



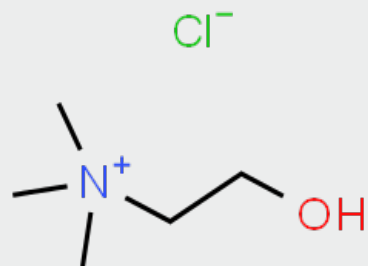
Quality Control of Choline as a Dietary Supplement by Liquid Chromatography Coupled to a Charged Aerosol Detector

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Pittcon March 1, 2020, Chicago, IL, USA

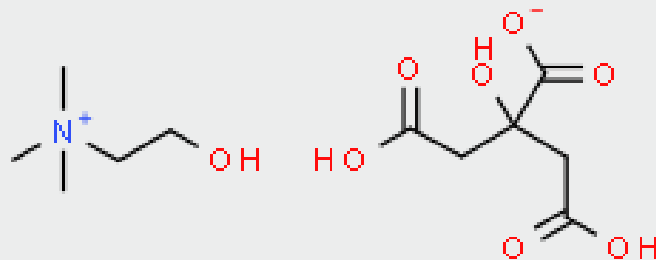
- Choline as dietary supplement
- Analytical considerations
- Mixed-mode chromatography with charged aerosol detection
- Results

- Essential nutrient involved in cell membrane integrity, lipid transport and metabolism, neurotransmission, etc.
- Food sources include animal-based products, beans and nuts
 - Free choline, phosphocholine and phosphatidylcholine (lecithin)
- Most people in US consume less than the amount considered to be necessary to ensure nutritional adequacy
- Use of dietary supplements is common
- Quality testing needed to ensure safety and effectiveness

