



Determination of Choline in Infant Formula and Other Food Samples by Ion Chromatography

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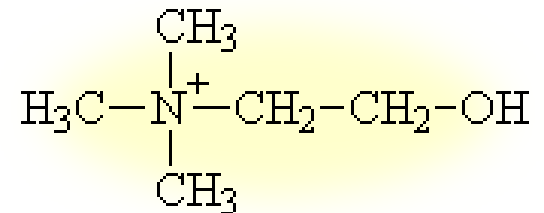
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Function of Choline

- Water-soluble essential micronutrient
- Maintenance of cell membrane integrity
- Support of methyl group metabolism
- Support of nervous system activity
- Lipid transport and metabolism
- Cell signaling



Choline in Foods

Choline in Infant Formula

- Critical in brain development of both fetus and infants
- Actively transported from maternal blood to milk
- U. S. Food and Drug Administration (FDA) regulation requires a minimum of 7.0 mg/100 Cal of choline in infant formula.



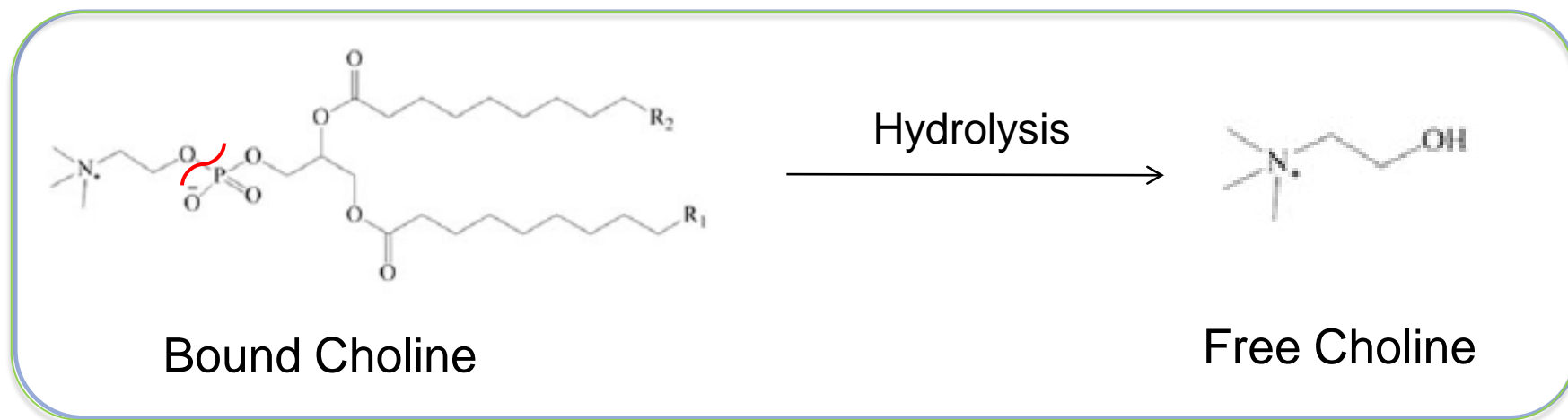
Source of Choline in Other Foods

- Meats (especially liver), fish, whole eggs, dairy products, whole grains, wheat germ, peanuts, soybeans, and vegetables such as Brussels sprouts and broccoli



Total Choline

- Choline is present in foods as free choline and in esterified form such as phosphocholine (Pcho), phosphatidylcholine (PtdCho), glycerophosphocholine (GPCCho), and sphingomyelin (SM).
- Total Choline = Free Choline + Bound Choline
- Acidic hydrolysis releases esterified forms of choline to give free choline.

