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

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Overview

Purpose: To develop an ion chromatography (IC) method for the determination of choline in infant formula, egg powder, and soy flour.

Method: Release choline from its esterified forms using acid hydrolysis and separate choline from common cations on a high-performance cation-exchange column using a Thermo Scientific™ Dionex™ ICS-5000 Reagent-Free™ IC (RFIC™) system with suppressed conductivity detection.

Results: This method provides a sensitive, selective, and reproducible alternative to AOAC Method 999.14 for choline determination in food samples.

Introduction

Choline is a water-soluble micronutrient vital to cell membrane integrity, support of methyl group metabolism, and nervous system activity.[1] It plays an important role during the perinatal period. It is transported from maternal blood into breast milk to assist in growth and brain development of breast-fed children.[1] In recognition of its nutritional importance, choline is a required additive in many infant formulas. It is also present as free choline in small quantities in a variety of foods and is frequently found in its esterified forms.[2] AOAC Method 999.14 describes the determination of choline in infant formula and milk using an enzymatic colorimetric method.[3,4] As a quaternary amine, choline can be separated from other mineral cations present in the samples on a cation-exchange column and detected by suppressed conductivity.[5,6] An RFIC system enables on-line generation of high-purity eluent, greatly enhancing the level of automation. The method not only proves to be a sensitive technique for choline determination, but also allows simultaneous determination of mineral cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) in the samples.

Methods

Sample Preparation

1.For Infant Formula and Adult Nutritional Powder: Weigh 2.5 g of powder in a microwave vessel and add 15 mL of 1.5 M HCl.

For Infant Formula and Adult Nutritional Ready-to-Feed (RTF): Weigh 8.5 g of the RTF liquid in a microwave vessel and add 8.5 g of 3.0 M HCl.

For Egg Powder and Soy Flour: Weigh 200 mg of whole egg powder or 400 mg of defatted soy flour into a 15 mL conical plastic tube with a cap. Add 10 mL of 1 M hydrochloric acid, cap, and mix by shaking until well dispersed.

2.Microwave the sample 20–30 min at 100 ° C (infant formula and adult nutritionals) or place in a 70 ° C water bath for 3 h (egg powder and soy flour).

3.Filter the hydrolysate through a 0.2 µm PES syringe filter.

4.Dilute the sample and inject (egg powder and soy flour) or dilute the sample and then treat with the Thermo Scientific™ Dionex™ OnGuard™ II A Cartridge (infant formulas and adult nutritionals).

Samples Tested

NIST SRM 1849a

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 RTF

NIST Egg Powder

NIST Soy Flour

Liquid Chromatography

Dionex ICS-5000 RFIC system, including:

- DP Dual Pump
- EG Eluent Generator
- DC Detector/Chromatography Compartment
- AS-AP Autosampler

Columns: Thermo Scientific™ Dionex™ IonPac™ CG19 Guard, 2 × 50 mm
Dionex IonPac CS19 Analytical, 2 × 250 mm

Eluent: 6.4 mM Methanesulfonic acid (MSA)

Eluent Source/Cartridge: Thermo Scientific™ Dionex™ EGC III MSA Eluent Generator with Thermo Scientific™ Dionex™ CR-CTC II Continuously-Regenerated Cation Trap Column

Flow Rate: 0.25 mL/min

Inj. Volume: 5 µL

Temperature: 30 °C

Detection: Suppressed conductivity with Thermo Scientific™ Dionex™ CSRS™ 300 (2 mm) Cation Self-Regenerating Suppressor 4 mA, recycle mode

Background Conductance: ~ 0.1 µS

Noise: ~ 0.1 nS/min peak-to-peak

System Backpressure: ~ 2350 psi

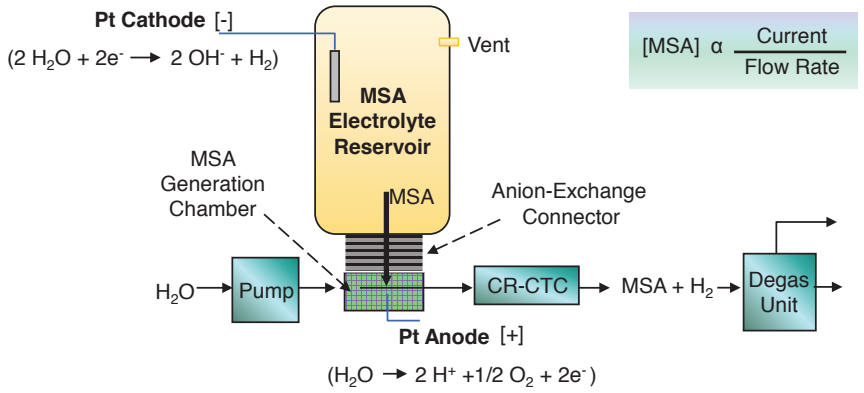
Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ 7.1 Chromatography Data System (CDS) software was used for chromatographic data collection and processing.