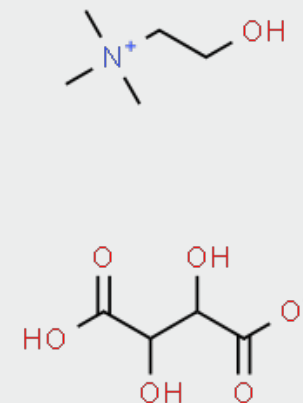
Choline – Analytical Considerations



Choline Chloride



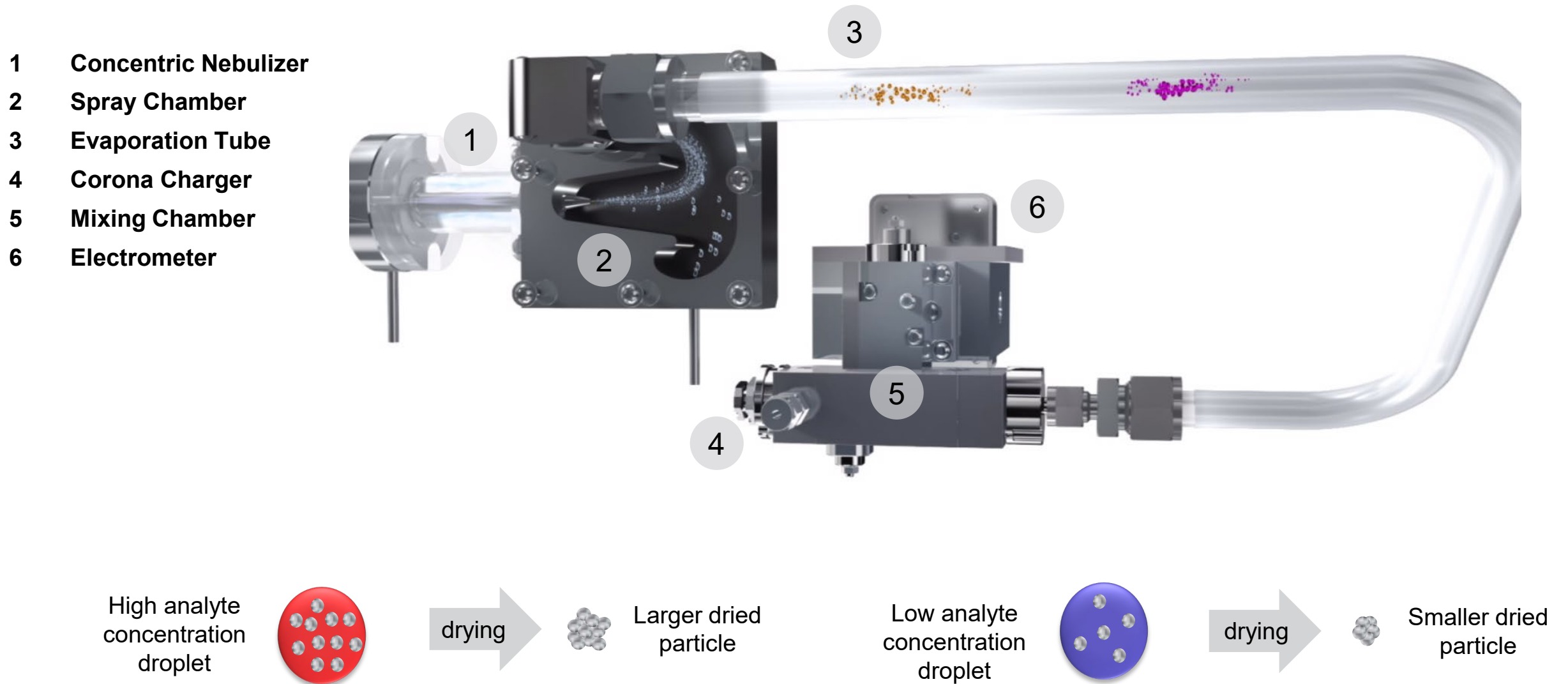
Choline Citrate



Choline Bitartrate

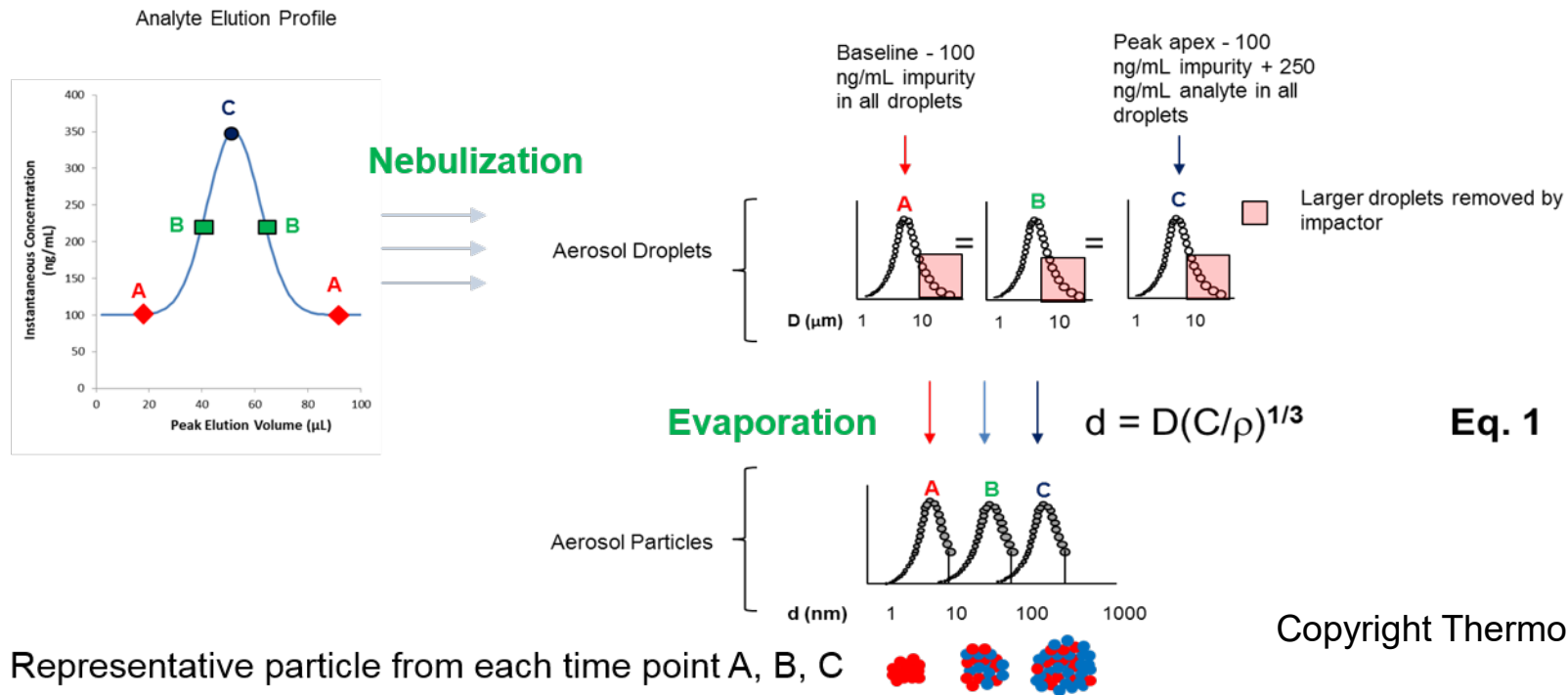
- Polar; cation and anions
- Weak or non-chromophores
- Goal = single method with direct detection (no derivatization)
 - Mixed mode (zwitterionic operated in HILIC mode)
 - Universal detection – Charged aerosol detector (CAD)
 - MS-compatible method