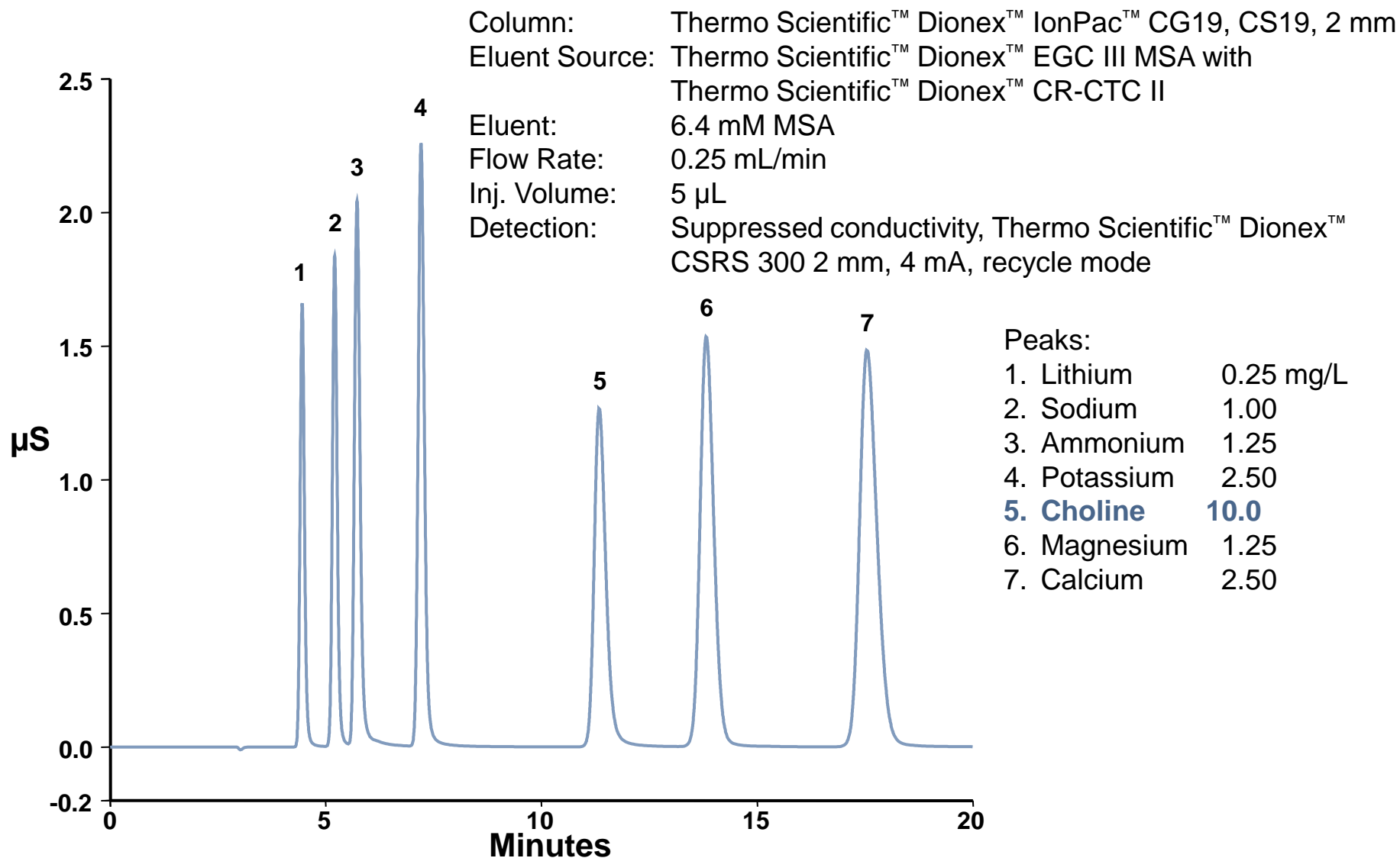


- Total choline is measured.

