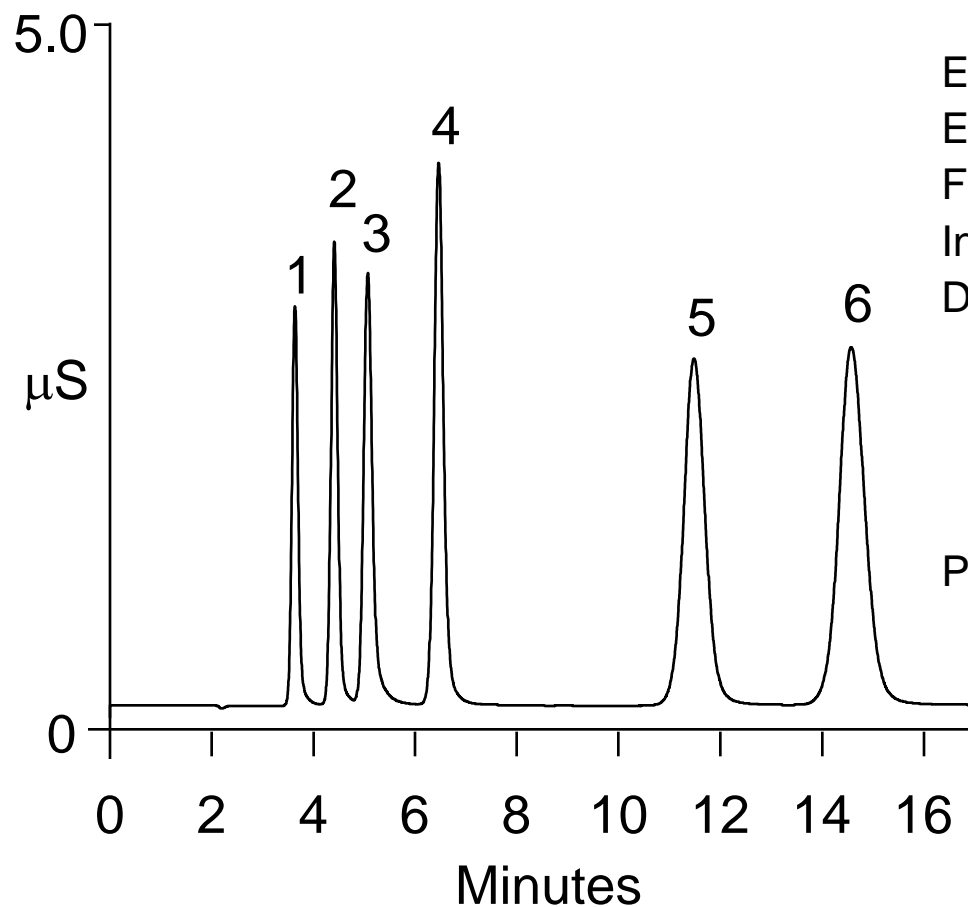
# The Role of Chemical Suppression (KOH)



# Hydroxide Eluent Generation



# Separation of the Common Cations



Column: Dionex IonPac CG12A, CS12A, 4 mm

Eluent: 18 mM Methanesulfonic acid

Eluent Source: Eluent Generator

Flow Rate: 1.0 mL/min

Injection: 25 mL

Detection: Suppressed conductivity, Thermo Scientific™ Dionex™ CSRS™-ULTRA cation self regenerating suppressor, recycle mode

Peaks:

1. Lithium	0.5 mg/L (ppm)
2. Sodium	2.0
3. Ammonium	2.5
4. Potassium	5.0
5. Magnesium	2.5
6. Calcium	5.0

# Common IC Detection Techniques

- **Conductivity:**
  - **Suppressed**
  - (Non suppressed)
- **UV detection:**
  - **Direct**
  - (Indirect)
  - **Post column derivatization**
- **Amperometry:**
  - Direct current amperometry (DC)
  - **Integrated amperometry (PAD and IPAD)**
- **Mass spectrometry**



# Some Ion Chromatography Analytes

- **Anions:** Chloride, sulfate, fluoride, **nitrite**, **nitrate**, bromide, iodide, bromate, chlorite, chlorate, perchlorate, sulfite, thiosulfate, cyanide, thiocyanate, cyanate, sulfide, benzoate, acetate, formate, silicate, glycolate, oxalate, iodate, lactate, trifluoroacetate, numerous other organic acids and inorganic anions, **carbohydrates**, amino acids
- **Cations:** **Lithium**, sodium, potassium, ammonium, **calcium**, magnesium, barium, strontium, methylamine, dimethylamine, trimethylamine, ethanolamine, diethanolamine, triethanolamine, choline, many transition metals, and numerous amines