Charged Aerosol Detection



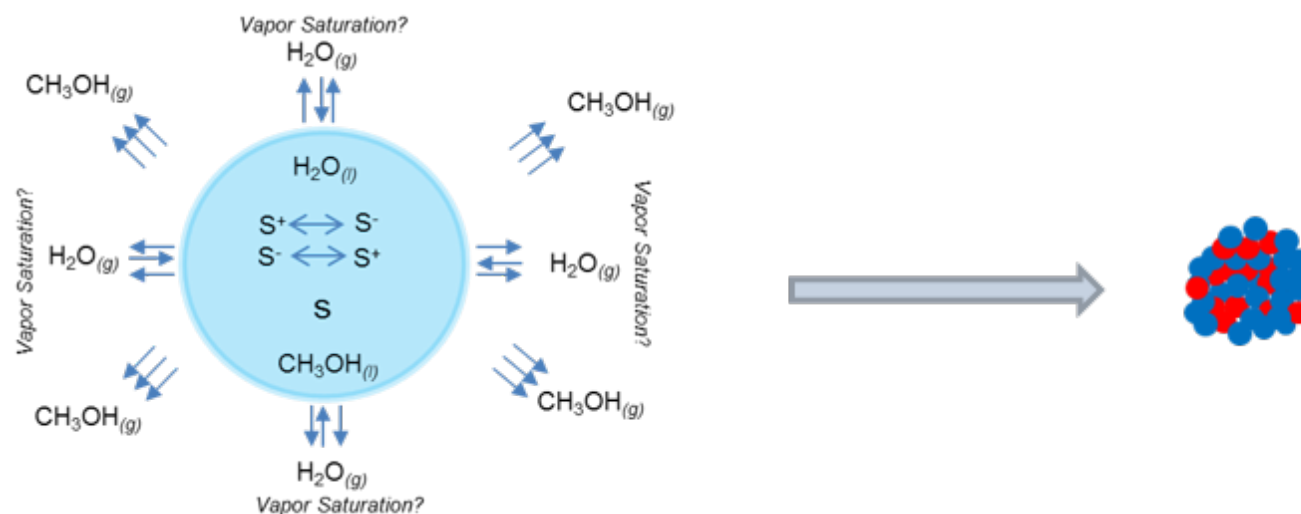
Spray Drying during Analyte Elution

- Droplet distribution ~ constant in isocratic conditions, changes during solvent gradients
- Diameter of each dried particle (d) depends on its initial droplet diameter (D) and its mass concentration (C) of non-volatile solute, corrected for 'bulk' density (ρ)
- Each dried particle consists of many molecules of: analyte (if present) + impurities



Ionized Analytes

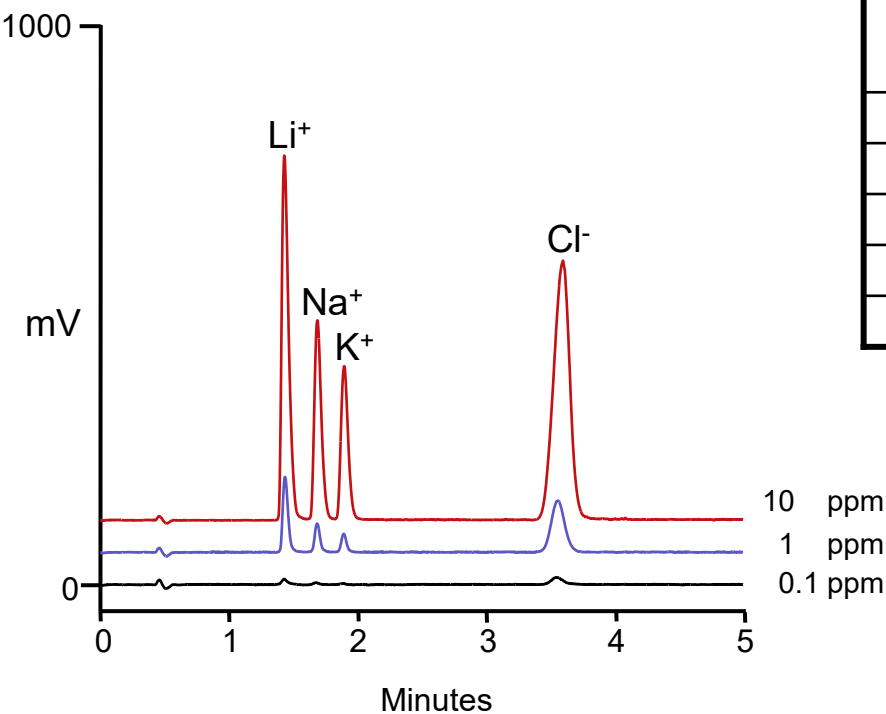
- Mobile Phase Additives (e.g., pH modifiers, buffers)
 - Ionic, typically at higher concentration than analytes
- Interactions between oppositely charged species are enhanced as droplets shrink during evaporation
- Ionic analytes exist as salt form within dried aerosol particles