Existing Methods-Determination of Total Choline

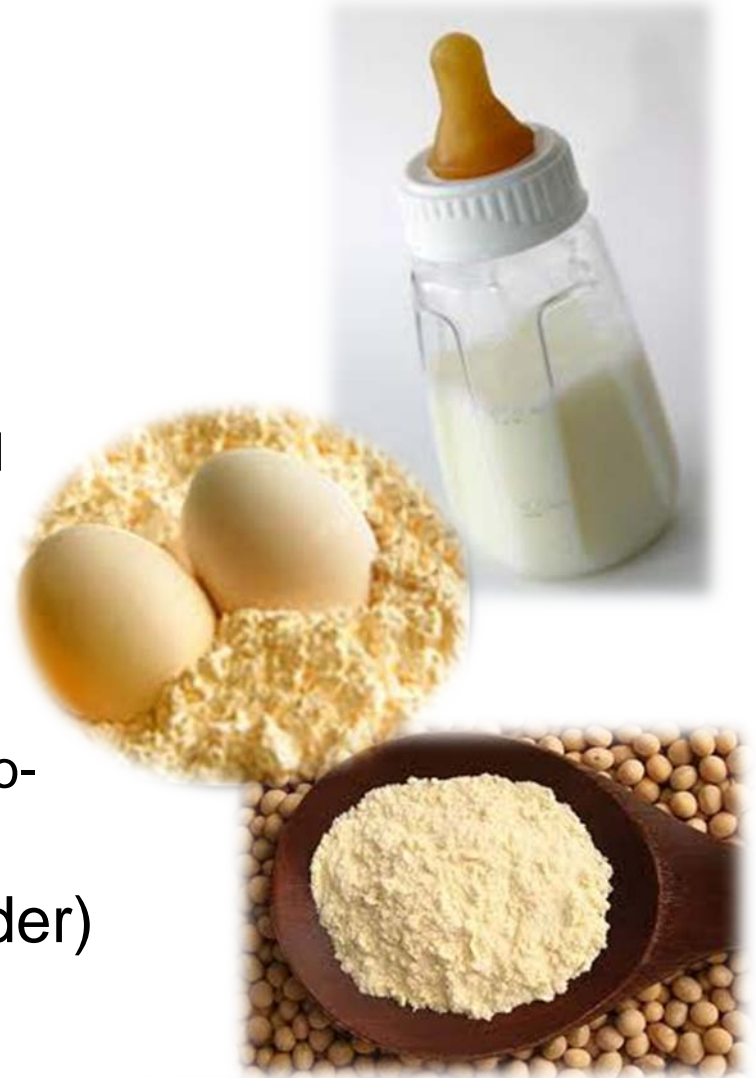
- Extraction-based approaches
 - Isolate the choline esters and quantify each individually.
- Hydrolysis-based approaches
 - Hydrolysis of esters to the free form
 - Classical method—precipitation of choline as a Reinecke salt in a colorimetric reaction (absorption at 520 nm)
 - Acid hydrolysis-enzymatic colorimetric assay (AOAC Official Method 999.14, 1999)
 - Other
 - Headspace GC/FID, headspace GC/MS, acid hydrolysis-enzymatic hydrolysis-LC/IDMS, basic hydrolysis-CE/UV, acid hydrolysis-electrochemical biosensor
 - Acid hydrolysis—ion chromatography (IC) with suppressed conductivity detection

Separation of 10 mg/L of Choline Standard and Six Common Cations



Samples Tested

- NIST SRM 1849a (Infant/Adult Nutritional Formula)
- Infant Formula
 - Infant Formula 1—Milk-Based Powder
 - Infant Formula 2—Soy-Based Powder
 - Infant Formula 3—Hydrolyzed Milk-Based Powder
- Adult Nutritionals
 - Adult Nutritional 1—Powder
 - Adult Nutritional 2—High-Protein Ready-to-Feed (RTF)
- NIST SRM 1845a (Whole Egg Powder)
- NIST SRM 3234 (Soy Flour)



