

# Choline in Infant Formula and Adult Nutritionals, a Single Laboratory Validation

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## Keywords

Dionex IonPac CS19 Column, Suppressed Conductivity Detection, Ion Chromatography, AOAC

## Introduction

Choline is a water-soluble quaternary amine essential to methyl metabolism, transmembrane signaling, and normal brain development. Choline is present in many foods and also exists in esterified and bound forms: acetylcholine, phosphocholine, phosphatidylcholine, glycerophosphocholine, and sphingomyelin. The adequate intake (AI) for infants ages 0–12 months ranges from 125 to 150 mg/day whereas the AI for adult men, pregnant women, and lactating mothers ranges from 450 to 550 mg/day and the AI for adult women who are not pregnant or lactating ranges from ~400 to 425 mg/day.<sup>1,2</sup> Although the body produces choline, a choline-rich diet is necessary to meet dietary needs. Therefore, infant formulas and adult nutritional products are fortified with choline.

The most widely accepted method for determining choline in infant formula and milk is AOAC Official Method 999.14.<sup>3,4</sup> This method is an enzymatic acid hydrolysis/colorimetric method. However, it is limited by the amount of vitamin C present in the sample (not for use with products containing more than 100 mg vitamin C per 100 g solid) and has only been validated for infant formula and milk powder products.

Dionex (now part of Thermo Scientific) Application Note 124 and the *Journal of AOAC International* publication by Laikhtman and Rohrer demonstrate that ion chromatography (IC) using a 4 mm Thermo Scientific™ Dionex™ IonPac™ CS12A Analytical column with manually prepared sulfuric acid eluent is a good technique

for the determination of choline in infant formula.<sup>5,6</sup> Improvements in IC technology, such as electrolytic eluent generation, have enabled significant enhancements to this method by providing lower noise, better sensitivity, and more stable retention times. Application Update (AU) 189 incorporates electrolytic generation of a methanesulfonic acid (MSA) eluent for a 2 mm Dionex IonPac CS19 column, which eliminates the need for manually prepared eluent and therefore enhances the ease of use, reproducibility, and sensitivity of the method. In addition, a modified sample preparation procedure increases sample throughput and improves recovery.<sup>7</sup>



In an effort to modernize methods used for the analysis of infant formulas and adult nutritionals, the International Formula Council (IFC) and AOAC International signed the Infant Formula Initiative of 2010. Therefore, a single laboratory validation (SLV) study was performed on commercially available matrices and was presented to the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) expert review panel (ERP) at the 2012 AOAC annual meeting. The ERP reviewed the data and concluded that it met the choline standard method performance requirements (SMPRs) established by the stakeholders and approved the method as an AOAC Official First Action Method (AOAC Method 2012.20).<sup>8,9</sup>

At the request of the ERP, a second and full SLV was performed on 17 SPIFAN matrices that included fortified and placebo products. This work discusses the results of that second SLV study and highlights the sample preparation improvements relative to AOAC Method 999.14. The samples were prepared by microwave-assisted acid hydrolysis to digest and release bound choline from powder and ready-to-feed (RTF) infant formula and adult nutritional samples, which decreased the hydrolysis reaction time 9-fold when compared to the traditional water bath hydrolysis reaction used in AOAC Method 999.14 and AU 189. Following hydrolysis, separation of choline from common cations was achieved using a Dionex IonPac CS19 column, followed by suppressed conductivity detection. Repeatability, recovery, linearity, limit of detection (LOD), and limit of quantification (LOQ) were determined according to the choline AOAC SMPR and AOAC SLV guidelines.<sup>10</sup>

## Goal

To develop a method to determine total choline (free and bound forms) in all forms of infant formula, adult nutritionals, and pediatric formulas (e.g., powder, RTF products, and liquid concentrates) with a sample preparation method that increases sample throughput