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Charged Aerosol Detection

Column: Thermo Scientific
Acclaim Trinity P1, 3 µm
Dimensions: 3.0 x 50 mm
Mobile Phase: 60/15/25 v/v/v
CH₃CN
0.1 M NH₄OAc, pH 5.2
D.I. H₂O
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: Corona™ ultra CAD
(nebulizer – 35 °C;
nitrogen – 35 psi;
filter – off)
Sample: Chloride salts dissolved in
D.I. H₂O



Concentration (ppm)	Li ⁺ (S/N)	Na ⁺ (S/N)	K ⁺ (S/N)
10	672	367	470
1	201	75	49
0.1	15	5.5	3
LOD (S/N = 3)	0.1 ng	0.3 ng	0.5 ng
LOQ (S/N = 10)	0.33 ng	1.0 ng	1.7 ng

Typically sub-ng to low ng limits of detection

Instrument Method

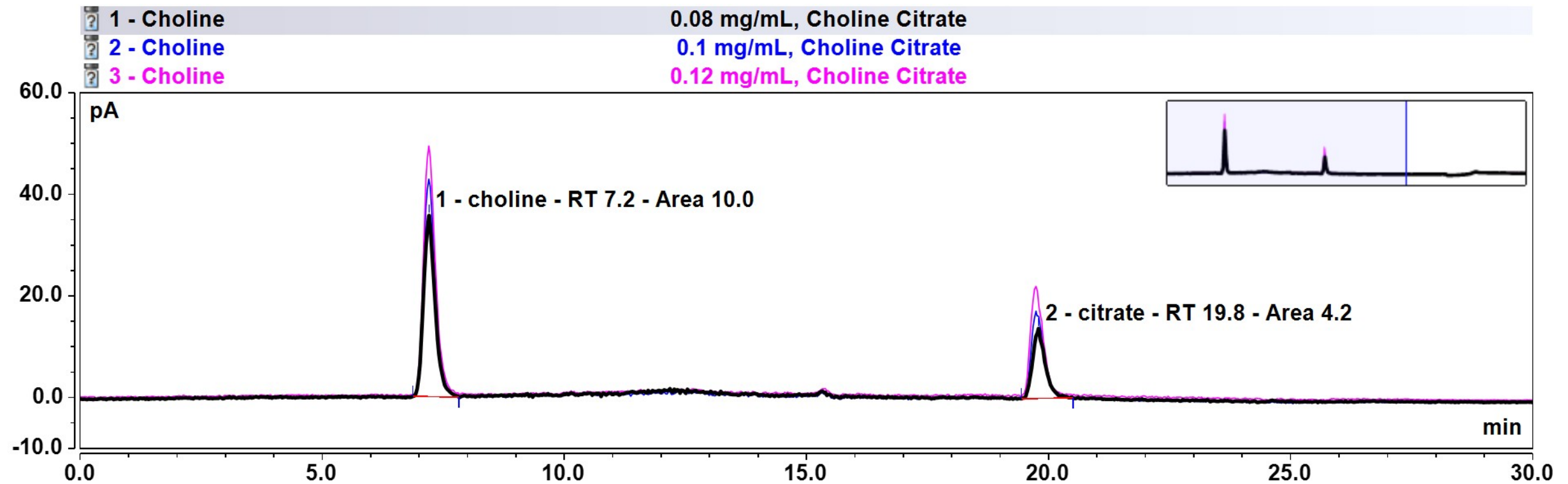
Parameter	Setting
Column	Zwitterionic HILIC*
Elution	Acetonitrile - aqueous ammonium acetate gradient*
Injection Volume	10 µL
Flow Rate	0.5 mL/min
Sample Diluent	Buffer and acetonitrile (30/70 v/v)
Column Temp.	30 °C still air mode, active preheater
Autosampler Temp.	10 °C
Detector	Vanquish Flex Charged Aerosol Detector (same as Corona Veo CAD)
	Filter Constant: 3.6 s Evaporator Temperature: 35 °C Data Collection Rate: 10 Hz Power Function Value: 1.00