Sample Preparation

Infant Formula, Egg Powder, and Soy Flour

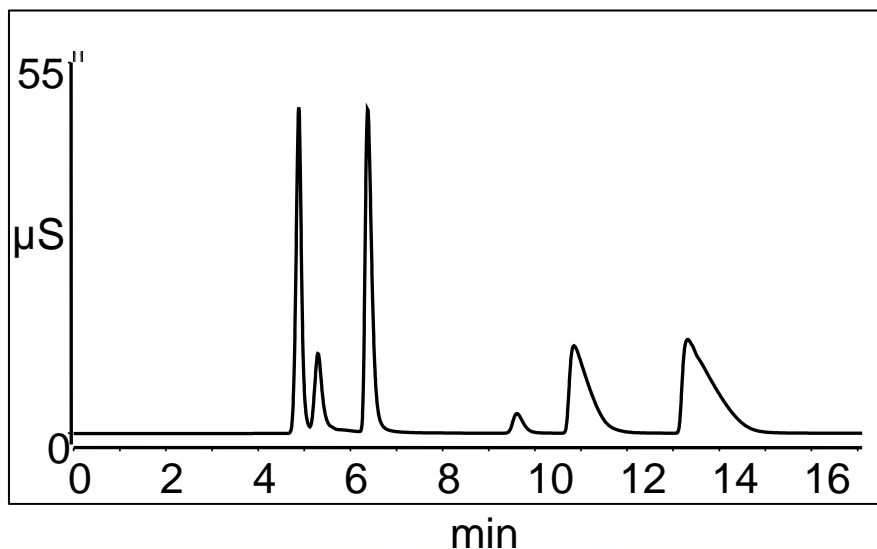
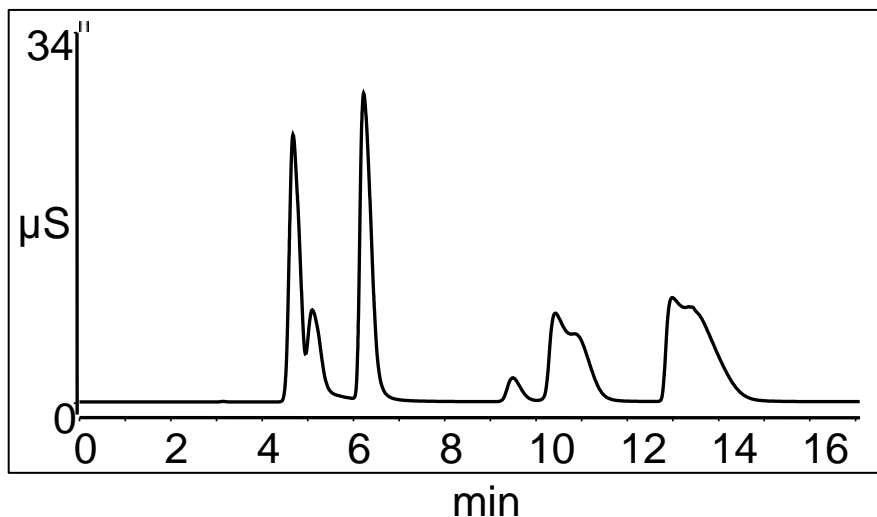
- 1) Dissolve 5 g of powder in 30 mL of 1 M HCl
- 2) Acid hydrolysis: **70 °C water bath for 3 hours**
- 3) Filter the hydrolysate through 0.2 µm PES syringe filter
- 4) Dilute the filtrate with DI water and inject

Infant Formulas and Adult Nutritional

- 1) Dissolve 2.5 g of powder in 15 mL of 1.5 M HCl
- 2) Acid hydrolysis: **microwave at 100 °C for 20–30 min**
- 3) Filter the hydrolysate through a 0.2 µm PES syringe filter.
- 4) Dilute the filtrate and then treat with the Thermo Scientific™ Dionex™ OnGuard™ II A Cartridge

Sample Preparation—Dionex OnGuard II A Treatment

- The cation-exchange sites on the Dionex IonPac CS19 column are protonated at low pH, resulting in a loss in peak efficiency.
- To reduce the excess amount of H^+ in the samples and obtain improved peak efficiency:
 - Treat the samples with proper dilution and/or
 - Treat the samples with a **Dionex OnGuard II A Cartridge** (pH is adjusted to approximately pH 6, particularly suitable for low-choline samples)