## Equipment

- Thermo Scientific Dionex ICS-5000\* system, including:
  - SP Single Pump or DP Dual Pump
  - DC Detector/Chromatography Compartment
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC III MSA Eluent Generator Cartridge (P/N 074535)
- Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software, Version 7.1
- CEM Corporation MARS 6 Microwave Reaction System, 230 V/60 Hz (CEM P/N 927500)
- CEM Corporation DuoTemp Control with Fiber-Optic Temperature Option (CEM P/N AVTC)
- CEM Corporation Easy Prep Vessel Full Starter Set, Pressure and Temperature: 100 mL (CEM P/N 910930)
- Helium or Nitrogen, 4.5 Grade (99.995%) or better (Praxair)
- Thermo Scientific Dionex Vial Kit, 10 mL Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Nalgene™ Narrow-Mouth Natural HDPE Packaging Bottles with Closure (P/N 312089-0008)
- Falcon™ 50 mL Conical Centrifuge Tubes, Polypropylene, 30 × 115 mm (Fisher Scientific P/N 14-432-22)
- Nalgene Syringe Filters, 25 mm Diameter Acrylic Housing, 25 mm, Pore Size: 0.2 µm, Sterile (Fisher Scientific P/N 194-2520)
- Thermo Scientific™ Nunc™ Serological Pipette, 10 mL (Fisher Scientific P/N 170356)
- Thermo Scientific™ Dionex™ OnGuard™ Sample Prep Workstation (P/N 039599)
- Dionex OnGuard Needle, 18 Gauge, 1.25 Luer (P/N 039996)
- Dionex OnGuard Sample Reservoir, 5 cc (P/N 041233)
- Dionex OnGuard Valve, Stopcock Luer (P/N 040896)
- Dionex OnGuard II A Cartridge, 2.5 cc (P/N 057092)
- AirTite All-Plastic Norm-Ject Syringes, 20 mL (Fisher Scientific P/N 14-817-33)

\* A Dionex ICS-5000+ HPIC™ IC system, capable of supporting high-pressure IC, or any other Dionex ICS system capable of eluent generation and using 2 mm columns can also be used for this application.

## Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 M $\Omega$ -cm resistance or better
- Hydrochloric Acid (HCl), Optima™ (Fisher Scientific P/N A466250)
- Combined Six Cation Standard-II (P/N 046070)
- Choline Bitartrate, >98.0% (Fisher Scientific P/N C0553)

## Samples

Determination of total choline was conducted for the samples provided in the SPIFAN SLV Test Materials Kit (Table 1). All powdered samples except National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1849a were reconstituted in DI water (25 g of powder in 200 g of DI water).

Table 1. SPIFAN SLV test materials kit.

Category	No.	Product Description	Container Size (g)	Lot No. Identification
Fortified	1	NIST SRM 1849a Powder	10	CLC10-b
	2	AN Milk Protein-Based Powder	100	11750017V3
	3	IF Milk-Based Partially Hydrolyzed Powder	360	1172572116
	4	IF Soy-Based Partially Hydrolyzed Powder	360	117257651Z
	5	AN Low-Fat Powder	250	00394RF00
	6	Child Formula Powder	250	00412RF00
	7	Infant Elemental Powder	250	00403RF00
	8	IF Milk-Based Powder	360	D04HTCVV
	9	IF Soy-Based Powder	730	E29JVLV
	10	IF Milk-Based RTF	60	SPIFAN Control Milk Form
	11	AN High-Protein RTF	250	00414RF00
	12	AN High-Fat RTF	250	00406RF00
Placebo	13	Child Formula Powder	250	00411RF00
	14	Infant Elemental Powder	250	00402RF00
	15	IF Milk-Based RTF	60	SPIFAN Blank Milk Form
	16	AN High-Protein RTF	250	00415RF00
	17	AN High-Fat RTF	250	00407RF00

AN = Adult Nutritional  
IF = Infant Formula  
RTF = Ready-to-Feed

## Conditions

Columns:	Dionex IonPac CG19 Guard, 2 × 50 mm (P/N 076029) Dionex IonPac CS19 Analytical, 2 × 250 mm (P/N 076028)
Eluent:	–5 to 13 min 6.4 mM MSA, step to 25 mM MSA at 13 min, 25 mM MSA from 13–17 min
Eluent Source:	Dionex EGC III MSA Eluent Generator Cartridge with Dionex CR-CTC II Continuously Regenerated Cation Trap Column
Flow Rate:	0.25 mL/min
Inj. Volume:	5 $\mu$ L
Temperature:	30 °C
Autosampler	Temperature: 10 °C
Detection:	Suppressed Conductivity, Thermo Scientific™ Dionex™ CSRS™ 300 Cation Self-Regenerating Suppressor, 2 mm (P/N 064557), Recycle Mode, –5–13 min 5 mA, 13–17 min 19 mA