# What IC Offers for Pharmaceutical Analysis

- Easy (direct) determination of analytes lacking chromophores
- Opportunity to have more automated assays compared to HPLC
- Usually requires no organic solvents
- Separation modes better suited for some analytes
- Counterion analysis of salt form drug substances to confirm ID and API content

# How IC has been Used in Pharmaceutical Analysis

- Assay
- Determination of impurities and degradation products – limit tests and related substances tests in drug substances and drug products
- Counterion analysis of salt form drug substances to confirm ID and API content
- Excipient analysis

## Example IC Methods in the USP-NF

- Assays of Kanamycin B and Amikacin in DS and DP monographs
- USP-NF <345> Assay for Citric Acid/Citrate and Phosphate
- Risedronate Sodium Assay
- Cefepime Hydrochloride—Limit of *N*-methylpyrrolidine
- Methacholine Chloride – Assay and limit of Acetylcholine Test
- Heparin Sodium – Organic Impurities Test
- Sodium Bicarbonate – Limit of Ammonia Test

# Revised <191> Identification Tests

- Published in 2015
- Eliminated flame tests
- Eliminated wet chemical tests that yielded poor results
- Added better wet chemical tests – EP harmonization too
- Added instrumentation options for identification tests – including ion chromatography and other forms of chromatography.

# Nitrite and Nitrate in Sodium Nitrite

- The method was published Pharmacopeia Forum 40(5) as part of a modernization proposal for the USP Sodium Nitrite monograph.
- Sodium nitrite part of the treatment for acute cyanide poisoning.
- The IC method assays nitrite and would replace a titration with potassium permanganate.
- The same method determines nitrate impurity.
- We replicated the proposed method in our laboratory, though we used eluent generation.

# IC Separation – Sodium Nitrite USP Monograph

Column: Dionex IonPac AS12A Analytical, 4 x 250 mm  
Dionex IonPac AG12A Guard, 4 x 50 mm

Eluent: 2.7 mM K<sub>2</sub>CO<sub>3</sub> /0.3 mM KHCO<sub>3</sub>

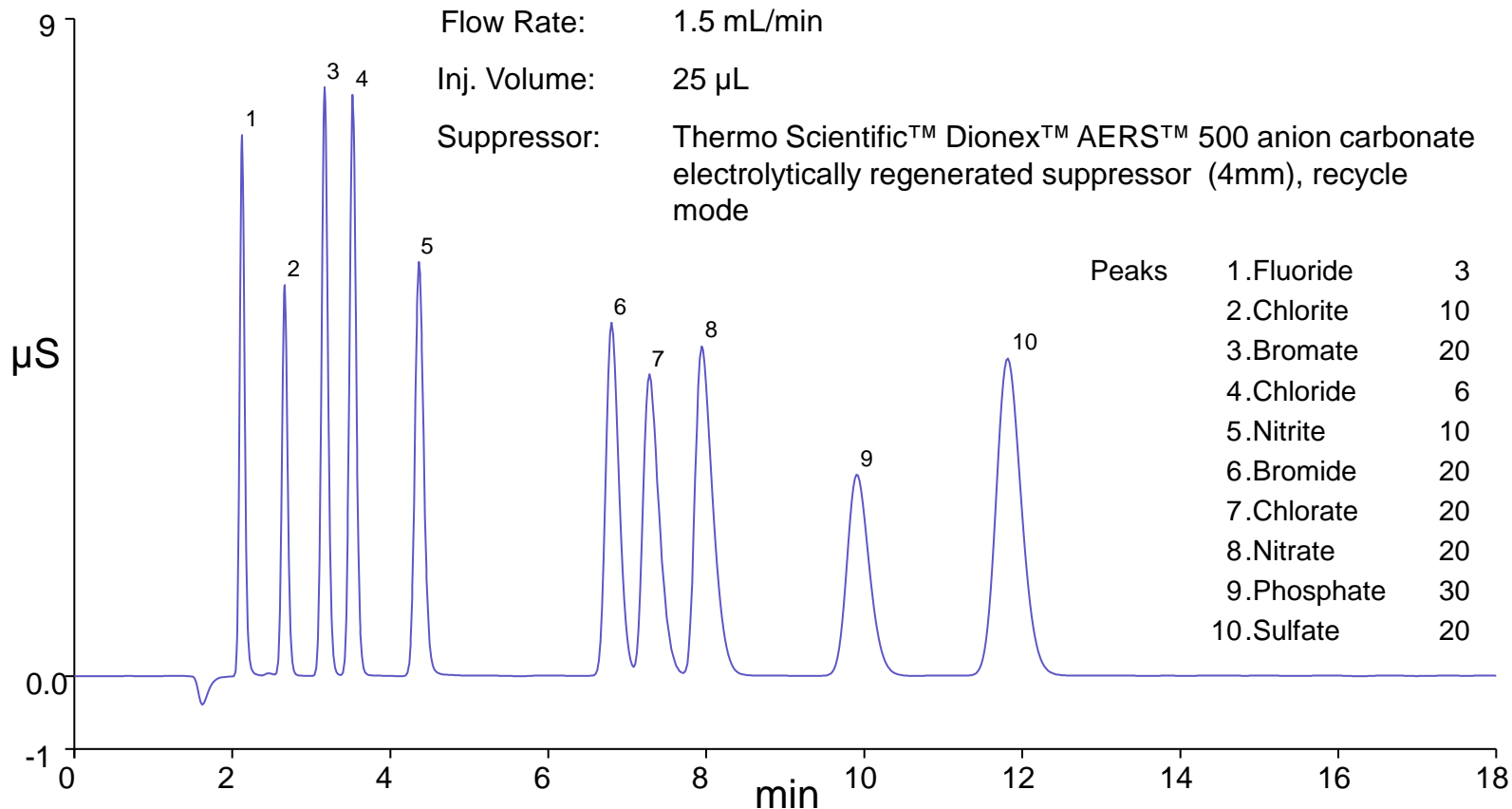
Eluent Source: Thermo Scientific™ Dionex™ Eluent Generator Cartridge  
EGC 500 K<sub>2</sub>CO<sub>3</sub> cartridge with EPM 500