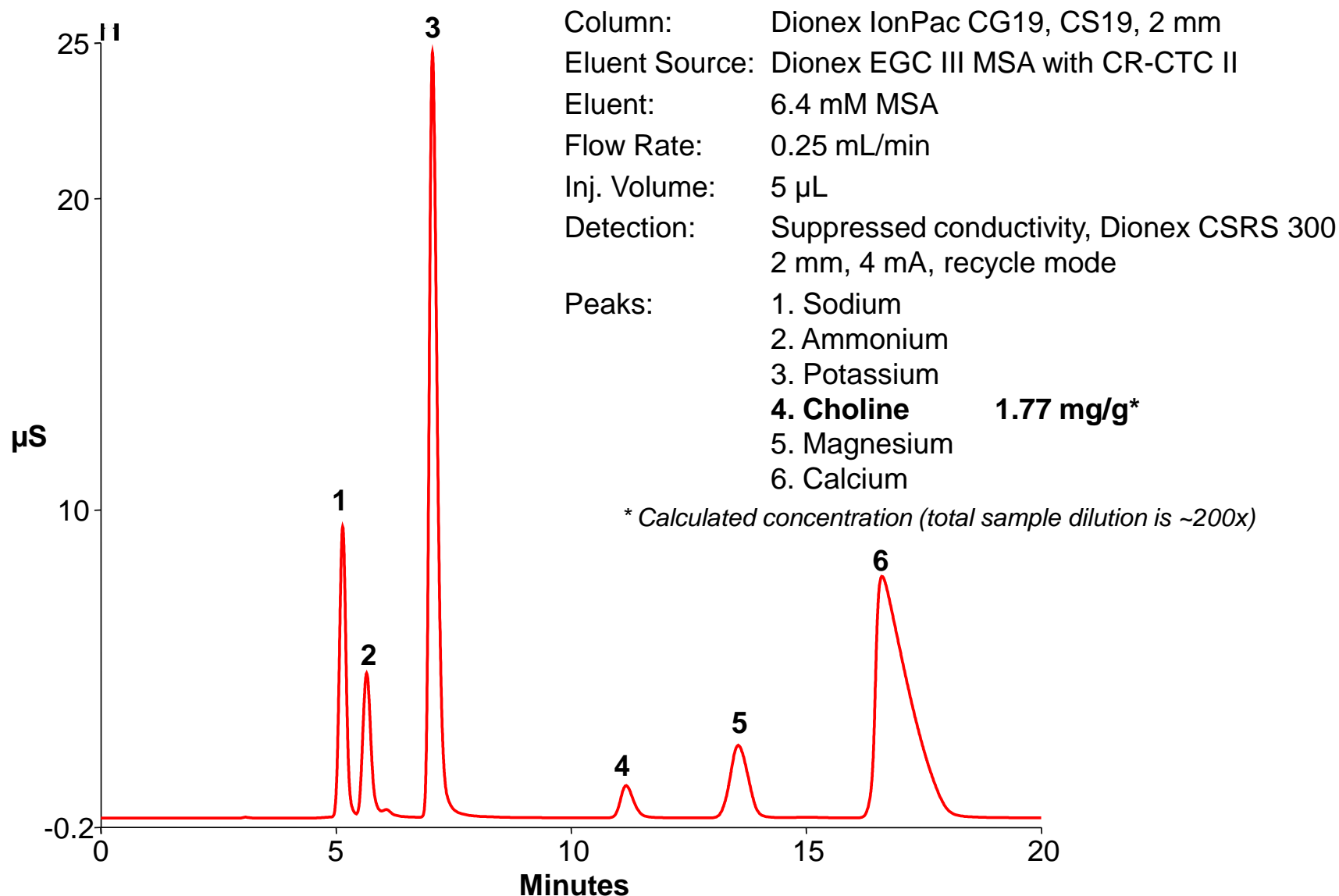
Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Analyte	Range (mg/L)	Linearity (r^2)	LOD* (mg/L)	LOQ** (mg/L)
Choline	0.06–75	0.9998	.003	.009

