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Ion Chromatography for Pharmaceutical Analysis

Jeffrey Rohrer, Ph. D.

Outline

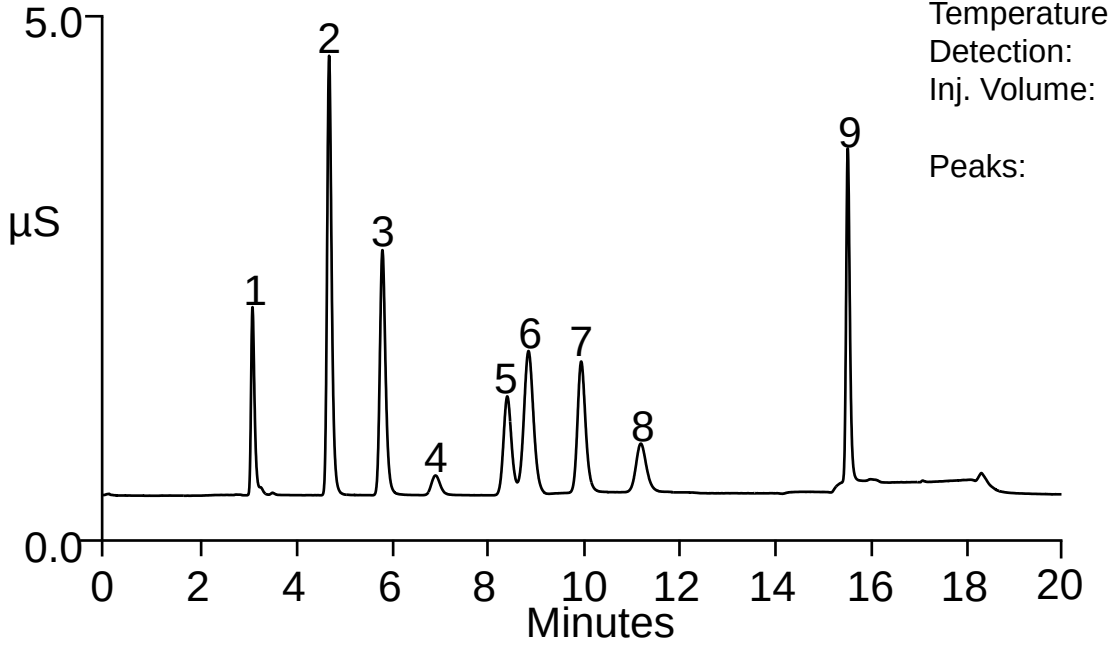
- Introduction to Ion Chromatography (IC)
- What IC Offers for Pharmaceutical Analysis
- Review of the Two IC Applications for Pharmaceutical Analysis
- How to Develop an IC for Pharmaceuticals Assay and an Example

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

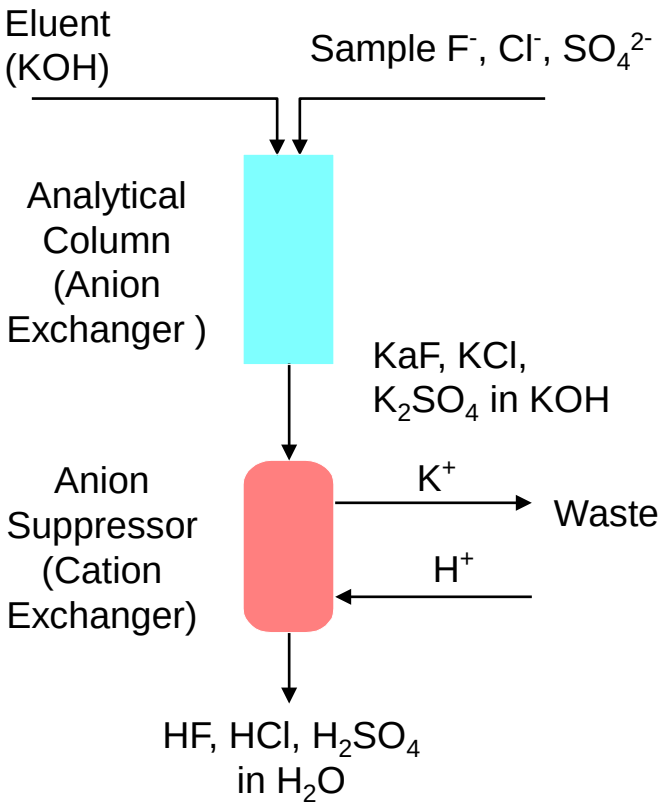


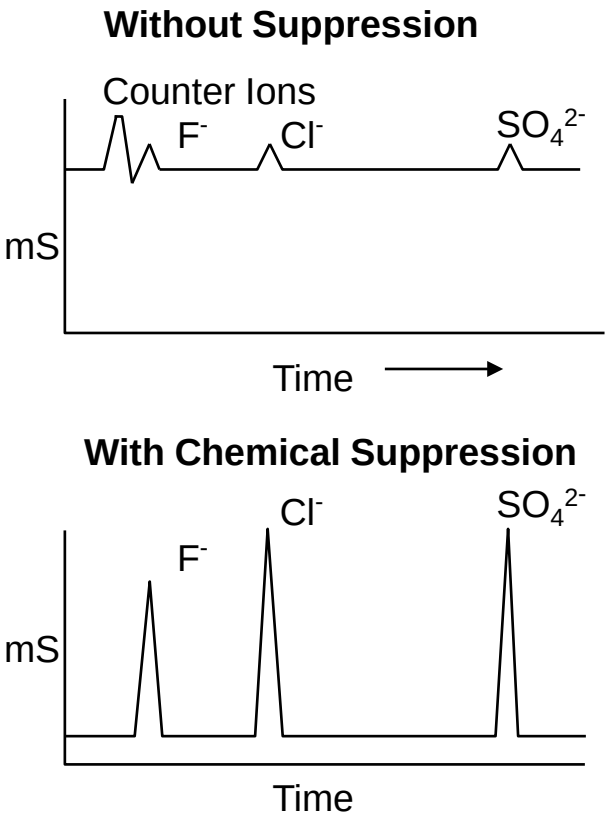
Eluent:	22 mM KOH for 0–6 min, 28 mM KOH from 6–12 min, 50 mM KOH for 12–15 min, 22 mM KOH from 15–20 min
Eluent Source:	EG50
Eluent Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	Suppressed conductivity
Inj. Volume:	5 µL