## System

Backpressure: ~2200 psi

## Background

Conductance: <0.2  $\mu$ S

Typical Noise: <0.2 nS

## Equilibration

Run Time: 17 min

## Preparation of Solutions and Reagents

### 20,000 mg/L Stock Choline Solution

Dry 7–10 g of choline bitartrate at 100 °C for 2–3 h. Weigh 4.86 g of dry choline bitartrate in a 125 mL polypropylene bottle and tare the balance. Add 100 g of DI water to make a 20,000 mg/L choline stock solution. Cap the bottle and shake to completely dissolve the solid material. The standard is stable for one week when stored at 4 °C.

### 10,000 mg/L Stock Choline Solution

Weigh 2.43 g of dry choline bitartrate in a 125 mL polypropylene bottle and tare the balance. Add 100 g of DI water to make a 10,000 mg/L choline stock solution. Cap the bottle and shake to completely dissolve the solid material. The standard is stable for one week when stored at 4 °C.

### 1,000 mg/L Stock Choline Solution

Weigh 0.243 g of dry choline bitartrate in a 125 mL polypropylene bottle and tare the balance. Add 100 g of DI water to make a 1,000 mg/L choline stock solution. Cap the bottle and shake to completely dissolve the solid material. The standard is stable for one week when stored at 4 °C.

### Choline Working Standard Solutions

Deliver the appropriate volume of the 1,000 mg/L stock solution into a 125 mL polypropylene bottle and bring to volume (by weight) with DI water.

For this application, calibration standards were prepared at 0.6, 5, 15, 30, 45, 60, and 75 mg/L. Aliquots were stored at  $-80^{\circ}\text{C}$  and thawed prior to use.

#### 1.5 M HCl

In a well-ventilated hood, weigh 435.6 g of DI water into a glass bottle and add 64.4 mL of ultrapure reagent-grade HCl. Cap and swirl to mix.

#### 3.0 M HCl

In a well-ventilated hood, weigh 371.4 g of DI water into a glass bottle and add 128.6 mL of ultrapure reagent-grade HCl. Cap and swirl to mix.

### Sample Preparation

#### 1. Reconstitute Powders

Reconstitute all powders except the NIST SRM 1849a in water prior to sampling.

- Place a 250 mL polypropylene bottle on the balance and tare it. Add 25 g of powder and record the weight. Add 200 g of DI water and record the total weight. Cap the bottle and shake until well dissolved. Reconstituted powder samples must be used immediately.

#### 2. Prepare the Microwave Vessel with Sample

- Place a 100 mL microwave vessel on the balance and tare it.
- For the NIST SRM 1849a powder, weigh 2.5 g of powder in the vessel and record the weight. Add 15 g of 1.5 M HCl and record the total weight.
- For reconstituted powders and RTF liquids, immediately prior to use, invert the bottle at least three times to ensure the sample is mixed before taking an aliquot; however, do not shake as this will cause bubbles in the sample. Add 10 g of the liquid (reconstituted powder or RTF liquid) to the microwave vessel and record the weight. Add 10 g of 3 M HCl and record the total weight.

#### 3. Run the Microwave Program

- Assemble the microwave vessels and place them in the rotor.
- Run the microwave program to completion (Table 2).
- Allow the vessels to cool to at least  $50^{\circ}\text{C}$ , then open in a well-ventilated hood.

Table 2. Microwave program.