* LOD = 3 S/N

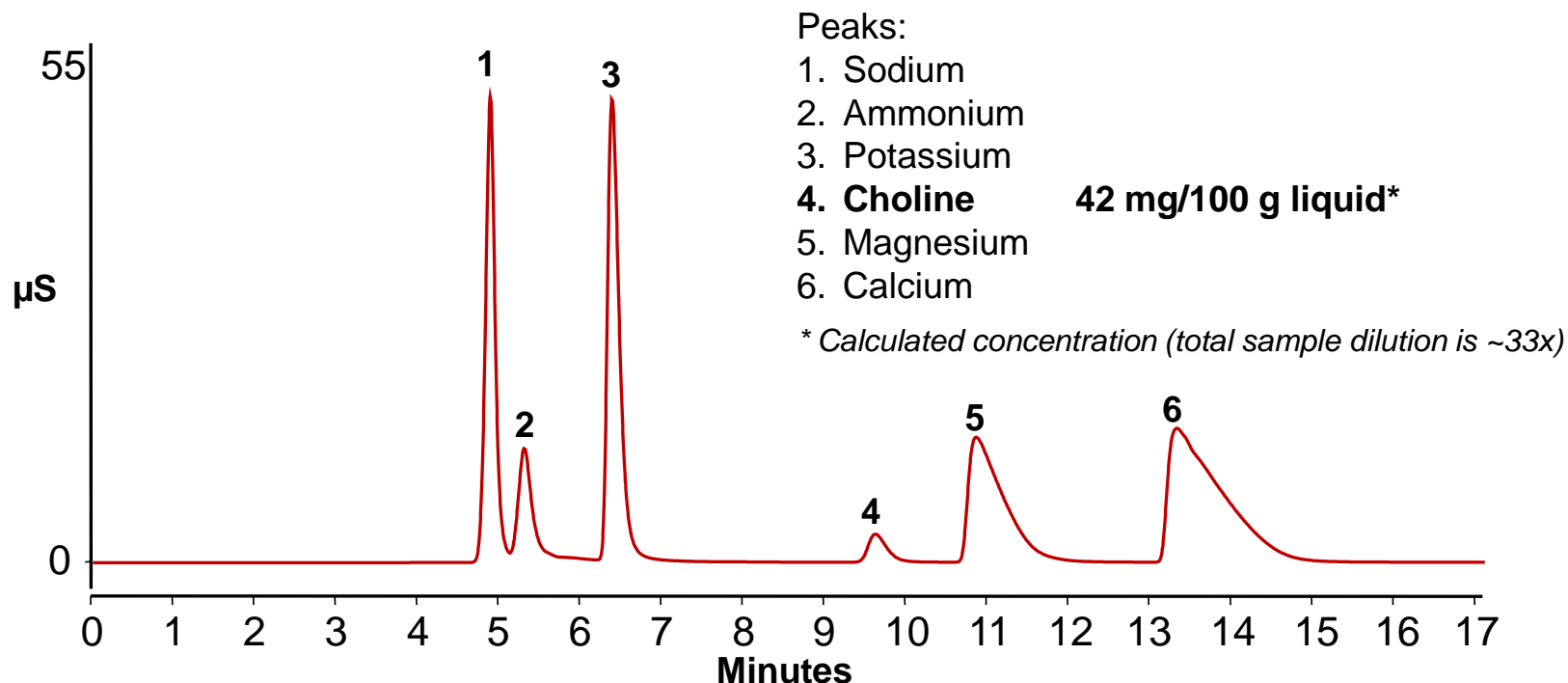
** LOQ = 10 S/N

Determination of Choline in Infant Formula

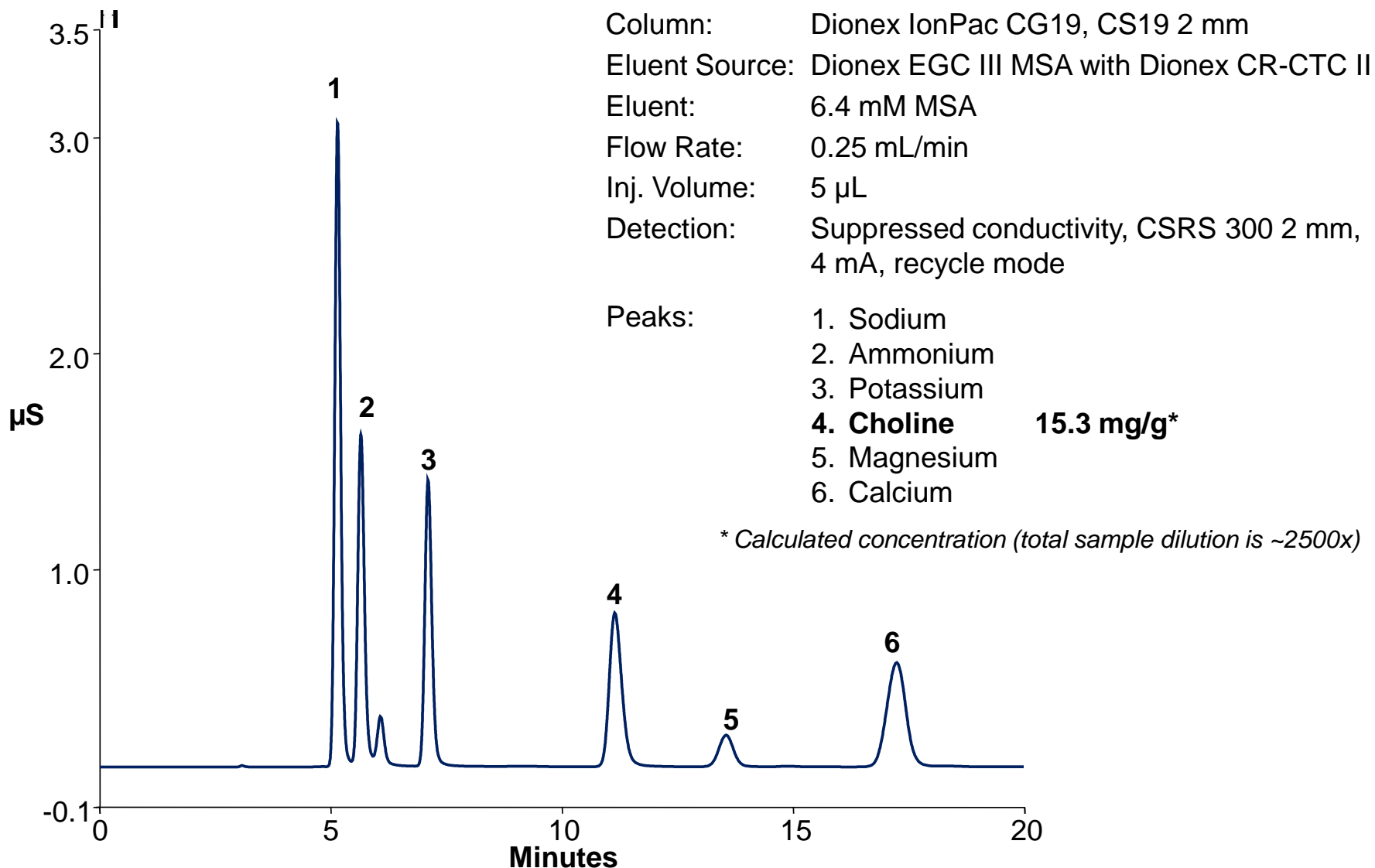


Determination of Choline in Adult Nutritional

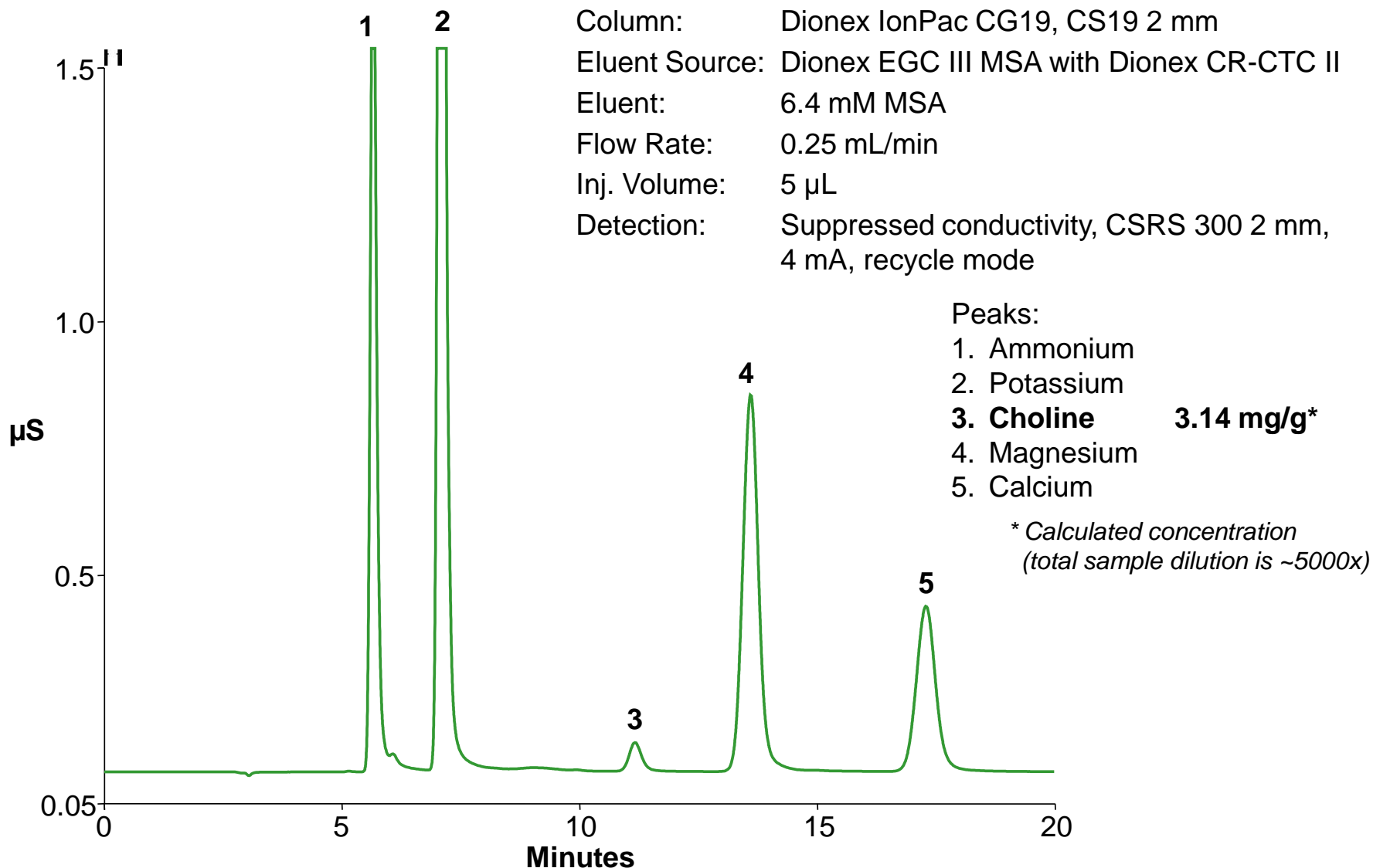
Column: Dionex IonPac CG19, CS19, 2 mm
Eluent: -5–13 min 6.4 mM MSA, 13 min step to 25 mM MSA, 13–17 min 25 mM
Eluent Source: Dionex EGC III MSA with Dionex CR-CTC II
Flow Rate: 0.25 mL/min
Inj. Volume: 5 μ L
Temperature: 30 $^{\circ}$ C
Detection: Dionex CSRS 300, 2 mm, recycle mode, -5–13 min 5 mA, 13.1–17 min 19 mA
Sample: Adult nutritional treated with a Dionex OnGuard II A Cartridge