Peaks:

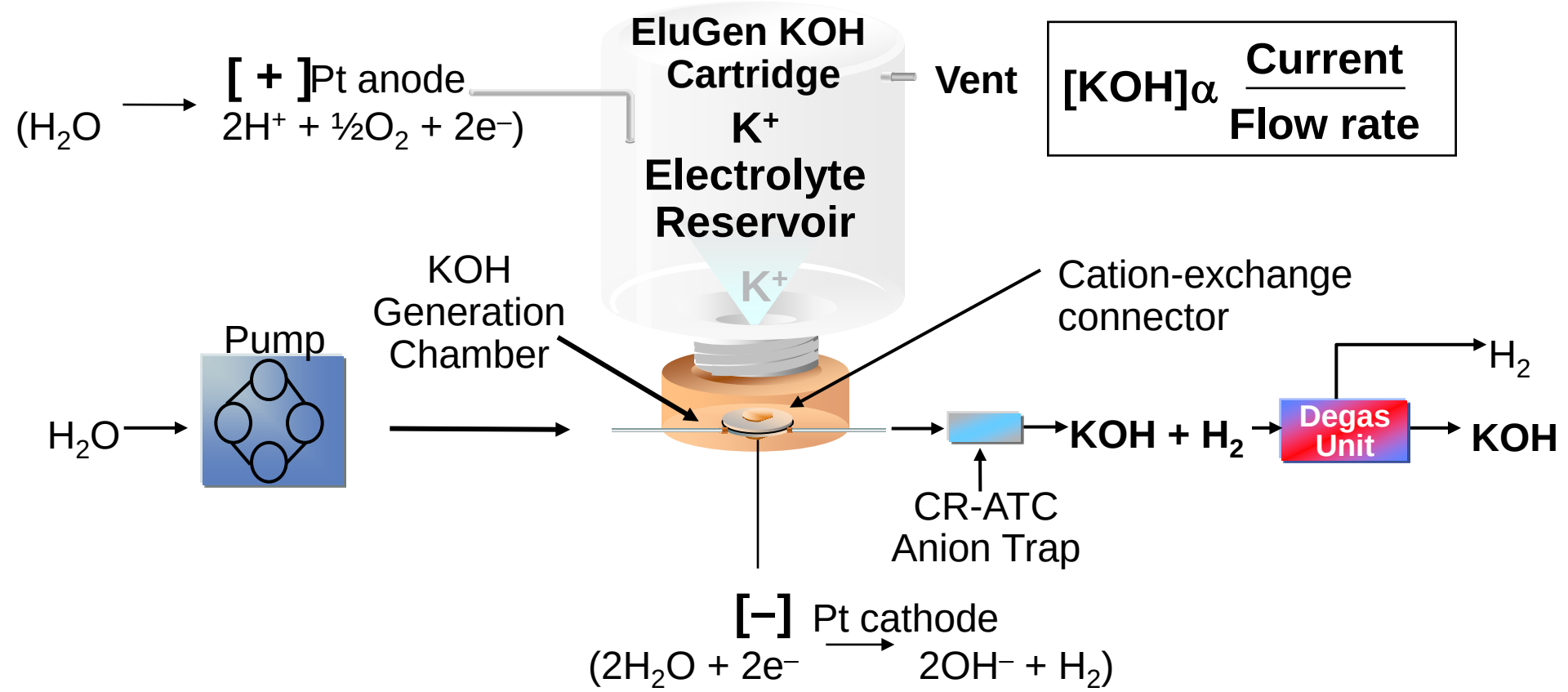
1.	Fluoride	2 mg/L
2.	Chloride	4
3.	Nitrite	10
4.	Carbonate	–
5.	Bromide	10
6.	Sulfate	10
7.	Nitrate	10
8.	Trifluoroacetate	10
9.	Phosphate	20

The Role of Chemical Suppression (KOH)

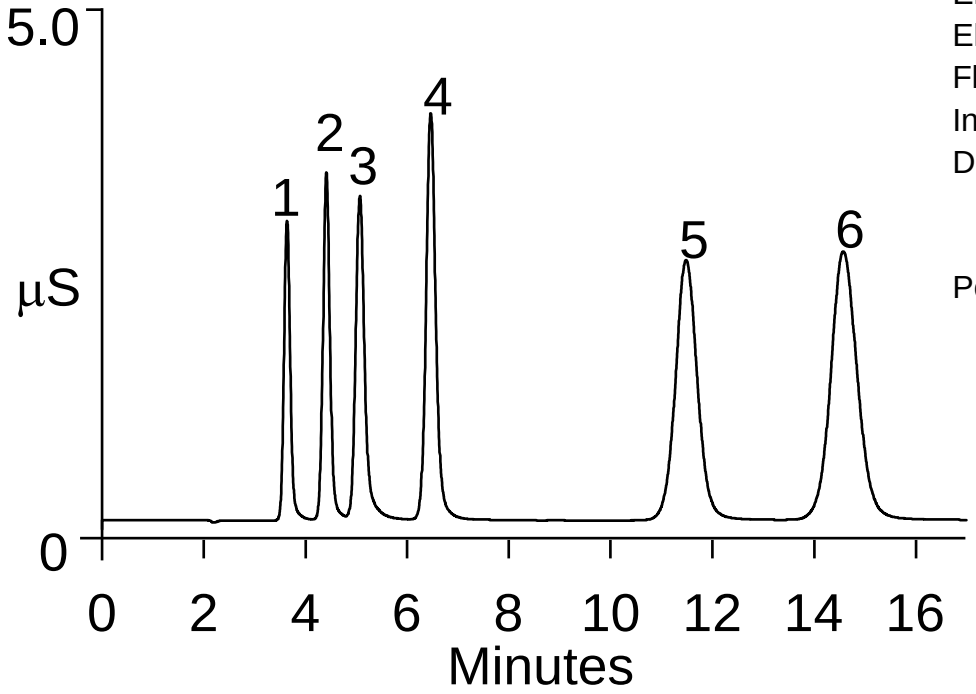




Hydroxide Eluent Generation



Separation of the Common Cations



Column:	Thermo Scientific™ Dionex™ IonPac™ CG12A, CS12A, 4 mm
Eluent:	18 mM Methanesulfonic acid
Eluent Source:	Eluent Generator
Flow Rate:	1.0 mL/min
Injection:	25 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ SC-CSRS™ ULTRA 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 amperomety (DC)
  - **Integrated amperometry (PAD and IPAD)**

