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APPLICATION NOTE

No. 1155

Determination of Chloride in Infant Formula and Adult Nutritionals

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Keywords: Dionex IonPac AS15 column, suppressed conductivity detection, ion chromatography, IC, AOAC, food and beverage

Goal

To develop an IC method to determine chloride in all forms of infant formula, adult nutritionals, and pediatric formulas (e.g., powder, Ready-to-Feed (RTF) products, and liquid concentrates)

Introduction

Chloride is an essential nutrient for growth and development in infants. Chloride deficiency can lead to hypochloremic metabolic alkalosis (HMA), a condition in which tissue fluids are too alkaline. In 1978 and 1979, two infant formulas, Neo-Mull-Soy and Cho-Free, were found to be deficient in chloride. The manufacturer of the formulas had reformulated these soy products by discontinuing the addition of salt (sodium chloride). The Centers for Disease Control received reports that HMA had developed in 141 children as a result of ingestion of these formulas. Most of the cases were due to the prolonged and exclusive use of one of the two soy formulas.[1,2] In August 1979, the two infant formulas were recalled following the reports of serious injuries among infants.

Consequently, in 1980, the Infant Formula Act (IFA) was passed.[3] This Act provided clear authority for the Secretary of Health and Human Services to promulgate quality control regulations and regulations requiring a declaration of the nutrient levels on the label of infant formulas. The Infant Formula Act of 1980 specifies minimum and maximum amounts of several nutrients. To meet these specifications, new and more stringent standard method performance requirements (SMPR) are being developed. 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. From this agreement the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) was formed.

This application note shows an ion chromatography (IC) determination of chloride in 19 SPIFAN matrices, including fortified and placebo products. In this determination, chloride is separated from other anions by anion-exchange chromatography and detected by suppressed conductivity. The results show that the method fulfills the SPIFAN standard method performance requirements (SMPRs). Repeatability, recovery, linearity, limit of detection (LOD), and limit of quantification (LOQ) were determined according to the chloride AOAC SMPR[4] and AOAC Single Lab Validation (SLV) guidelines.[5]

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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 KOH Eluent Generator Cartridge; P/N 074532
- Thermo Scientific™ Dionex™ CR-ATC II Continuously Regenerated Anion Trap Column; P/N 060477
- Thermo Scientific™ Dionex™ AERS™ Anion Electrolytically Regenerated Suppressor (2 mm); P/N 082541
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software, Version 7.1
- Thermo Scientific™ Dionex™ Vial Kit, 10 mL Polystyrene with Caps and Blue Septa; P/N 074228
- Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-3 membrane; P/N UFC900396

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Sodium Chloride >99.5% (Fisher Scientific Cat.No. BP358-1)

Consumables

- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)
- AirTite All-Plastic Norm-Ject Syringes, 5 mL, sterile (Fisher Scientific P/N 14-817-28)

Conditions	
System	Dionex ICS-5000+ Reagent-Free HPIC System
Columns	Thermo Scientific™ Dionex™ IonPac™ AS15, Analytical, 2 x 250 mm (P/N 053941) Dionex IonPac AG15, Guard, 2 x 50 mm (P/N 053943)
Column Temperature	30 °C
Eluent Source	Thermo Scientific™ Dionex™ EGC III KOH cartridge with high pressure CR-ATC
Eluent Condition	Gradient: 5 mM KOH (-7–0 min), 5 mM KOH (0–10 min), 5–35 mM KOH (10–12 min), 35 mM KOH (12–16 min), 60 mM KOH(16–30 min)
Flow Rate	0.4 mL/min
Injection Volume/Loop Size	2.5 µL
Inject Mode	Push full
Loop Overfill Factor	5
Detection	Suppressed conductivity with Thermo Scientific™ Dionex™ AERS 500 Electrolytically Regenerated Suppressor, recycle mode
Suppresser Current	60 mA
System Backpressure	~2350 psi
Background Conductance	~0.2–0.6 µS
Noise	0.3–0.8 nS/min peak-to-peak
Run Time	37 min

Preparation of Solutions and Reagents

5000 mg/L Stock Chloride Solution

Weigh 0.825 g of dry sodium chloride in a 125 mL polypropylene bottle and tare the balance. Add 100 g of DI water to make a 5000 mg/L chloride 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.