** Details to be published in USP Pharmacopeial Forum (PF Online)*



Thermo Scientific™ Vanquish™
UHPLC System with CAD

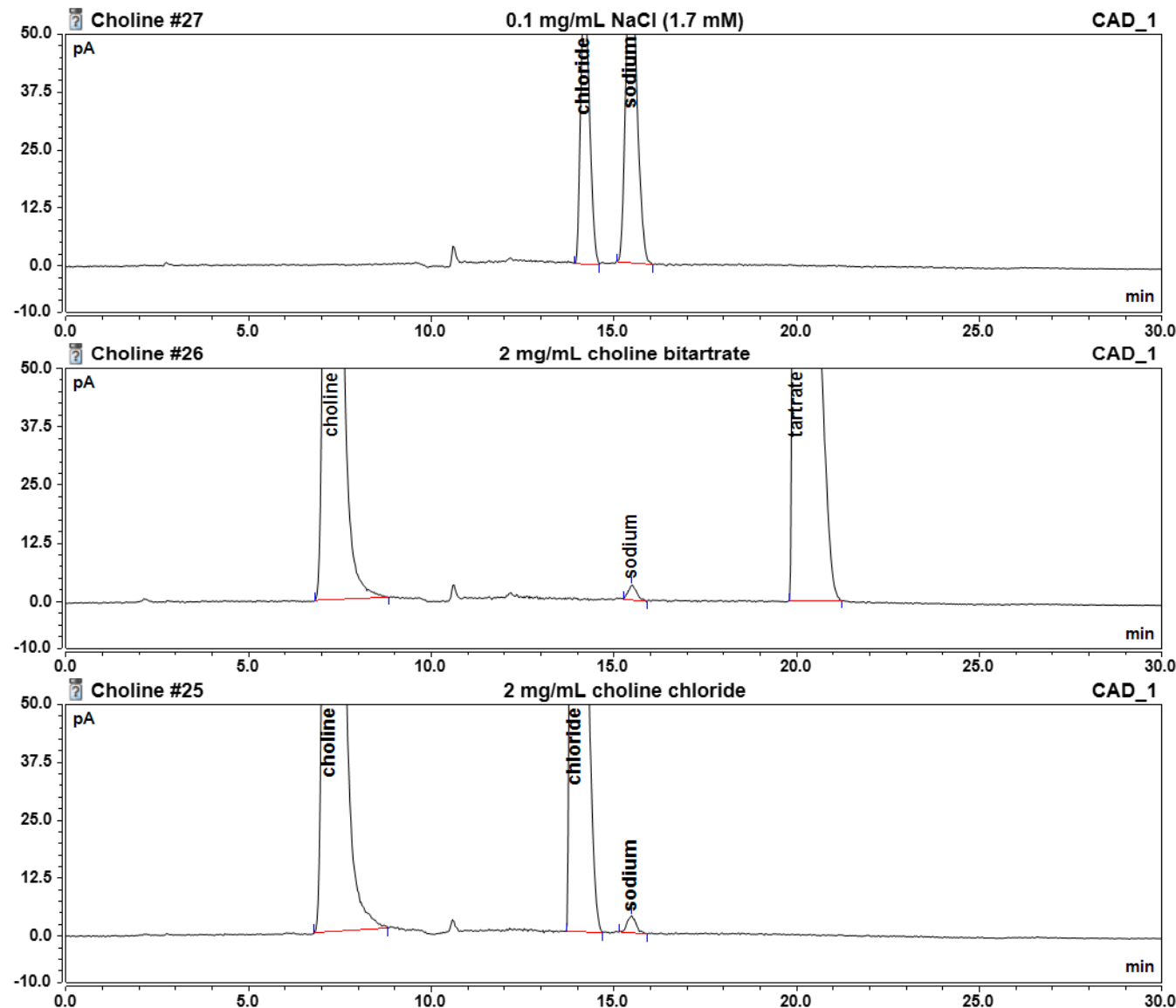
Choline Citrate



- Choline detected as acetate salt
- Citrate detected as ammonium salt

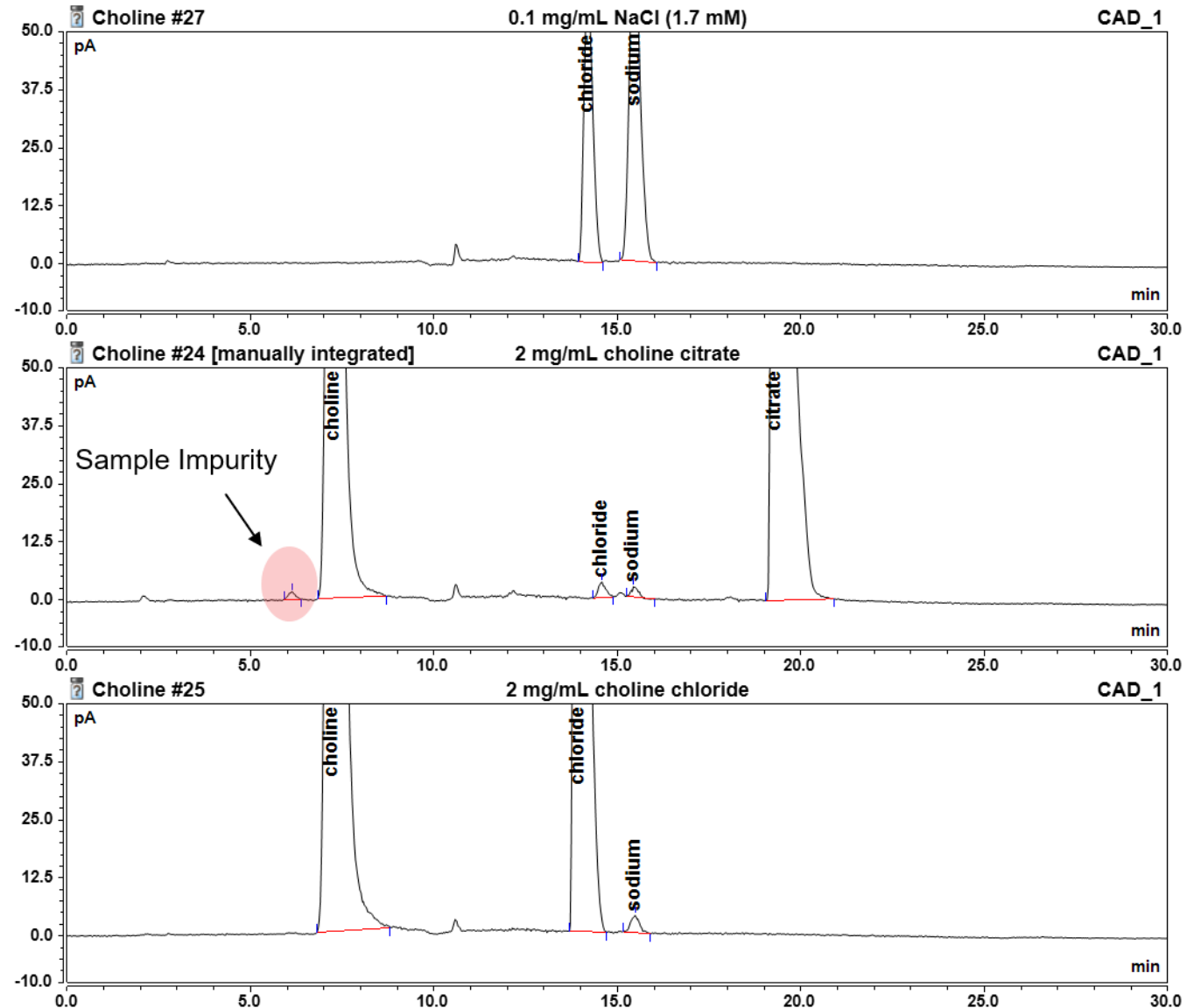
Impurities and Artifacts

- Sodium present in choline bitartrate and choline chloride samples
- Identified based on RT using standards
- Observed in blank when using glass sample vials
- Plastic containers to minimize
- Sodium detected as acetate salt
- Chloride detected as ammonium salt
 - Without ammonium additive, chloride is not detected by CAD (HCl is too volatile)