- **Mass spectrometry**

HPAE-PAD

High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

Basics of HPAE

- Carbohydrates are separated as oxyanions at high pH (>12)
- These separations require hydroxide eluents
- If the carbohydrate is **charged**, acetate or another strong eluent must be added to the hydroxide eluent to elute the carbohydrate

Basics of PAD

- Carbohydrates are detected on a Au working electrode (WE) at high pH by PAD
- PAD applies a series of potentials (a waveform) to a WE and the carbohydrate is detected by its oxidation at 1 potential
- The waveform is applied at a frequency of 2 Hz, i.e. two times a second
- **Therefore carbohydrates are separated and detected without derivatization, i.e. a direct analysis**

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
- Counter ion 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 for drug substances and drug products
- Counter ion 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.

Example #1

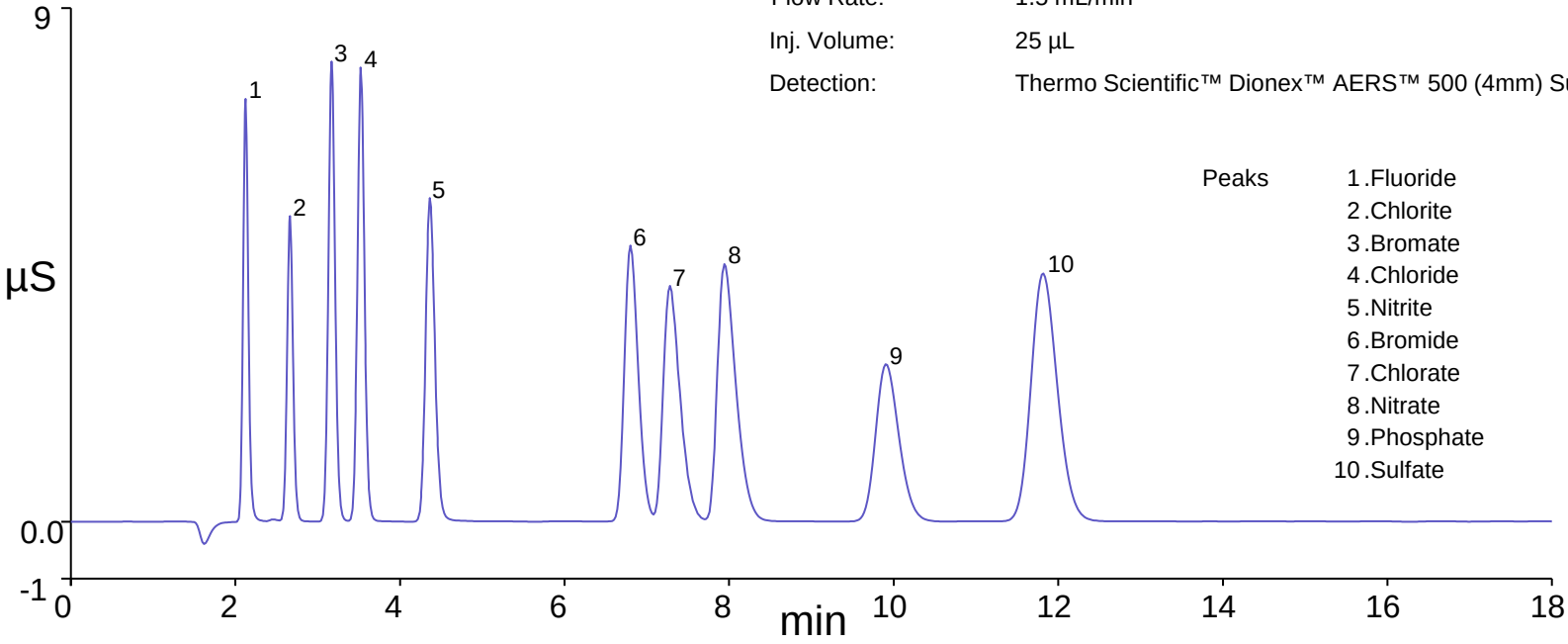
Anion IC Used for an Assay and a Limit Test of a Drug Substance

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:	Thermo Scientific™ Dionex™ IonPac™ AS12A Analytical, 4 x 250 mm Thermo Scientific™ Dionex™ IonPac™ AG12A Guard, 4 x 50 mm
Eluent:	2.7 mM K_2CO_3 /0.3 mM $KHCO_3$
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 K_2CO_3 cartridge with EPM 500
Temperature:	Ambient (~24 ˚C)
Flow Rate:	1.5 mL/min
Inj. Volume:	25 µL
Detection:	Thermo Scientific™ Dionex™ AERS™ 500 (4mm) Suppressor, 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	