Step	Time (min)	Temp ( $^{\circ}\text{C}$ )	Power (W)	
1	Ramp	14	100	900–1050
2	Hold	10	100	900–1050
3	Vent	15	—	—

#### 4. Filter and Dilute

- Transfer the sample to a 50 mL conical centrifuge tube and cap.
- Shake the contents just before filtering  $\sim 3$  mL of the hydrolysate through a  $0.2\ \mu\text{m}$  filter disk into a clean 50 mL conical centrifuge tube with cap.
- Transfer 0.75 g of the filtrate into a clean 50 mL conical centrifuge tube and record the weight. Bring to a total weight of 12.5 g with DI water and record the total weight.

#### 5. Dionex OnGuard II A Treatment

- Neutralize the sample using a Dionex OnGuard II A Cartridge. Prepare the cartridge for use by rinsing it with 15 mL of DI water. Connect the cartridge to a 20 mL syringe and load the syringe with the sample prepared in Step 4c. Push the first 6 mL of neutralized sample into a waste container at a flow rate of 2 mL/min. Collect the next 3 mL for injection in a 10 mL sample vial. Up to 12 samples can be prepared in parallel using the Dionex OnGuard Sample Prep Workstation.
- Samples are stable for three days when stored in a capped polypropylene vial at  $4^{\circ}\text{C}$ .

### Validation Protocol

Follow the SPIFAN SLV recommended guidelines (Final Version 4).<sup>7</sup>

#### System Suitability

Verify check standards daily at the lowest point and midpoint of the analytical range throughout the batch run. Recalibrate the system when the percent error of the check standards is  $>5\%$ .

#### Linearity/Calibration Fit

Perform three separate experiments using independently prepared standards at seven concentration levels that span the desired working range.

In this study, the relative error of back-calculated concentrations within the desired working range was  $<5\%$ .

## LOD/LOQ

Calculate the LOD as blank mean + 3× the standard deviation and the LOQ as blank mean + 10× the standard deviation.

## Precision Studies

Analyze samples selected for precision studies in duplicate on each of six days using multiple instruments. Prepare fresh reagents each day.

## Accuracy

- Analyze the NIST SRM 1849a in duplicate over six days and compare results to the reported NIST-certified value.
- Determine spike recovery from unfortified products (i.e., placebos). Spike each selected sample at 50 and 100% of the amounts found in the unfortified products and analyze in duplicate on each of three days. Use the overall mean of the unspiked unfortified samples for calculating recoveries.

## Results and Discussion

### Separation and Detection

Microwave-assisted hydrolysis was used to digest and release bound choline from infant formula and adult nutritional samples (powder and RTF). Following hydrolysis, choline was separated from common cations using a Dionex IonPac CS19 column and detected by suppressed conductivity. Figure 1 shows a typical chromatogram of a 5 µL injection for a 20-fold dilution of a Combined Six Cation Standard-II + choline. The sample was acidified during preparation to promote release of the bound choline. Because the cation exchange sites on the column protonate at low pH, there was a loss in peak efficiency, as shown in Figure 2B. Therefore, the sample required treatment with a Dionex OnGuard II A Cartridge to adjust the sample to ~pH 6. Figure 2A demonstrates improved peak efficiencies after sample neutralization.

Figure 1. A 20× dilution of a Combined Six Cation Standard-II + 5 mg/L choline.