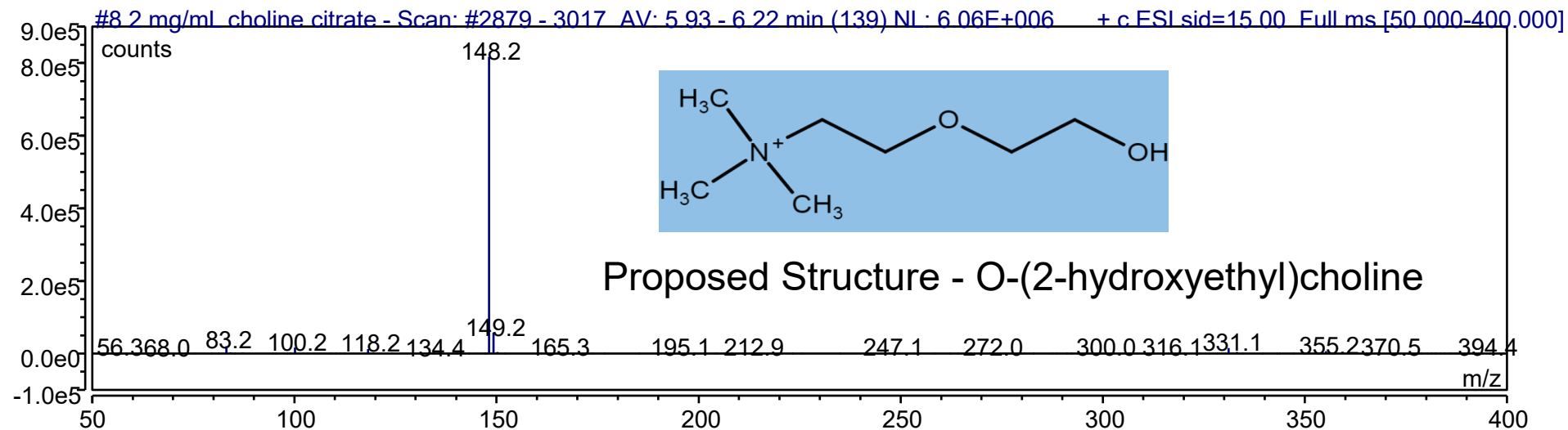
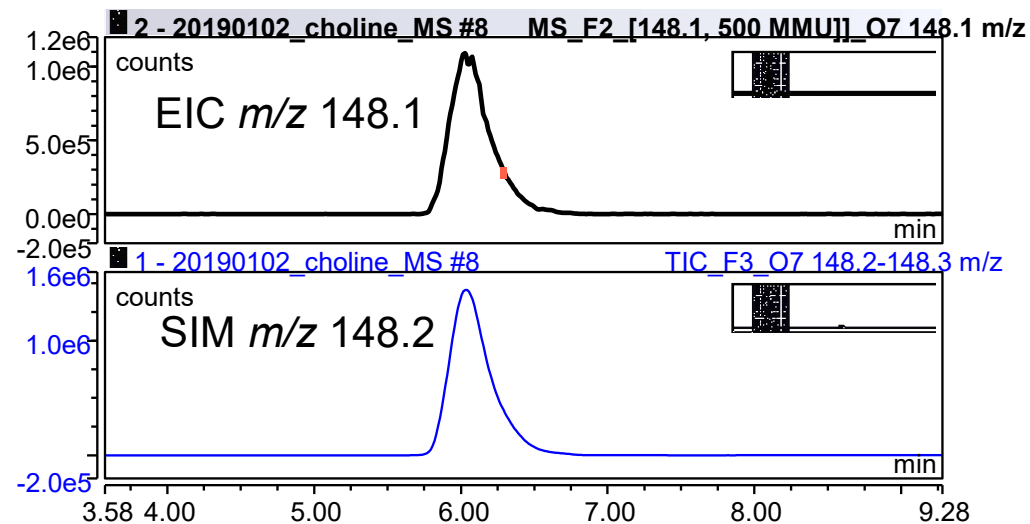
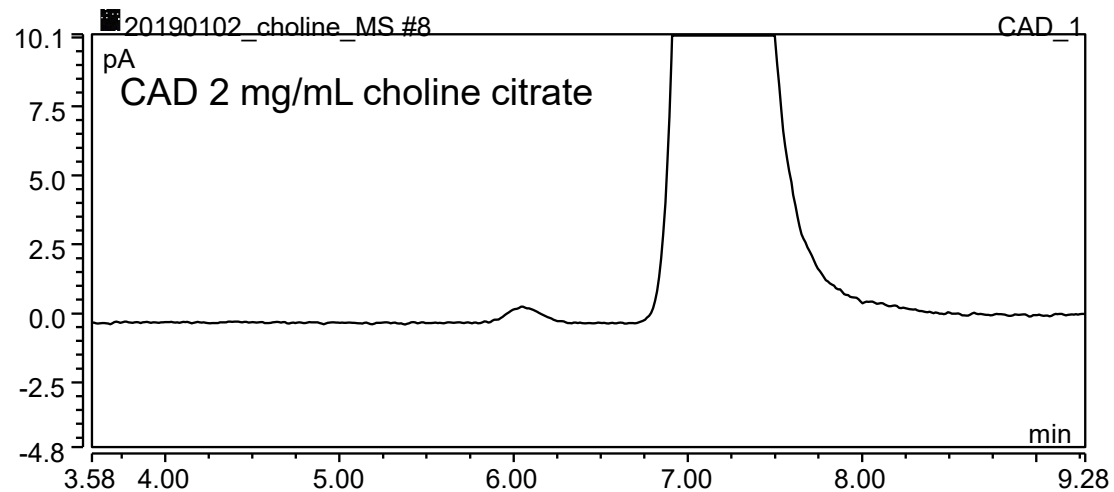


Impurities and Artifacts

- Sodium and chloride in choline citrate sample
- Sodium in choline chloride sample
- Sample impurity in choline citrate sample (RT ~ 6.0)
 - Studied using flow split to Thermo Scientific™ Vanquish™ ISQ EM Single Quadrupole Mass Spectrometer



MS Data for Choline Citrate Sample Impurity RT ~ 6 min



Choline Citrate Validation Summary

Choline Citrate

Parameter	Choline - Day 1, 2	Citrate - Day 1, 2
Precision 0.1 mg/mL (% RSD, n = 6)	1.83, 1.23	1.33, 1.76
Linearity (r^2) (0.08, 0.1 and 0.12 mg/mL)	0.99997, 0.99784	0.99778, 0.99918
Recovery (%) at level:		
80%	98.3, 100.2	94.2, 105.5
100%	100.8, 99.4	98.1, 102.2
120%	99.1, 96.7	99.8, 98.7