Temperature: Ambient (~24 °C)

Flow Rate: 1.5 mL/min

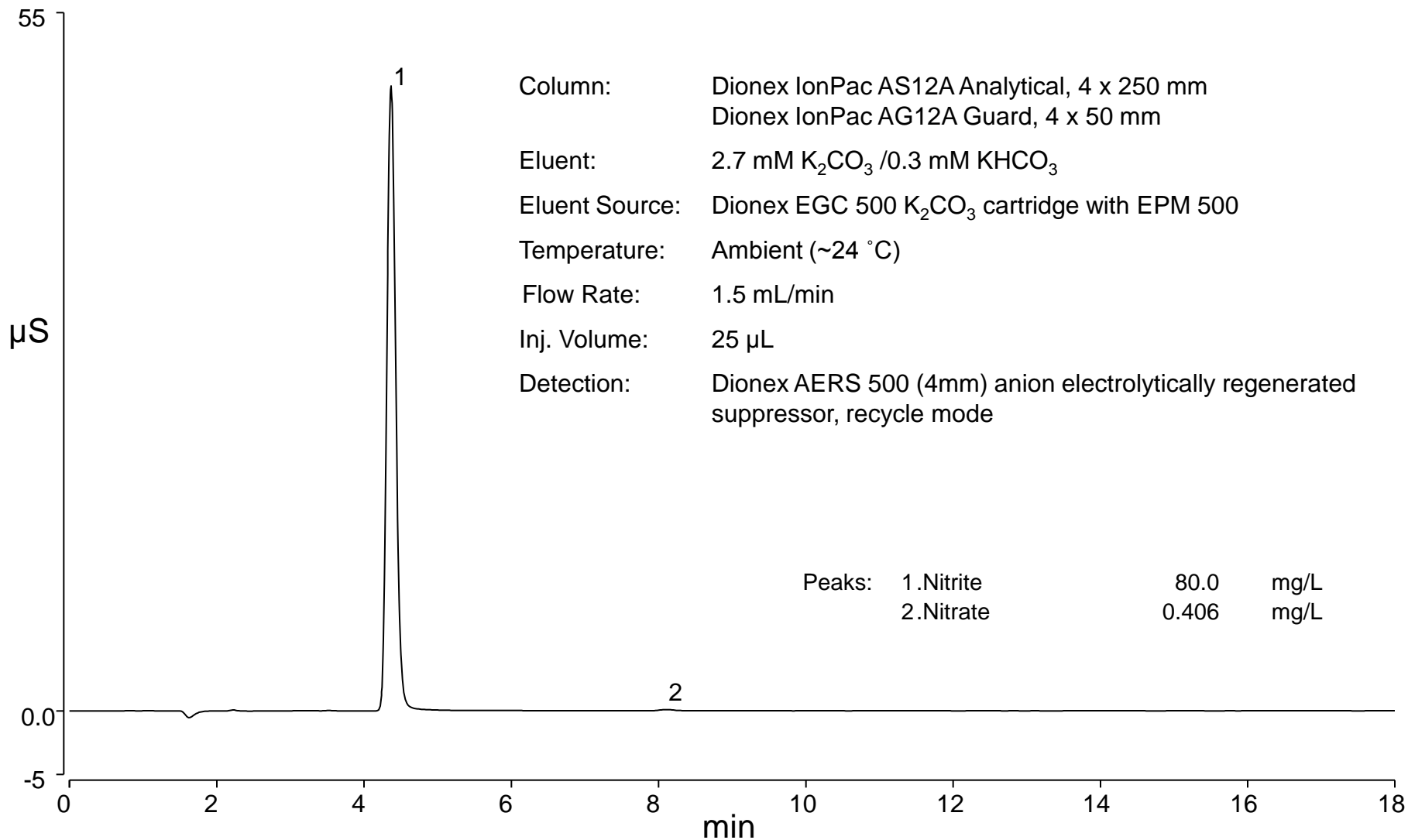
Inj. Volume: 25 µL

Suppressor: Thermo Scientific™ Dionex™ AERS™ 500 anion carbonate  
electrolytically regenerated suppressor (4mm), recycle  