Electrolytical Generation of Eluent

Eluents were generated using a Dionex EGC III MSA Cartridge (Figure 1). This module automatically generates high-purity MSA eluent on line utilizing only DI water as the carrier, eliminating the need to manually prepare the MSA eluent. The Chromeleon™ CDS software will track the amount of MSA used and calculate the remaining lifetime.

FIGURE 1. Eluent generation using RFIC technology.



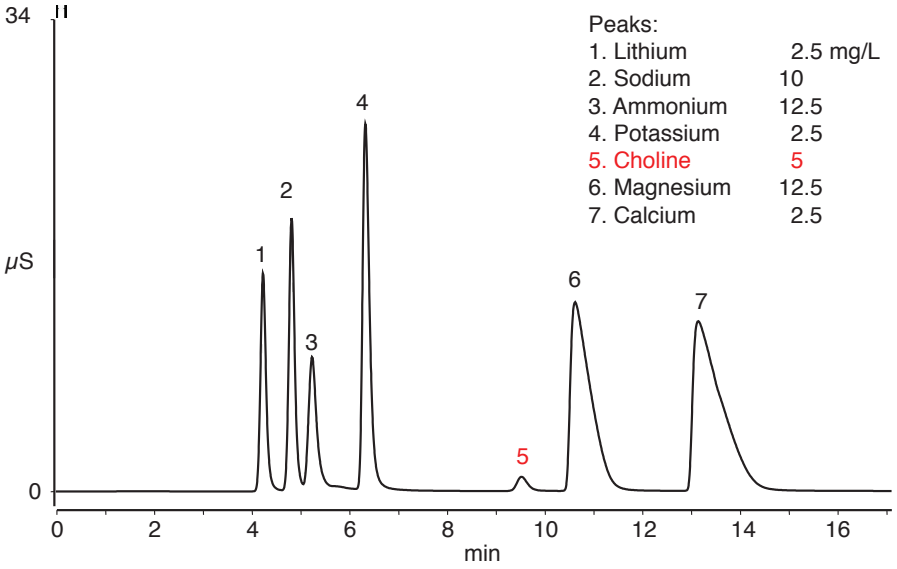
Degassed DI water is delivered to the eluent generator by the pump. Current is applied to the electrodes to generate MSA eluent. The eluent is then passed through the Dionex CR-CTC II column to remove any extraneous cationic contaminants from the degas unit prior to use. The CDS software precisely controls the current to accurately and reproducibly generate MSA at the desired concentrations.

Results

Separation

The Dionex IonPac CS19 column offers a selectivity that is optimized for small hydrophilic amines. Choline, ammonium, and group I and II cations are separated and eluted with excellent peak efficiencies and symmetries with a simple isocratic eluent.

FIGURE 2. Separation of choline and six common cations.



Dionex OnGuard II A Cartridge Sample Treatment

- Because the cation-exchange sites on the Dionex IonPac CS19 column protonate at low pH, there is a loss of column capacity causing a loss of peak efficiency.
- By treating the sample with a Dionex OnGuard II A Cartridge, the pH is adjusted to approximately pH 6 and improved peak efficiencies are obtained.

FIGURE 3. Peak efficiencies with and without Dionex OnGuard II A treatment.

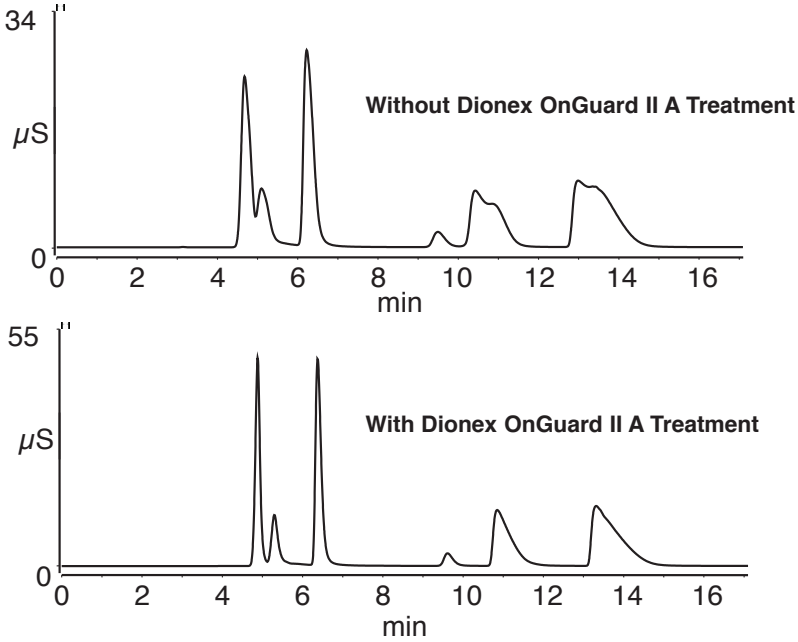


TABLE 1. Summary of linearity, limit of detection (LOD), and limit of quantitation (LOQ).