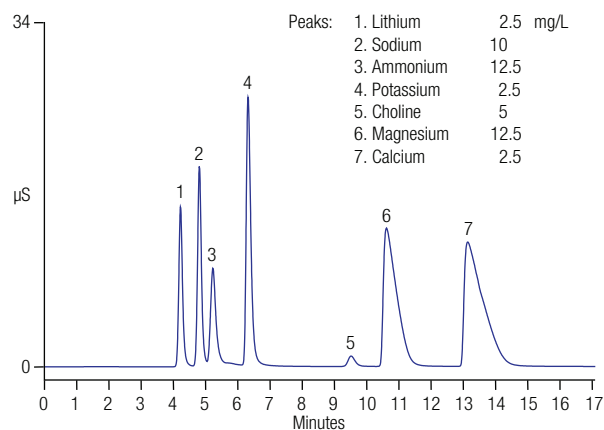
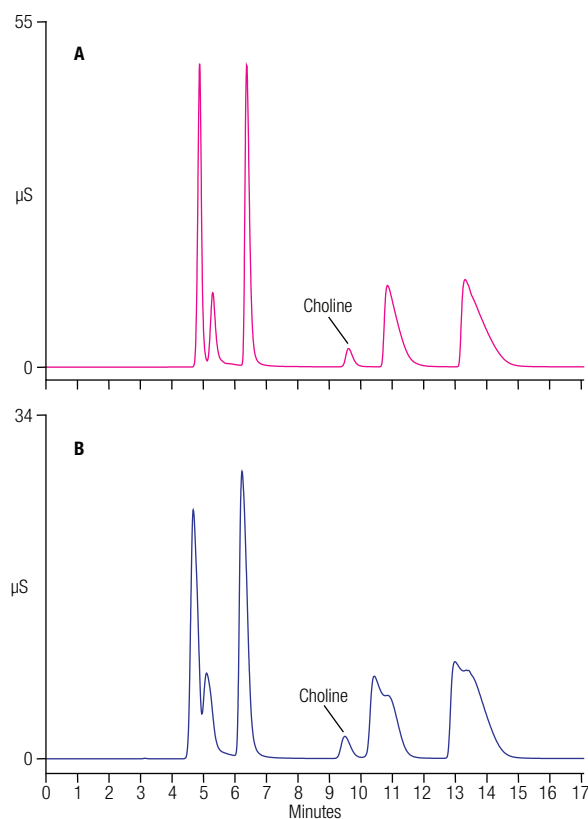


Figure 2. The same infant formula sample (A) with and (B) without Dionex OnGuard II A treatment.



### Linearity, LOD, and LOQ

To determine linearity, calibration standards were injected in triplicate over seven concentration levels covering the range of 0.6–75 mg/L choline (2–250 mg/100 g). To establish stability of the analytical curve, three independent experiments were conducted using independently prepared standards. The calibration results show that the detection of choline is linear over the concentration range, with a coefficient of determination >0.9999. Calibration errors at each level of the calibration curve were <5%. Check standards at the lowest point and midpoint of the analytical range were checked daily throughout the batch run. The system was recalibrated when the percent error of the check standards was >5%.

$$\text{Error, \%} = \frac{\text{calculated concentration} - \text{actual concentration}}{\text{actual concentration}} \times 100$$

The LOD was first estimated as 3× the signal-to-noise ratio (S/N) and the LOQ as 10× the S/N. The system baseline noise for the LOD and LOQ was determined by measuring peak-to-peak noise between 3.4 and 3.9 min over five injections of a prepared sample. The estimated LOD and LOQ for choline in a sample were 0.003 and 0.009 mg/100 g, respectively. Spiked blanks containing these amounts were prepared to calculate the final LOD and LOQ. The LOD is defined as blank mean + 3× standard deviations and the LOQ is defined as blank mean + 10× standard deviations. Using these calculations, LOD and LOQ are 0.009 and 0.012 mg/100 g, respectively. These estimated values are well below the required sample LOD and LOQ of 0.7 and 2 mg/100 g, respectively, as specified in the choline SMPR.

Table 3. Choline validation data<sup>a</sup> for intermediate precision,  $n = 2$ , mg/100 g.