mode



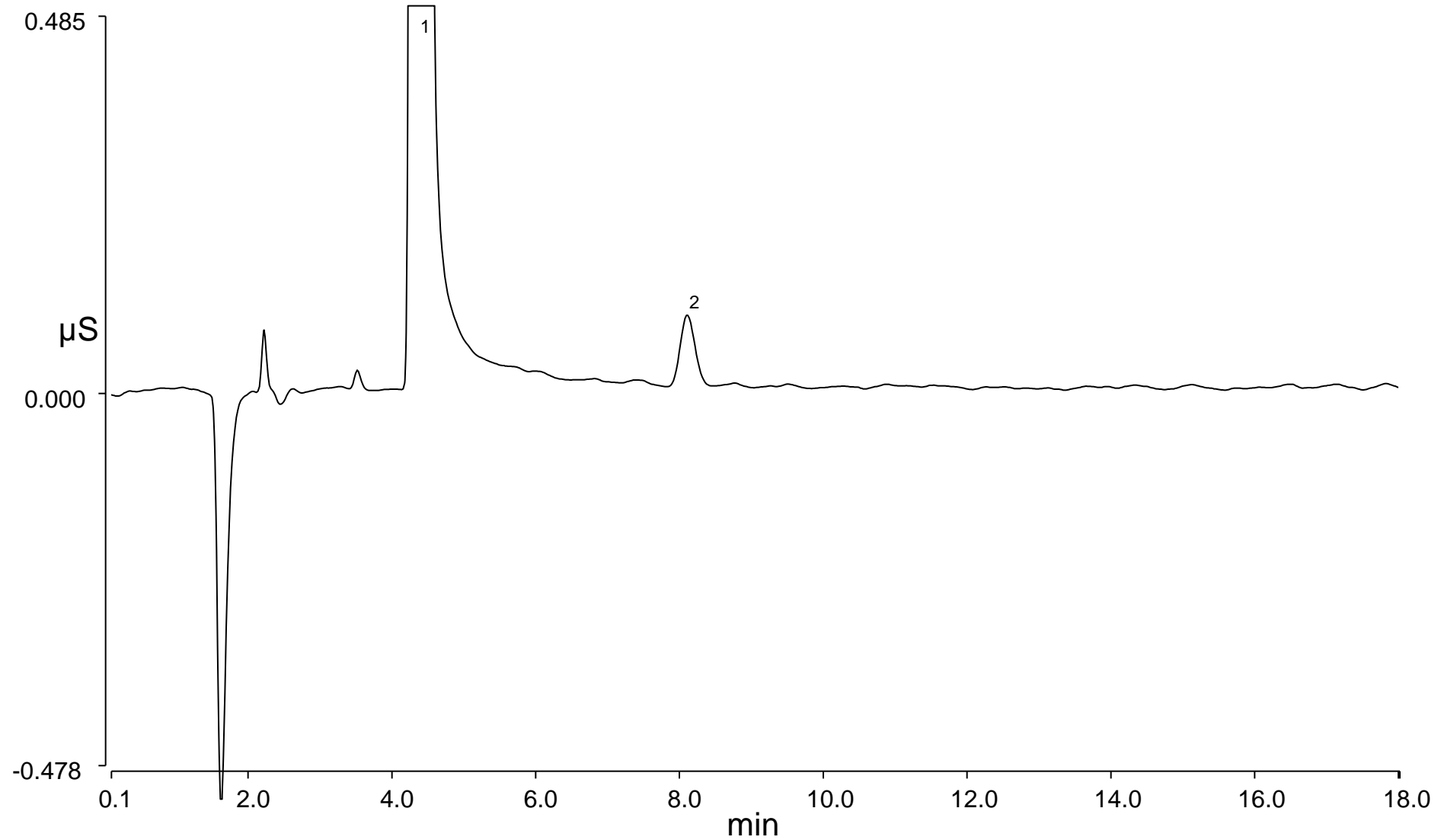
Peaks	1.Fluoride	3	mg/L
	2.Chlorite	10	
	3.Bromate	20	
	4.Chloride	6	
	5.Nitrite	10	
	6.Bromide	20	
	7.Chlorate	20	
	8.Nitrate	20	
	9.Phosphate	30	
	10.Sulfate	20	