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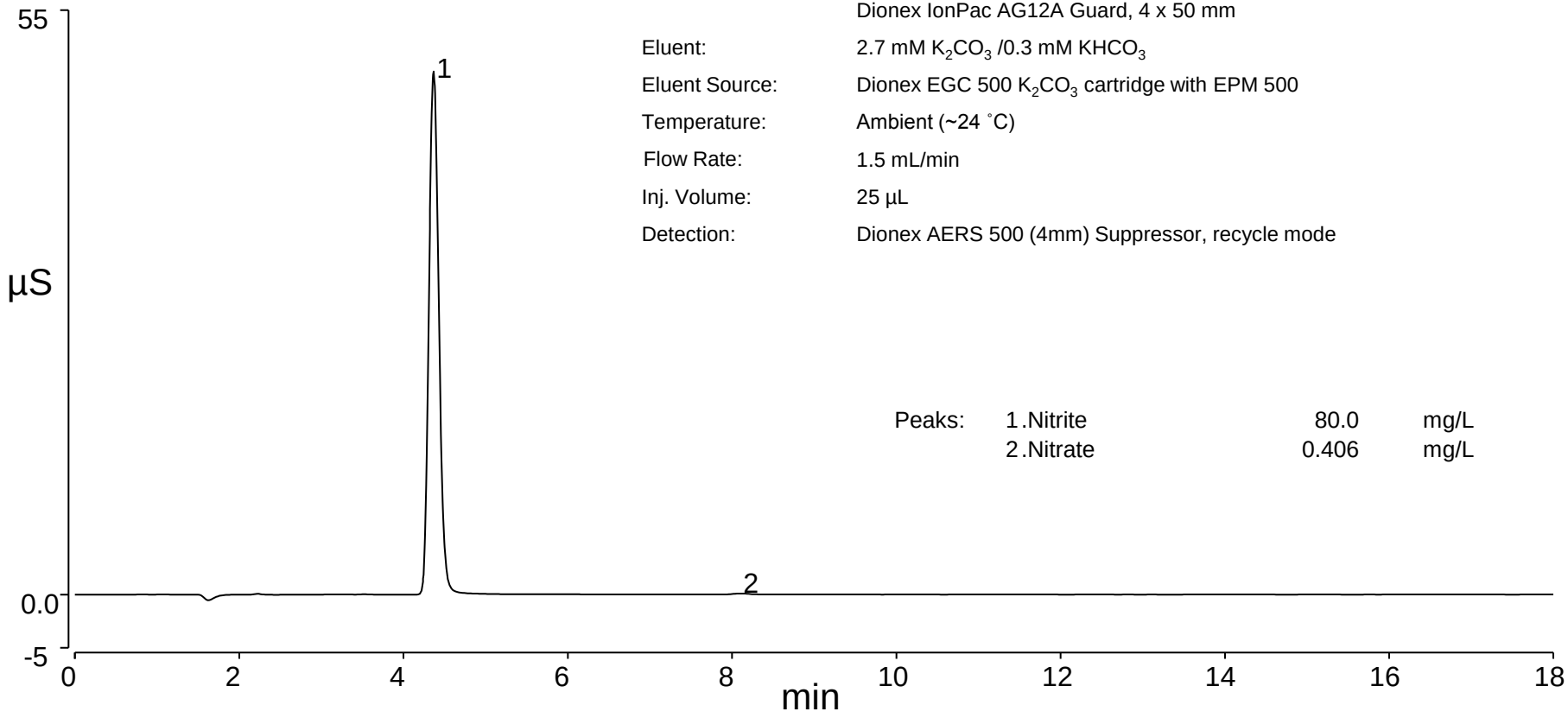
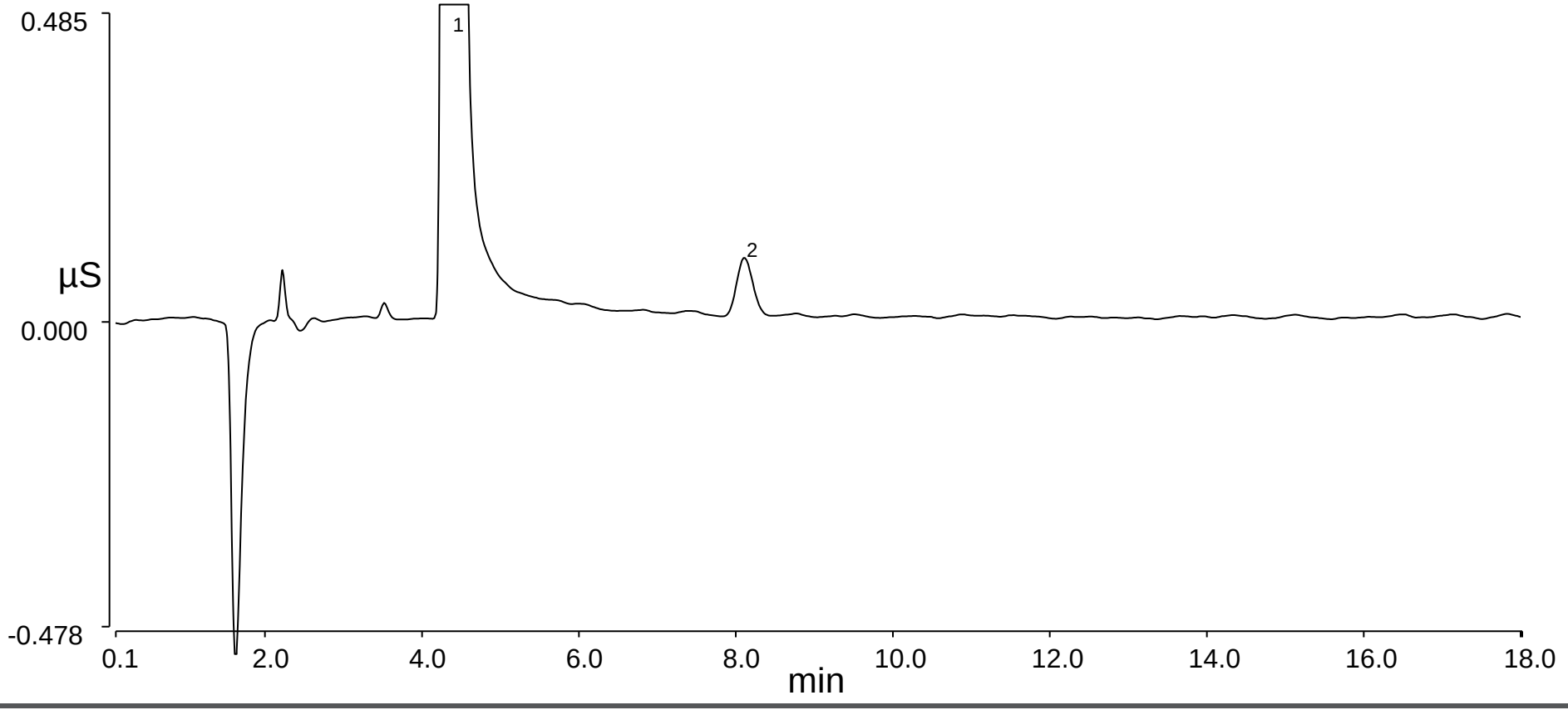
Sodium Nitrite Assay by the Proposed USP Monograph