No.	Product Description	Day					
		1	2	3	4	5	6
1	NIST SRM 1849a Powder	10.1	10.2	10.0	10.1	10.4	10.3
	RSD	0.1	1.8	2.5	1.8	2.2	0.5
2	AN Milk Protein-Based Powder	3.02	2.95	2.96	2.96	3.02	3.07
	RSD	3.27	3.68	1.75	1.06	2.30	6.49
3	IF Milk-Based Partially Hydrolyzed Powder	17.3	17.3	16.7	16.9	17.2	17.3
	RSD	3.3	2.5	0.4	1.2	0.7	1.5
4	IF Soy-Based Partially Hydrolyzed Powder	16.3	16.7	16.1	16.3	16.5	16.4
	RSD	2.8	2.7	1.6	1.1	1.0	3.2
5	AN Low-Fat Powder	16.7	16.9	16.3	16.4	16.6	16.9
	RSD	2.8	3.0	1.7	1.2	0.6	2.6
6	Child Formula Powder	4.92	4.99	4.88	4.86	5.11	4.97
	RSD	4.68	4.31	1.06	0.59	0.17	0.43
7	Infant Elemental Powder	7.82	7.80	7.53	7.56	7.68	7.78
	RSD	3.32	2.97	0.90	0.50	1.15	0.27
8	IF Milk-Based Powder	16.2	15.8	15.5	15.4	15.7	15.9
	RSD	0.9	3.7	1.7	0.9	2.2	0.6
9	IF Soy-Based Powder	18.1	17.7	17.5	17.4	17.8	18.4
	RSD	0.2	2.7	2.2	1.6	1.3	3.0
10	IF Milk-Based RTF	20.0	19.7	20.2	19.5	20.7	20.3
	RSD	0.0	2.7	0.0	2.7	2.2	0.9
11	AN High-Protein RTF	46.6	46.2	47.3	45.3	47.2	46.7
	RSD	0.4	2.6	1.1	4.9	0.6	1.9
12	AN High-Fat RTF	51.5	51.4	52.2	49.4	51.5	50.8
	RSD	2.5	0.4	1.9	4.1	0.4	2.8

AN = Adult Nutritional

IF = Infant Formula

RTF = Ready-to-Feed

<sup>a</sup>Analyzed on multiple instruments in duplicate over six days, each preparation injected three times

### Precision

Repeatability was evaluated on all 12 fortified AOAC SLV test material matrices using multiple instruments. Samples were prepared in duplicate over six days and intermediate and overall reproducibility were calculated. Choline concentration in these samples ranged from 3.00 to 51.5 mg/100 g. Intermediate precision was <6.5 and 4.7% for samples containing choline at 2 and 20 mg/100 mg, respectively. Overall precision was <3.0% for all matrices. Repeatability data are summarized in Tables 3 and 4.

Table 4. Choline validation data<sup>a</sup> for overall precision,  $n = 12$ , mg/100 g.

No.	Product Description	Average Amount	±	RSD
1	NIST SRM 1849a Powder	10.2	0.2	1.8
2	AN Milk Protein-Based Powder	3.00	0.09	3.0
3	IF Milk-Based Partially Hydrolyzed Powder	17.1	0.3	2.0
4	IF Soy-Based Partially Hydrolyzed Powder	16.4	0.3	2.1
5	AN Low-Fat Powder	16.6	0.4	2.1
6	Child Formula Powder	4.96	0.13	2.7
7	Infant Elemental Powder	7.70	0.16	2.1
8	IF Milk-Based Powder	15.8	0.4	2.3
9	IF Soy-Based Powder	17.8	0.4	2.5
10	IF Milk-Based RTF	20.1	0.5	2.5
11	AN High-Protein RTF	46.6	1.1	2.4
12	AN High-Fat RTF	51.1	1.3	2.5

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<sup>a</sup>Analyzed on multiple instruments in duplicate over six days, each preparation injected three times

### Precision over the analytical range

To show precision over the analytical range, selected samples were spiked with 0, 2, 20, 100, and/or 200 mg/100 g choline (Table 5). These samples showed that the method is capable of measuring choline over the range of the assay.

### Accuracy

#### Trueness against reference material

The NIST SRM 1849a was analyzed in duplicate over six days. The amount of choline present in the sample over the six days ( $n = 12$ ) was 10.2 mg/100 g  $\pm$  0.2 mg/100 g calculated as the reconstituted powder with a RSD of 1.8. This is a 93.4% recovery of the NIST-certified value of 10.9 mg/100 g  $\pm$  1.1 mg/100 g reconstituted powder.

### Choline spiked recoveries

To evaluate accuracy, recovery of choline was determined by spiking duplicate preparations at 50 and 100% of the choline amounts found in the fortified products directly into the placebo product (reconstituted powder or RTF liquid), then continuing to follow the extraction procedure. This method demonstrated good recovery of choline from the five matrices ranging from 92.8 to 101%, with RSDs <6.7 for one product containing <2 mg/100 g choline and <3.9 for all other products (Table 6).

$$\text{Recovery, \%} = \frac{C_{\text{spiked sample}} - C_{\text{unspiked sample}}}{C_{\text{analyte added}}} \times 100$$

Table 5. Precision over the analytical range<sup>a</sup> of placebo products,  $n = 6$ , mg/100 g.