Chloride Working Standard Solutions

Deliver the appropriate volume of the 5000 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 5, 20, 50, 200, 500, 1000, and 2000 mg/L. Aliquots were stored at –40 °C and thawed prior to use.

Samples

Samples were provided by the SPIFAN (SPIFAN SLV Test Materials Kit (Table 1)).

Sample Preparation

Reconstituted Powders

Step 1: Place a 20 mL polypropylene bottle on the balance and tare it. Add 2.50 g of powder and record the weight.

Step 2: Add 20.0 g of DI water and record the total weight. Cap the bottle and shake until well dissolved (2–3 min). Reconstituted powder samples must be used immediately.

Step 3: Transfer 12 mL reconstituted powder sample to a 50 mL Amicon Ultra-15 Centrifugal filter device and cap. Centrifuge for 60 min at 5000 rpm at 20 ºC.

Step 4. Collect 5–6 mL of filtrate (from step 3) and pass through a 0.2 µm filter before analysis.

Ready-to-Feed Formula

Step 1: Transfer 12 mL of RTF to a 50 mL Amicon Ultra-15 Centrifugal filter device and cap. Centrifuge for 60 min at 5000 rpm at 20 °C.

Step 2. Collect 5–6 mL of filtrate (from step 1) and pass through a 0.2 µm filter before analysis.

Table 1. SPIFAN II SLV test materials kit.

Category	Sample No.	AOAC SLV Test Material
Fortified	1	NIST SRM 1849a Powder
	2	IF Milk-Based Partially Hydrolyzed Powder
	3	IF Soy-Based Partially Hydrolyzed Powder
	4	Toddler Formula Milk-Based Powder
	5	IF Milk-Based Powder
	6	AN Low Fat Powder
	7	Child Formula Powder
	8	Infant Elemental Powder
	9	IF FOS/GOS-Based Powder
	10	IF Milk-Based Powder
	11	IF Soy-Based Powder
	12	IF Milk Based RTF
	13	AN High Protein RTF
	14	AN High Fat RTF
Placebo	15	Child Formula Powder
	16	Infant Elemental Powder
	17	IF Milk Based RTF
	18	AN High Protein RTF
	19	AN High Fat RTF

Adult Nutritional High Fat and High Protein Formula

Step 1: Place a 20 mL polypropylene bottle on the balance and tare it. Add 10 g of liquid and record the weight.

Step 2: Add 10.0 g of DI water and record the total weight. Cap the bottle and shake until well dissolved (2–3 min).

Step 3: Transfer the 12 mL of solution from step 2 to a 50 mL Amicon Ultra-15 Centrifugal filter device and cap. Centrifuge for 60 min at 5000 rpm at 20 °C.

Step 4. Collect 5–6 mL of filtrate (from step 3) and pass through a 0.2 µm filter before analysis.

Validation Protocol

SPIFAN SLV recommended guidelines were followed.

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.

Precision studies: Analyze samples selected for precision studies on each of six days. Prepare fresh reagents each day.

Accuracy:

a. Analyze the NIST SRM 1849a over six days using multiple instruments and compare results to the reported NIST-certified value.

b. Determine spike recovery from unfortified products (i.e., placebos). Spike each selected sample at 50 and 100% of the amounts found in the fortified 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

Ultracentrifugal filter devices were used to filter and remove the fats and proteins from the sample. Following filtration, chloride was separated from other anions using a Dionex IonPac AS15 column and detected by suppressed conductivity. Figure 1 shows a chromatogram of a 2.5 µL injection of the SRM 1849a sample. The retention time for chloride was 15.40 min.

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

To determine linearity, calibration standards were injected at seven concentrations covering the range of 5–2000 mg/L. To establish stability of the analytical curve, three independent experiments were conducted using independently prepared standards. The calibration results show that the detection is linear over the concentration range, with a coefficient of determination > 0.9995. 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%.

Error % = (calculated concentration – actual concentration)/ (actual concentration)*100

The LOD was 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 6.0 and 7.0 min over five injections of a prepared sample. The estimated LOD and LOQ for chloride were 0.006 and 0.02 mg/100 g respectively. These estimated values are well below the required sample LOD and LOQ of 1.6 and 5 mg/100 g, respectively, as specified in the chloride SMPR.