# Sodium Nitrite Assay by the Proposed USP Monograph





# Enlarged to View the Nitrate Peak



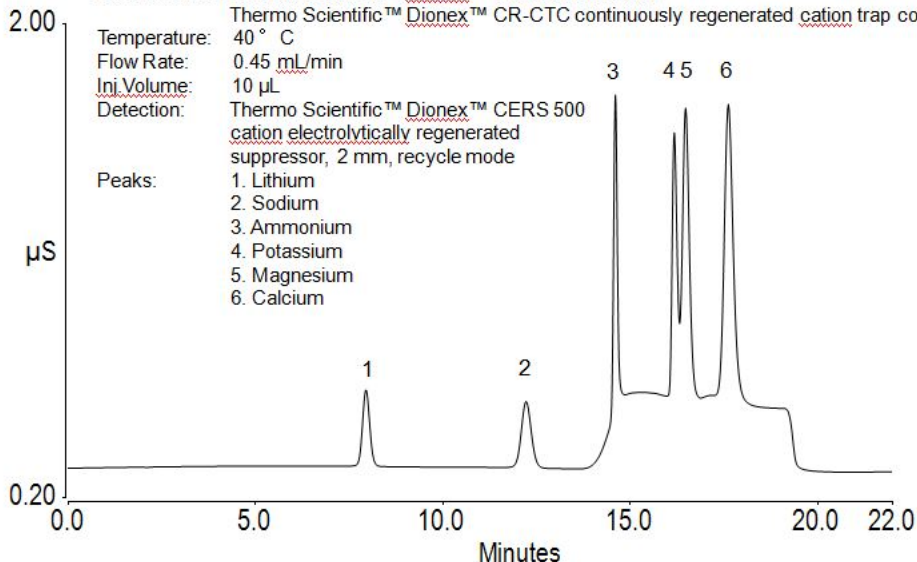
# Assay of Lithium in Lithium Hydroxide

- The method to assay lithium was developed in our lab for a proposal to modernize the USP Lithium Hydroxide monograph.
- The same method was developed to allow the measurement of calcium that is also required in the LiOH monograph.
- Our work has been reported in Application Note 1144.

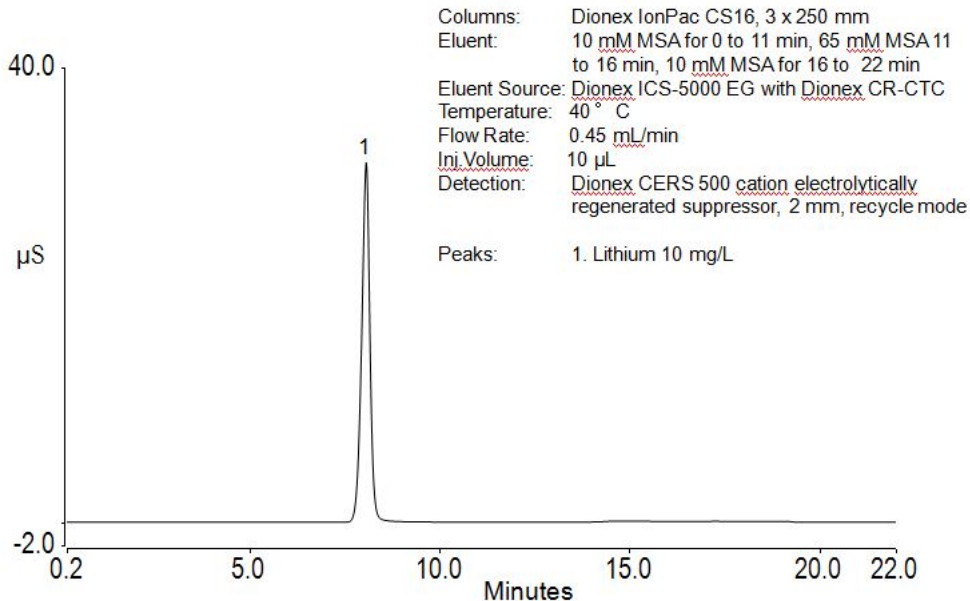
# Separation of Six Common Cations

Columns: Dionex IonPac CS16, 3 x 250 mm  
Eluent: 10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min  
Eluent Source: Thermo Scientific™ Dionex™ ICS-5000 EG with  
Thermo Scientific™ Dionex™ CR-CTC continuously regenerated cation trap column  
Temperature: 40° C  
Flow Rate: 0.45 mL/min  
Inj. Volume: 10 µL  
Detection: Thermo Scientific™ Dionex™ CERS 500  
cation electrolytically regenerated  
suppressor, 2 mm, recycle mode