Column:	Dionex IonPac AS12A Analytical, 4 x 250 mm Dionex IonPac AG12A Guard, 4 x 50 mm
Eluent:	2.7 mM K_2CO_3 /0.3 mM $KHCO_3$
Eluent Source:	Dionex EGC 500 K_2CO_3 cartridge with EPM 500
Temperature:	Ambient (~24 ˚C)
Flow Rate:	1.5 mL/min
Inj. Volume:	25 µL
Detection:	Dionex AERS 500 (4mm) Suppressor, recycle mode

Peaks:			
	1.Nitrite	80.0	mg/L
	2.Nitrate	0.406	mg/L

Enlarged to View the Nitrate Peak



Example #2

Cation IC Used for an Assay and Limit Test of a Drug Substance

Assay of Lithium in Lithium Hydroxide

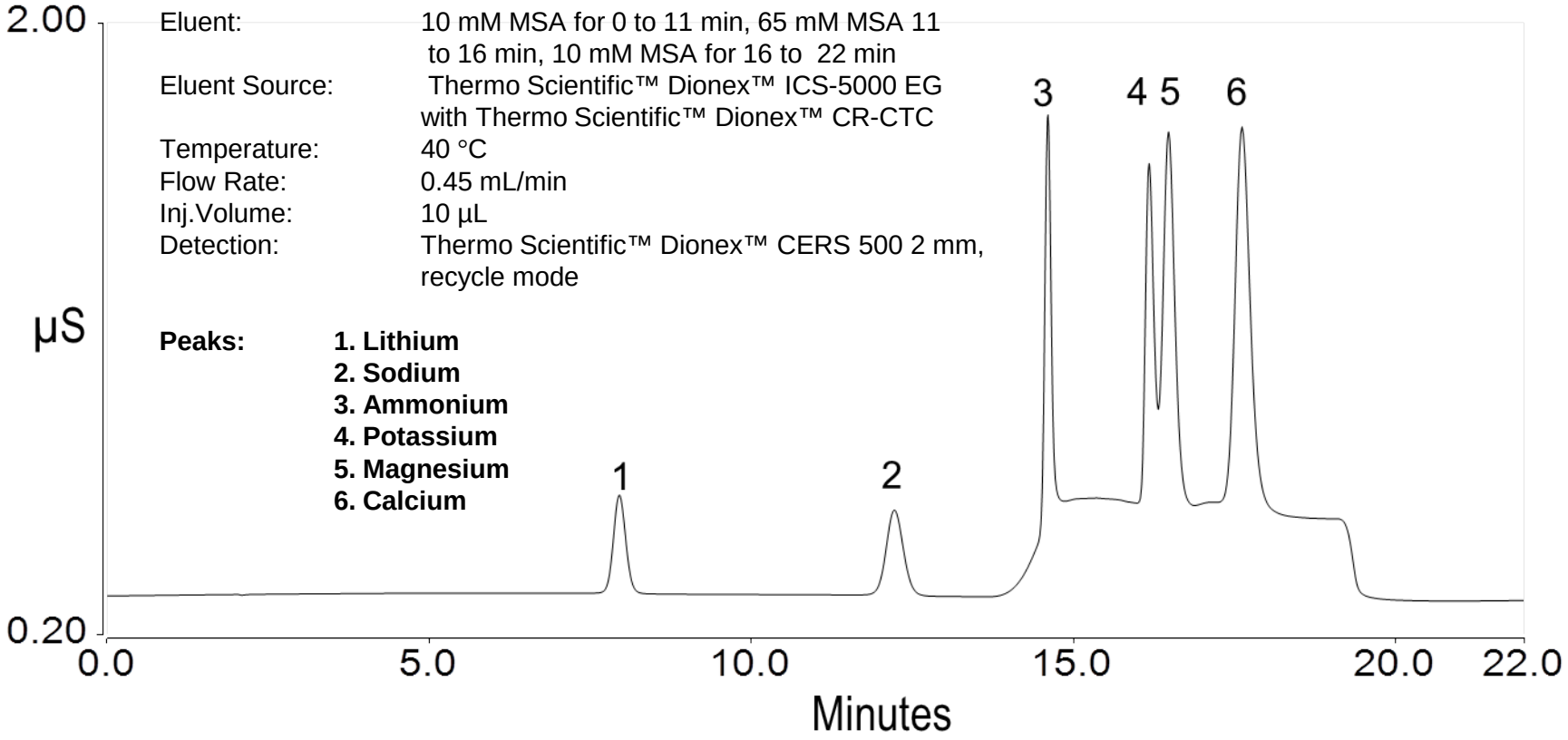
- The method to assay lithium was developed in our lab for a proposal to modernize the USP lithium hydroxide monograph
- The same method was developed to allow the measurement of calcium that is also required in the LiOH monograph
- Our work has been reported in Application Note 1144

Separation of Six Common Cations

Columns:	Thermo Scientific™ Dionex™ IonPac™ CS16, 3x 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
Temperature:	40 °C
Flow Rate:	0.45 mL/min
Inj.Volume:	10 µL
Detection:	Thermo Scientific™ Dionex™ CERS 500 2 mm, recycle mode

Peaks:

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



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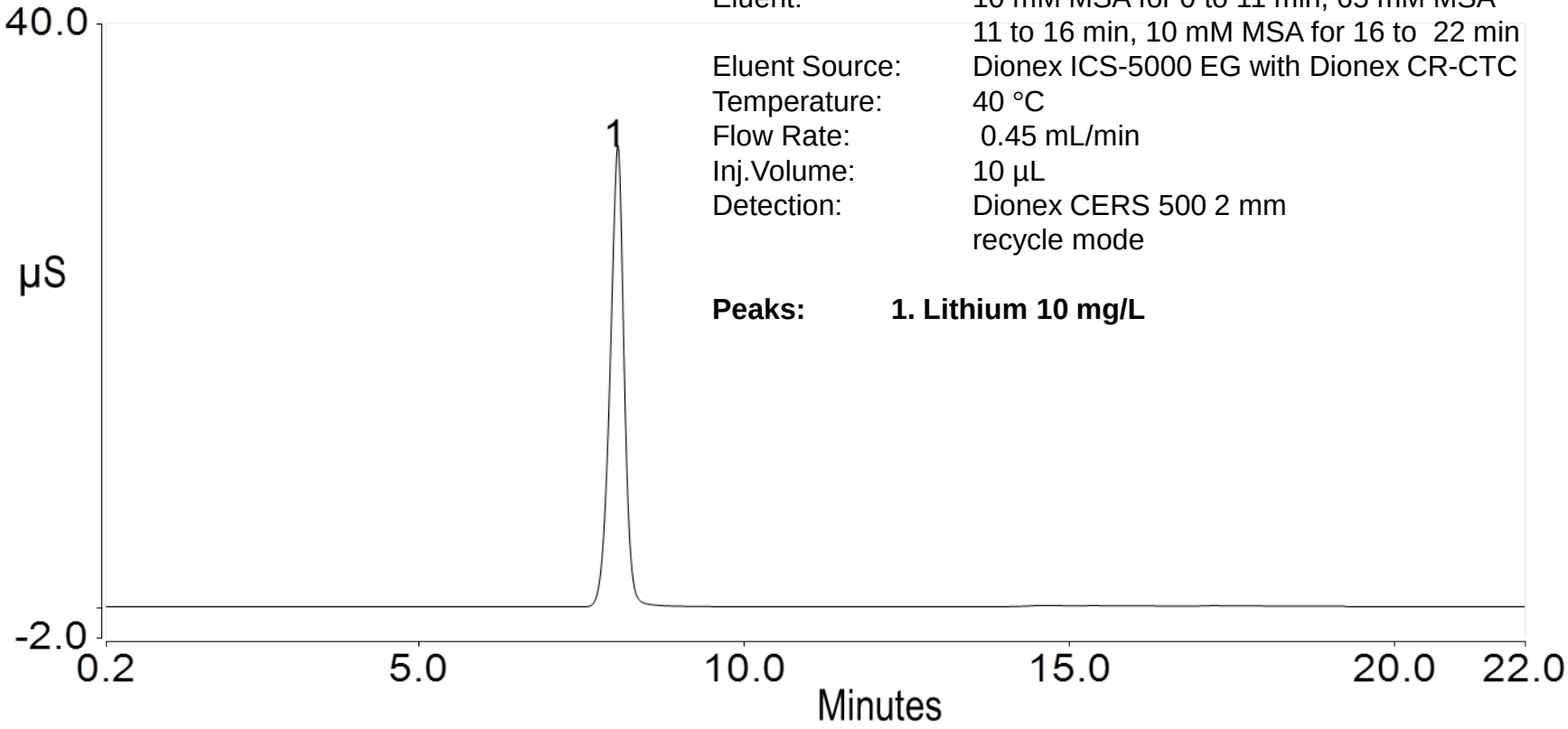
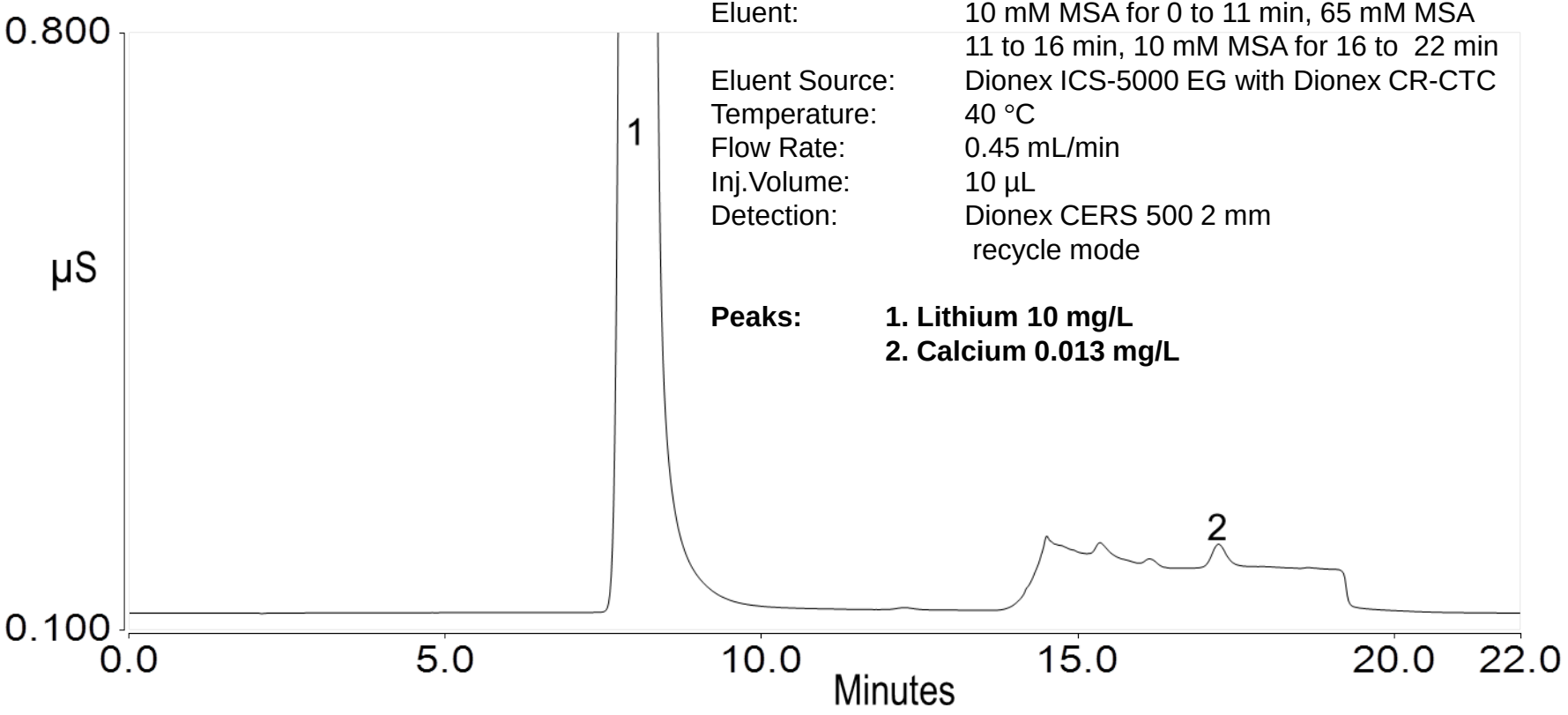
Determination of 10 mg/L Lithium in 10 mM Acetic Acid