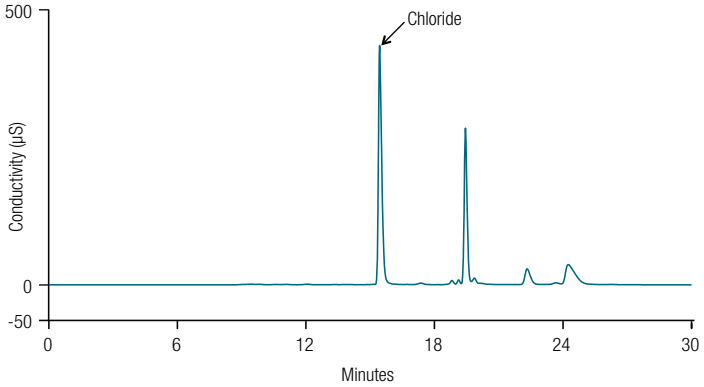

Figure 1. Chromatogram of SRM1849a showing the chloride peak.

Precision

Repeatability was evaluated on all 14 fortified AOAC SLV test material matrices including the NIST SRM 1849a. Samples and standards were prepared fresh twice every day over six days and analyzed to calculate the intermediate (n=2) and overall reproducibility (n=12). Chloride concentration in these samples ranged from 346 to 842 mg/100 g. Intermediate precision and overall precision were <2.5% and <3.2% respectively for all matrices. Repeatability data are summarized in Tables 2 and 3.

Accuracy

Trueness against reference material

The NIST SRM 1849a was analyzed in duplicate over six days. The amount of chloride present in the sample over the six days (n = 12) was 7454 mg/kg ± 142 mg/kg calculated as the reconstituted powder with a RSD of 1.9% (Table 4). This is a 106% recovery of the NIST-certified value of 7010 mg/kg reconstituted powder.

Table 2. Chloride validation data for intermediate precision, n = 2, mg/100 g powder.*

No.	AOAC SLV Test Material	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
		Avg	RSD	Avg	RSD	Avg	RSD	Avg	RSD	Avg	RSD	Avg	RSD
1	NIST SRM 1849a Powder	755	0.71	733	2.45	728	1.43	751	0.88	739	1.95	757	0.79
2	IF Milk-Based Partially Hydrolyzed Powder	452	1.28	443	1.08	437	0.29	435	0.73	443	0.26	434	0.45
3	IF Soy-Based Partially Hydrolyzed Powder	456	0.78	442	0.84	444	0.12	438	0.28	445	1.01	445	0.83
4	Toddler Formula Milk-Based Powder	534	0.38	533	2.14	537	0.67	524	0.81	520	0.62	531	0.58
5	IF Milk-Based Powder	407	0.89	392	0.88	397	0.7	382	0.49	392	0.37	390	0.31
6	AN Low Fat Powder	441	0.44	441	0.93	434	0.29	425	0.87	417	0.44	437	0.48
7	Child Formula Powder	503	0.88	487	1.17	482	1.21	484	1.09	480	0.16	476	0.28
8	Infant Elemental Powder	412	0.82	400	1.35	403	0.88	394	0.65	395	0.59	398	0.68
9	IF FOS/GOS-Based Powder	365	1.53	358	1.53	363	1.36	350	0.08	346	0.62	354	0.83
10	IF Milk-Based Powder	469	2.54	466	0.56	442	0.33	440	1.00	438	0.91	441	1.42
11	IF Soy-Based Powder	598	0.59	581	1.33	584	0.82	572	0.01	568	0.51	570	0.87
12	IF Milk Based RTF	388	0.96	377	1.77	370	0.25	368	0.89	363	0.72	369	0.75
13	AN High Protein RTF	770	1.47	735	0.39	735	0.39	738	0.78	738	0.82	739	0.68
14	AN High Fat RTF	842	1.02	813	0.61	830	0.9	804	0.42	800	1.16	797	0.55

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

*RTF formulas are assumed to be prepared in the same way as the powder formulas, i.e., 2.5 g of powder dissolved in 20.0 g of DI water.