Determination of Choline in Whole Egg Powder



Determination of Choline in Soy Flour



Method Validation

Analysis of National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) Samples

Sample	Average Sample Concentration (mg/g)	RSD	Choline Content Determined by NIST* (mg/g)	% Recovery
Infant Formula ¹	1.04	0.02	1.09	95
Whole Egg Powder ²	15.3	0.92	14.1	108
Soy Flour ²	3.13	0.57	2.49	126

* Choline contents in SRM 3234 Soy Flour samples and SRM 1845a Whole Egg Powder provided by NIST as a result of participation in DSQAP exercises

¹ Average of nine sample preparations

² Average of three sample preparations

Choline Recoveries from Different Sample Matrices

Sample Name	Amount Found (mg/L)	Spiked Amount % Native Amount	Recovery (%)
Infant Formula, Milk-Based Powder	14.9	50	102
		100	100
Adult Nutritional, Powder	12.2	50	98.3
		100	97.5
Adult Nutritional, High Protein, RTF	7.9	50	97.5
		100	98.7
Whole Egg Powder	6.2	100	98.3
		120	92.1
Soy Flour	0.6	100	98.2
		110	98.1

Method Precisions

Sample	Day	Intraday Peak Area RSD	Between-Day Peak Area RSD
NIST 1849a SRM	1	1.0	1.9
	2	2.7	
	3	0.2	
Infant Formula, Soy-Based	1	2.3	2.5
	2	1.0	
	3	2.3	
Infant Formula, Milk-Based	1	4.9	3.2
	2	1.1	
	3	0.9	
Infant Formula, Hydrolyzed	1	0.4	1.5
	2	1.7	
	3	1.2	
Adult Nutritional, Powder	1	0.6	0.7
	2	1.0	
	3	0.3	
Adult Nutritional, High-Protein, RTF	1	1.4	1.2
	2	1.2	
	3	0.8	

Samples were prepared in triplicate over three days.

Summary

- High-performance cation-exchange chromatography separates choline and other cations with excellent peak shape and efficiency, allowing simultaneous determination of choline and other cations present in the samples.
- Suppressed conductivity detection allows simple and robust determination of choline in different food samples with high sensitivity.
- This IC method is applicable for the determination of choline in a variety of complex matrices with excellent accuracy and precision:
 - Infant Formula
 - Adult Nutritional
 - Whole Egg Powder
 - Soy Flour

Thank you for your attention!