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 2 mm recycle mode

Peaks: **1. Lithium 10 mg/L**

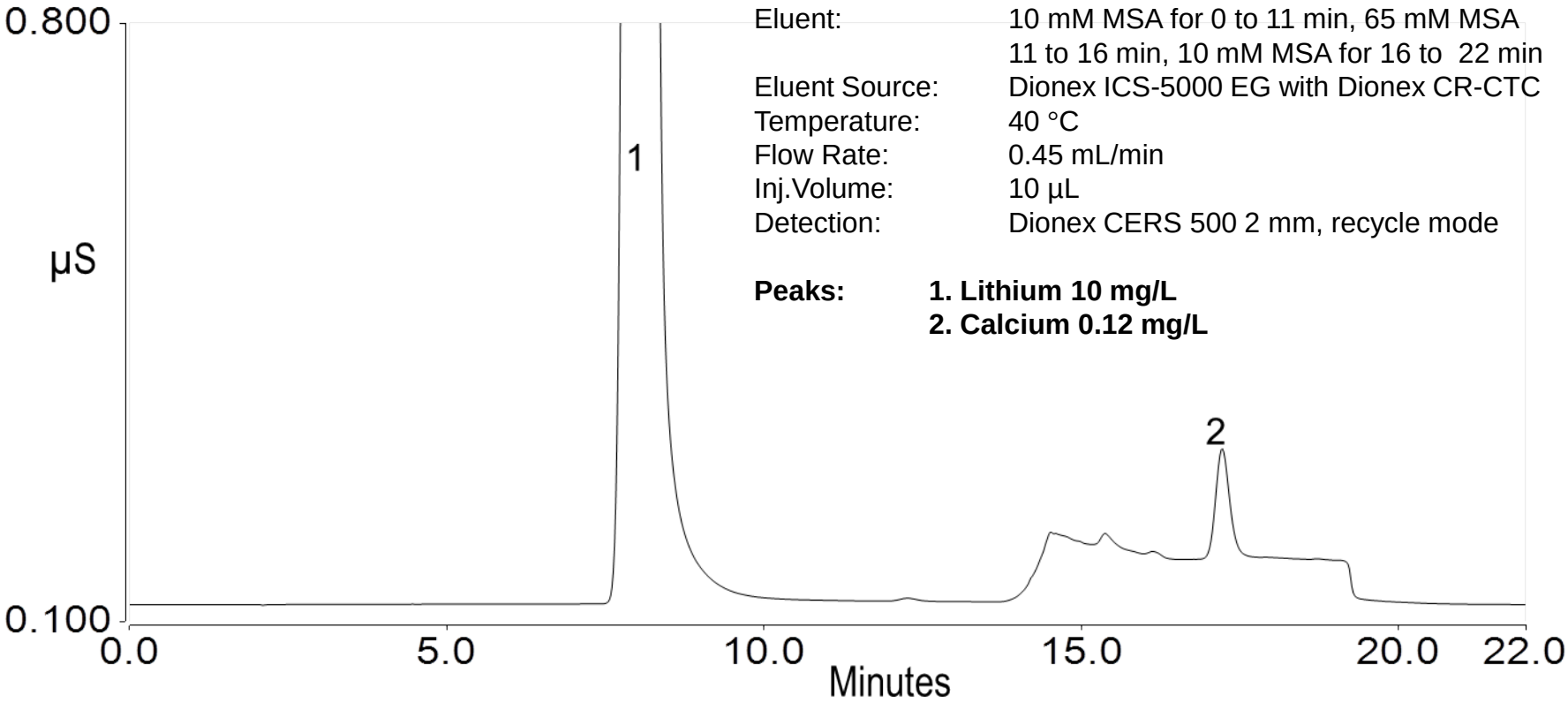
Enlarged to View the Calcium Peak



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 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



Columns:	Dionex IonPac CS16A, 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 2 mm, recycle mode

Peaks:
1. Lithium 10 mg/L
2. Calcium 0.12 mg/L

Developing an IC Method for Pharmaceutical Analysis

- Question 1: Has someone already done this or similar analysis?
  - Web/Literature search
  - Search AppsLab https://appslab.thermofisher.com/

- If yes, does it apply to my sample and can it be improved?
  - Check the column used and see if Thermo Fisher Scientific has a better column or a different format

- If no, Question 2: Is my analyte an anion, cation, carbohydrate, or other compound amenable to IC?