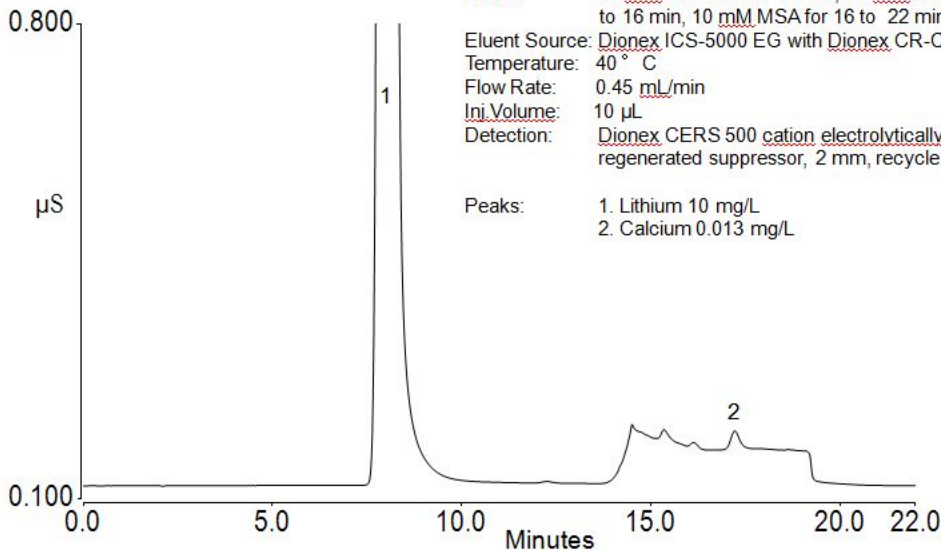
Peaks:  
1. Lithium  
2. Sodium  
3. Ammonium  
4. Potassium  
5. Magnesium  
6. Calcium



# Determination of 10 mg/L Lithium in 10 mM Acetic Acid



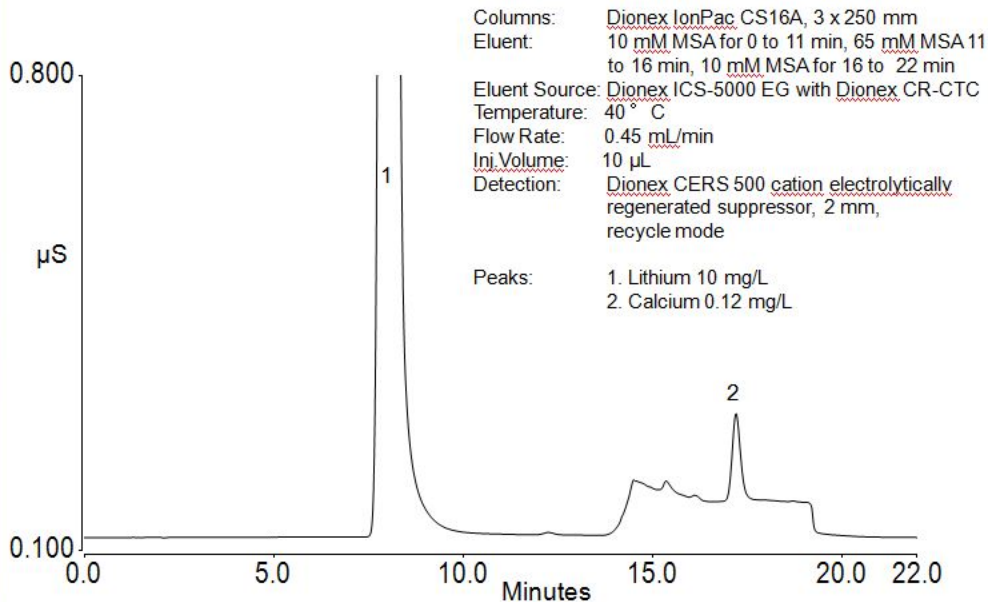
# Enlarged to View the Calcium Peak



Columns: Dionex IonPac CS16, 3 x 250 mm  
Eluent: 10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min  
Eluent Source: Dionex ICS-5000 EG with Dionex CR-CTC  
Temperature: 40 ° C  
Flow Rate: 0.45 mL/min  
Inj. Volume: 10 μL  
Detection: Dionex CERS 500 cation electrolytically regenerated suppressor, 2 mm, recycle mode