Table 3. Chloride validation data for overall precision, n = 12, mg/100 g powder.*

	AOAC SLV Test Material	Average amount	±	RSD
1	NIST SRM 1849a Powder	742	12.2	1.64
2	IF Milk-Based Partially Hydrolyzed Powder	441	6.76	1.53
3	IF Soy-Based Partially Hydrolyzed Powder	445	5.96	1.34
4	Toddler Formula Milk-Based Powder	530	6.47	1.22
5	IF Milk-Based Powder	393	8.23	2.09
6	AN Low Fat Powder	433	9.54	2.2
7	Child Formula Powder	485	9.27	1.91
8	Infant Elemental Powder	400	6.67	1.67
9	IF FOS/GOS-Based Powder	356	7.33	2.06
10	IF Milk-Based Powder	449	14.2	3.16
11	IF Soy-Based Powder	579	11.11	1.92
12	IF Milk Based RTF	373	8.65	2.32
13	AN High Protein RTF	744	15.1	2.03
14	AN High Fat RTF	814	18.05	2.22

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

*RTF formulas are assumed to be prepared in the same way as the powder formulas, i.e., 2.5 g of powder dissolved in 20.0 g of DI water.

Table 4. Precision results for NIST SRM1849.

NIST SRM 1849a Powder	Chloride (mg/Kg)
1	7600
2	7677
3	7461
4	7207
5	7351
6	7203
7	7562
8	7468
9	7493
10	7289
11	7611
12	7527
Mean (n=12)	7454
SD	142
% RSD	1.90

Chloride-spiked recoveries

All placebo analyses show low concentrations of chloride compared to fortified samples except the infant elemental formula which showed the same concentration of chloride in the placebo and the fortified product.

To evaluate accuracy, recovery of chloride was determined by spiking duplicate preparations at 50 and 100% of the chloride amounts found in the fortified products directly into the placebo product (reconstituted powder or RTF liquid), then continuing to follow the sample preparation procedure. These recovery experiments were performed over three days.

Recovery % = (C spiked sample-C unspiked sample)/ (C analyte added)*100

Table 5 contains recovery results for chloride on the five placebo samples respectively. The percent chloride recovered ranged from a low of 94.7% to a high of 106%. The percent relative standard deviations (RSD) ranged from a low of 0.46% to a high of 3.33%. The overall mean recovery is 100% (RSD of 1.53%) for 50% spiked level and 101% (RSD 1.62%) for 100% spiked level. With the exception of two data points, 94.7% and 106%, all data meet the criteria specified in the SMPR (95–105% recovery).

Table 5. Recovery of chloride spikes added to SPIFAN SLV placebo products, n = 6, mg/100 g powder.*

No.	Product Description	Amount found in Fortified Product [a]	Amount found in Placebo Product [b]	RSD	Placebo Spiked at 50% of the Fortified amount [b]		Placebo Spiked at 100% of the Fortified amount [b]	
					Recovery %	RSD	Recovery %	RSD
15	Child Formula Powder	485	23.1	2.08	102	3.33	106	1.82
16	Infant Elemental Powder	400	392	1.31	103	1.57	105	0.62
17	IF Milk Based RTF	373	204	1.51	98.4	0.87	96.3	0.46
18	AN High Protein RTF	744	183	3.07	94.7	1.20	95.4	3.01
19	AN High Fat RTF	814	176	3.80	104	0.70	100	2.19

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

[a] Prepared in duplicate over six days, each preparation injected three times

[b] Prepared in duplicate over three days, each preparation injected three times

*RTF formulas are assumed to be prepared in the same way as the powder formulas, i.e., 2.5 g of powder dissolved in 20.0 g of DI water.

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Conclusion

The reported IC method can determine chloride accurately in 19 SPIFAN samples. The Dionex IonPac AS15 column separates chloride and other anions in the sample with excellent efficiency, allowing determination of chloride. 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 chloride in all samples with high sensitivity. This method demonstrates good precision and accuracy while meeting all method requirements outlined by the chloride SMPR except two data points, 94.7% and 106%, where percent recovery of chloride is slightly out of range (95–105%).

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