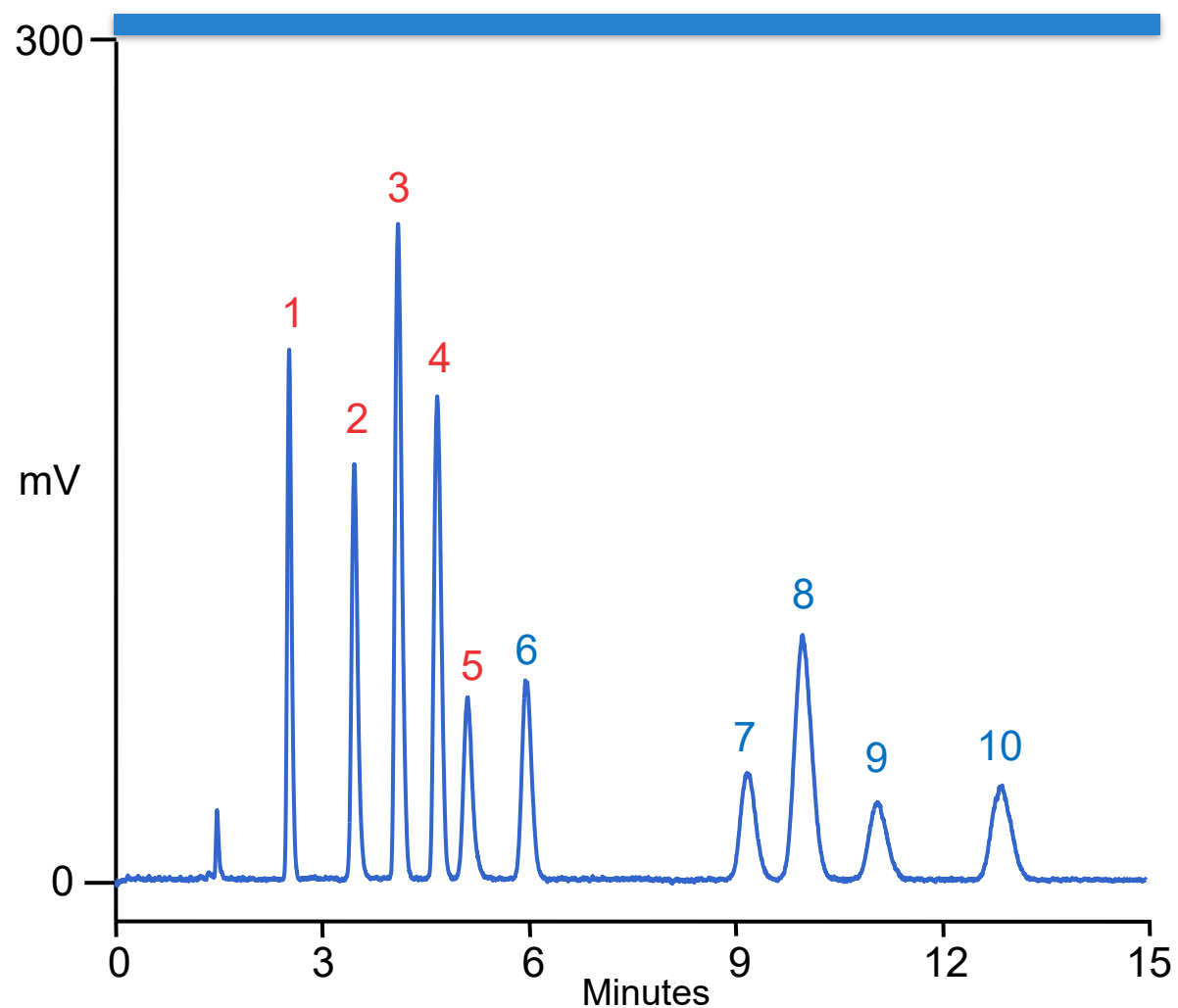
- Mixed mode chromatography with CAD allows simultaneous analysis of anions and cations
- Analytes detected as salts formed with mobile phase additives
- Low or sub-ng on-column limits of detection
- Directly compatible with MS, which facilitated impurity identification
- Zwitterionic HILIC method was applied to choline citrate, choline chloride and choline bitartrate samples
- Acceptable precision, linearity and recovery obtained



Thank you

ThermoFisher
S C I E N T I F I C

Pharmaceutical-related Anions and Cations – Mixed Mode Separation



Column: Thermo Scientific™ Acclaim™ Trinity P1, 3 μ m
Dimensions: 3.0 \times 100 mm
Mobile Phase: 60/40 v/v CH₃CN/20 mM (total) NH₄OAc, pH5
Temperature: 30 $^{\circ}$ C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 μ L
Detection: Corona® *ultra*™ (Gain = 100 pA; Filter = med; Neb Temp = 30 $^{\circ}$ C)

Peaks: (50 to 100 ppm)
1. Choline
2. Tromethamine
3. Sodium
4. Potassium
5. Meglumine
6. Mesylate
7. Nitrate
8. Chloride
9. Bromide
10. Iodide

PP73732-EN 0720S