Peaks: 1. Lithium 10 mg/L  
2. Calcium 0.013 mg/L

# Lithium Sample Spiked with Calcium at the USP Limit



# Determination of the Sialic Acid Content of Glycoconjugates

- Common assay for therapeutic glycoproteins.
- One method is an IC (HPAE-PAD) method and it is described in USP General Chapter <129>.

- Sialic acids are released from glycoproteins by either mild acid hydrolysis or by treatment with a neuraminidase.
- Samples are then dried to remove the acid.
- Samples are injected onto the HPAE-PAD system.
- For neuraminidase digestions the sample is either injected or diluted and injected.



# Separation of Sialic Acids using HPAE-PAD

Column: Thermo Scientific™ Dionex™ CarboPac™ PA20 guard, 3 x 30 mm Dionex CarboPac PA20, 3 x 150 mm  
Eluent: 70-300 mM acetate in 100 mM NaOH from 0- 7.5 min, 300 mM acetate in 100 mM NaOH from 7.5-9.0 min, 300-70 mM acetate from 9.0-9.5 min. 7 min of equilibration at 70 mM acetate in 100 mM NaOH

Temperature: 30 ° C

Flow Rate: 0.5 mL/min

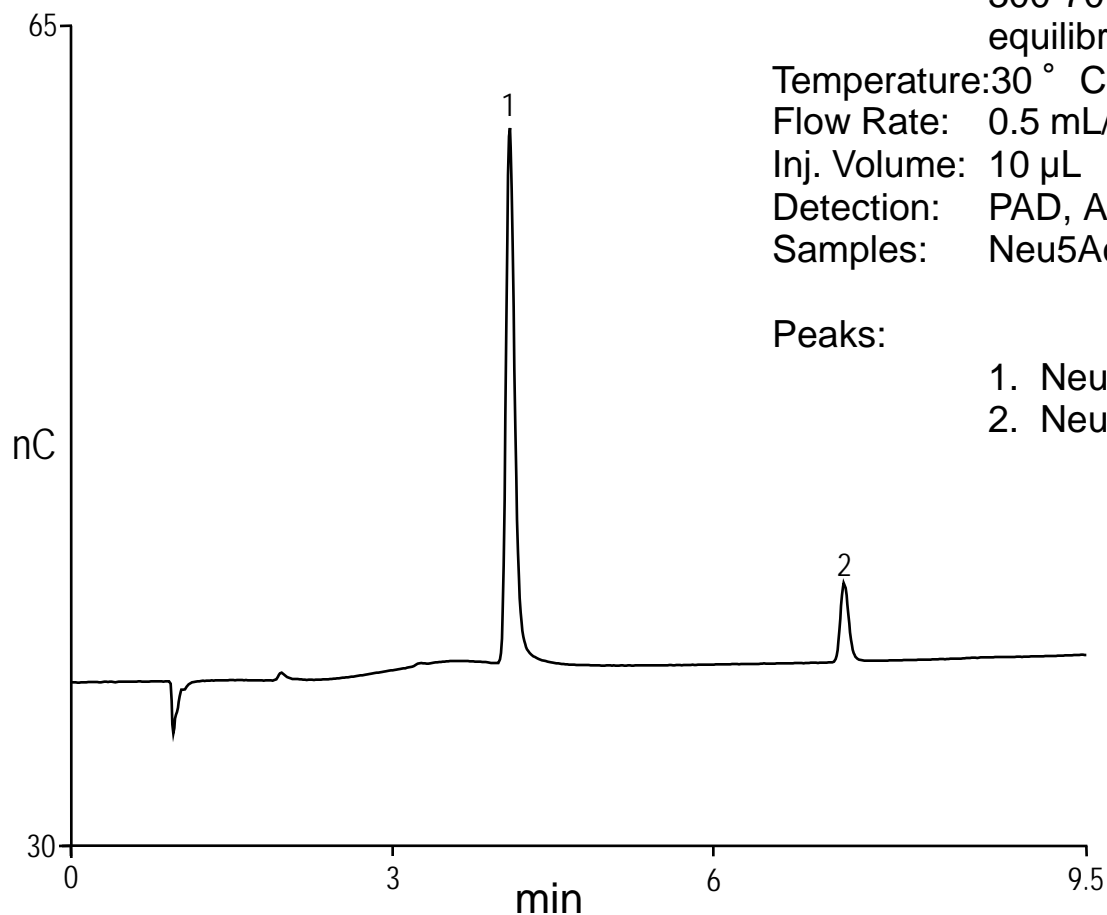
Inj. Volume: 10 µL

Detection: PAD, Au (Disposable)