No.	Product Description	Average Amount Found	RSD	Spiked to Contain					
				2.5 mg/100 g		20 mg/100 g		100 mg/100 g	
				Average Amount Found	RSD	Average Amount Found	RSD	Average Amount Found	RSD
13	Child Formula Powder	0.94	5.93	2.38	4.63	23.2	1.47	98.6	1.53
15	IF Milk-Based RTF	12.1	1.72	—	—	—	—	108	2.8
17	AN High-Fat RTF	5.08	2.55	—	—	—	—	100 <sup>b</sup>	1.0

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<sup>a</sup>Analyzed on multiple instruments in duplicate over six days, each preparation injected three times

<sup>b</sup>Due to limited sample amount,  $n = 2$

Table 6. Recovery of choline spikes added to SPIFAN SLV placebo products<sup>a</sup>,  $n = 6$ , mg/100 g.

No.	Product Description	Amount Found in Fortified Product <sup>b</sup>	Native Amount Found in Placebo Product <sup>c</sup>	RSD	Placebo Spiked at 50% of the Fortified Amount <sup>c</sup>		Placebo Spiked at 100% of the Fortified Amount <sup>c</sup>	
					Recovery (%)	RSD	Recovery (%)	RSD
13	Child Formula Powder	4.96	0.94	5.93	94.0	6.69	95.5	3.19
14	Infant Elemental Powder	7.70	0.55	3.44	92.8	0.80	95.4	3.25
15	IF Milk-Based RTF	20.1	12.1	1.72	101	3.81	98.2	3.90
16	AN High-Protein RTF	46.6	5.11	4.27	98.0	3.09	97.1	1.90
17	AN High-Fat RTF	51.1	5.08	2.55	98.8	2.68	99.6	3.27

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<sup>a</sup>Analyzed on multiple instruments

<sup>b</sup>Prepared in duplicate over six days, each preparation injected three times

<sup>c</sup>Prepared in duplicate over three days, each preparation injected three times

Table 7. Choline spiked recoveries in placebo products<sup>a</sup> over the analytical range,  $n = 6$ .

No.	Product Description	Spiked to Contain							
		2.5 mg/100 g		20 mg/100 g		100 mg/100 g		200 mg/100 g	
		Recovery %	RSD	Recovery %	RSD	Recovery %	RSD	Recovery %	RSD
13	Child Formula Powder	98.2	6.7	98.8	1.53	99.2	1.64	100	0.74
15	IF Milk-Based RTF	—	—	—	—	97.8	4.02	97.7	2.75
17	AN High-Fat RTF	—	—	—	—	98.3 <sup>b</sup>	0.49	101 <sup>b</sup>	2.00

AN = Adult Nutritional  
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<sup>a</sup>Analyzed on multiple instruments in duplicate over three days, each preparation injected three times

<sup>b</sup>Due to limited sample amount,  $n = 2$

### Choline spiked recoveries over the range

To show spiked recovery over the analytical range, several placebo products were spiked to contain 2.5, 20, 100, and/or 200 mg/100g choline. Recoveries ranged from 97.7 to 101% with RSDs <6.7 for one product containing <2 mg/100 g choline and <4 for all other products (Table 7).

### Conclusion

This newly developed IC method can determine choline in 17 SPIFAN samples from a SLV study. The Dionex IonPac CS19 column separates choline and other cations in the sample with excellent efficiency, allowing simultaneous determination of choline and other cations present in the samples. The Reagent-Free™ IC (RFIC™) system requires only a source of degassed DI water for generation of high-purity eluent, thus simplifying operation while increasing precision and accuracy. Suppressed conductivity detection allows simple, robust, and accurate determination of choline in all samples with high sensitivity. In addition, microwave-assisted digestion for hydrolysis of the bound choline to free choline significantly decreases sample preparation time. This method demonstrates good precision and accuracy while meeting all method requirements outlined by the choline SMPR.

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