Developing an IC Method for Pharmaceutical Analysis

- Question 3: Are there compounds of the same charge in the sample?
- If yes, will need a column to resolve those compounds
- If yes, and the concentration of the other compounds are high relative to the analyte of interest, then a high-capacity column will be needed.
- Question 4: How can my analyte be detected &what sensitivity do I need?
  - Detection choice impacts the column and mobile phase chosen

Example

Designing an Assay for Kanamycin Sulfate and Amikacin

Developing an IC Method: Kanamycin B and Amikacin

- Question 1: Has someone already done this or similar analysis?
  - Yes – a USP-NF method – But let's pretend not
- Question 2: Is my analyte an anion, cation, carbohydrate, or other compound amenable to IC?
  - Carbohydrate and some aminoglycosides have been analyzed by HPAE-PAD
- Question 3: Are there compounds of the same charge in the sample?
  - Yes – there are 2 analytes, but one low and the other high

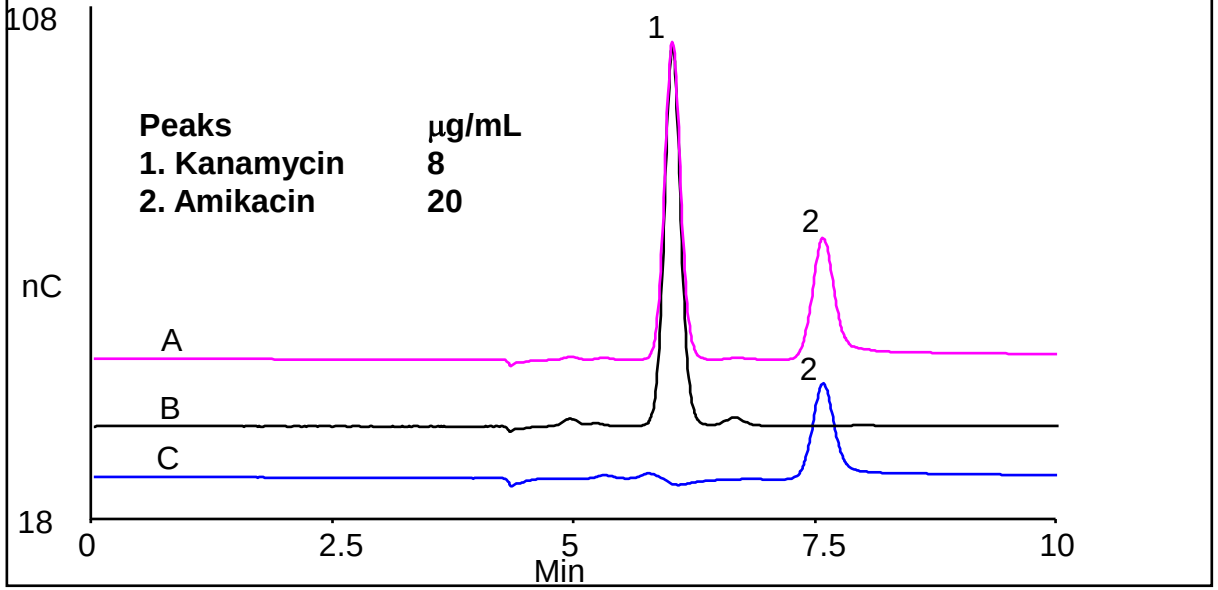
Developing an IC Method: Kanamycin B and Amikacin

- Question 4: How can my analyte be detected &what sensitivity do I need?
  - Detection by pulsed amperometry (PAD)
  - No requirement for the amount of the compound at lower concentration

- The solution:
  - As a carbohydrate – HPAE-PAD
  - Because they are even weaker anions than typical carbohydrates – a high pH and therefore high capacity column is required – CarboPac MA1

Separation of Amikacin and Kanamycin

Column:	Thermo Scientific™ Dionex ™CarboPac™ MA1, Analytical (4 x 250 mm)
	Thermo Scientific™ Dionex ™CarboPac MA1, Guard (4 x 50 mm)
Eluent:	115 mM NaOH
Flow Rate:	0.5 mL/min
Temperature:	30 °C
Inj. Volume:	20 µL (full loop)
Detection:	PAD (Au)



- This method used for assay for both compounds in drug substance and drug products – 7 monographs

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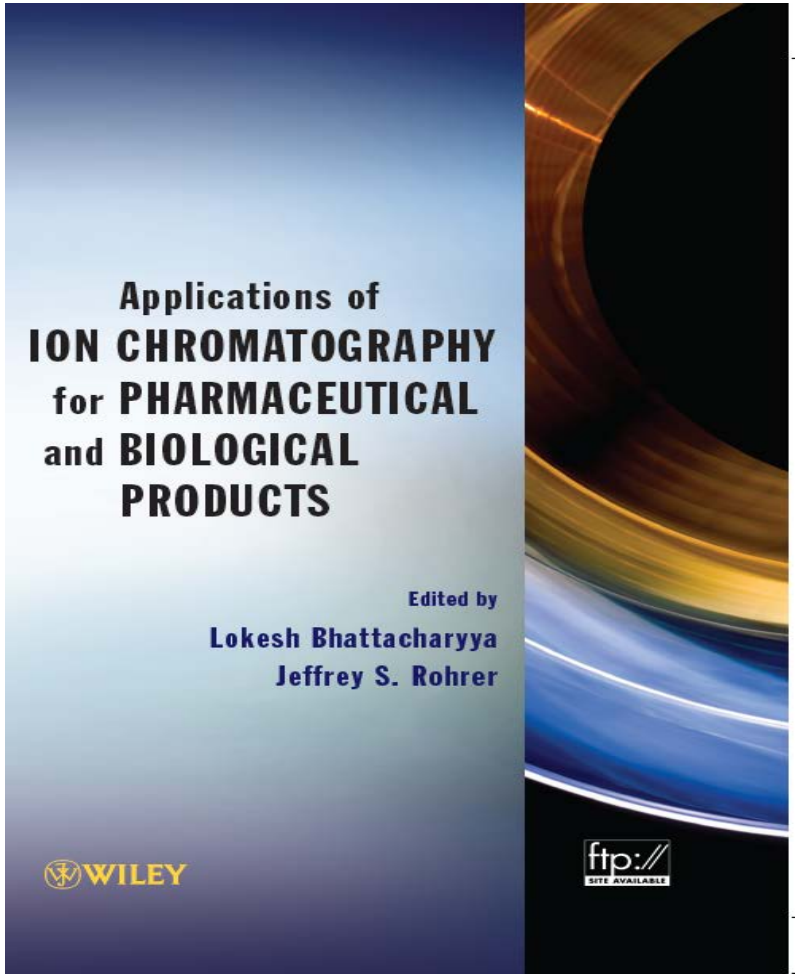
Conclusions

- 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

Acknowledgements

- Jingli Hu – Sodium Nitrite
- Sachin Patil – Lithium Hydroxide
- Lipika Basumallick – Kanamycin and Amikacin



Thank you for your attention!