Samples: Neu5Ac and Neu5Gc standards

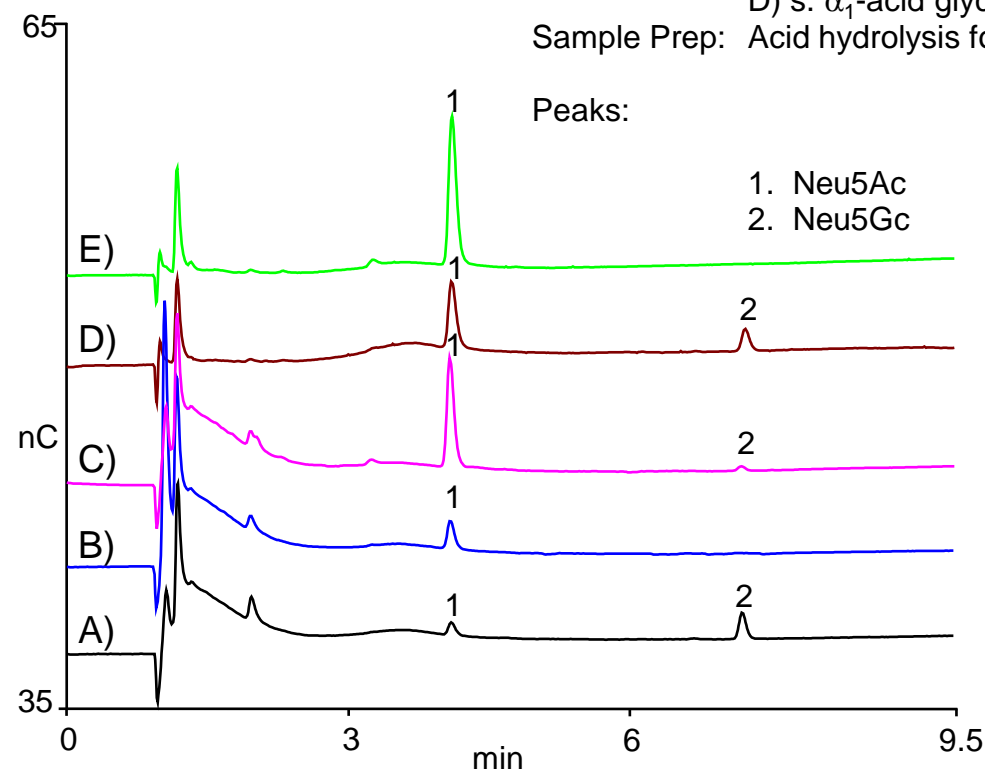
Peaks:

1. Neu5Ac	75	pmol
2. Neu5Gc	5.8	



# Separation of Glycoprotein Acid Hydrolyzates

Column: Dionex CarboPac PA20 guard, 3 x 30 mm  
 Dionex CarboPac PA20, 3 x 150 mm  
 Eluent: 70-300 mM acetate in 100 mM NaOH from 0-7.5 min, 300 mM acetate in 100 mM NaOH from 7.5-9.0 min, 300-70 mM acetate from 9.0-9.5 min. 7 min of equilibration at 70 mM acetate in 100 mM NaOH  
 Temperature: 30 ° C  
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 10 µL  
 Detection: PAD, Au (Disposable)  
 Samples: A) b. apo-transferrin, B) h. transferrin, C) fetuin, D) s.  $\alpha_1$ -acid glycoprotein, E) h.  $\alpha_1$ -acid glycoprotein  
 Sample Prep: Acid hydrolysis followed by lyophilization and dissolution



Peaks:	A)	B)	C)	D)	E)
1. Neu5Ac	1.7	4.4	18	15	37 pmol
2. Neu5Gc	2.1	ND	0.39	2.6	ND

A 10% signal offset has been applied.  
 ND = Not Detected

# Conclusions

- IC is finding greater application in pharmaceutical laboratories to develop methods for drug products and drug substances.
- IC methods have a greater degree of automation compared to other chromatographic techniques.
- IC is one of the techniques being used to modernize pharmacopeia methods.

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Applications of  
**ION CHROMATOGRAPHY**  
for **PHARMACEUTICAL**  
and **BIOLOGICAL**  
**PRODUCTS**

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Thank you for your attention!