Analyte	Range (mg/L)	Linearity (r^2)	LOD* (mg/L)	LOQ** (mg/L)
Choline	0.06–75	0.9998	0.003	0.009

* LOD = 3 S/N
** LOQ = 10 S/N

Sample Analysis

The method was validated for choline by determining the intra- and interday precision and the recovery, which was determined from spiking known concentrations of choline in different samples at varied concentrations. To determine the recovery, appropriate amounts of choline standard was added to the solid samples and the total amount of choline was determined following the same hydrolysis, filtration, and separation processes.

FIGURE 4. Determination of choline in an adult nutritional treated with a Dionex OnGuard II A Cartridge

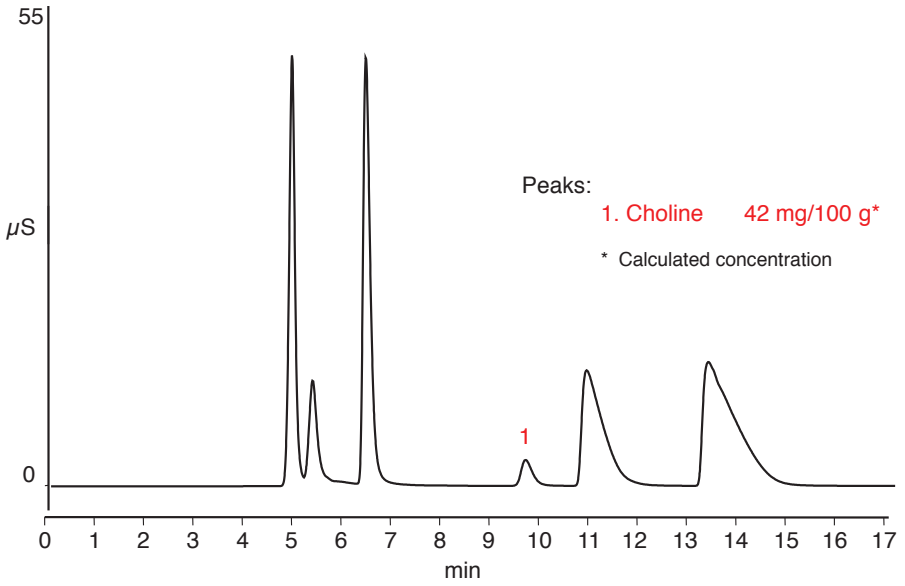


TABLE 2. Choline spiked recoveries.

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
Egg Powder	6.2	100	98.3
		120	92.1
Soy Flour	0.6	100	98.2
		110	98.1

TABLE 3. Choline determination in three replicates of infant formula, egg powder, and soy flour 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
Egg Powder[2]	15.3	0.92	14.1	108
Soy Flour[2]	3.13	0.57	2.49	126

[1] Average of nine sample preparations
[2] Average of three sample preparations

Method Precisions

The intraday peak area precisions were investigated based on the extraction of choline from three replicates of the selected food samples followed by five successive injections of each replicate in the IC system. The between-day peak area precisions were examined by analyzing one sample with five successive injections on each of three consecutive days.

TABLE 4. Intraday and between-day peak area precisions of choline.

Samples	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	

Conclusion

- The Dionex IonPac CS19 column separates choline and other cations in the sample with excellent peak efficiency, allowing simultaneous determination of choline and other cations present in the samples.
- The RFIC system requires only a source of degassed DI water for generation of high-purity eluent, simplifying operation while increasing precision and accuracy.
- Suppressed conductivity detection allows simple and robust determination of choline in different food samples with high sensitivity.

References

1. Phillips, M. M. Analytical Approaches to Determination of Total Choline in Foods and Dietary Supplements. *Anal. Bioanal. Chem.* **2012**, *403*, 2103–2112.
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5. Laikhtman, M.; Rohrer, J. Determination of Choline in Infant Formula by Ion Chromatography. *J. AOAC Int.* **1999**, *82*, 1156–1162.
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PN70581_E 08/16S