

Determination of chlorate and perchlorate in powdered infant formula using ion chromatography-mass spectrometry

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Goal

To develop an IC-MS method for the determination of chlorate and perchlorate in powdered infant formula

Introduction

Food can be contaminated by chlorate and perchlorate during different stages of production. Perchlorate can be present in food via the use of fertilizers, while chlorate can be present due to the use of chlorinated disinfectants during food production. The dairy industry often uses hypochlorite and chlorine dioxide as sanitizing agents. Studies have confirmed that the use of these types of sanitizing agents could result in elevated chlorate levels in final products.¹

Recent reports by the European Food Safety Authority (EFSA) found that current levels of chlorate in drinking water and foods were too high and could negatively impact iodine uptake, especially among infants and children. Chlorate originates from chlorine disinfectants widely and legally used in water treatment and food processing, with drinking water being overwhelmingly the main contributor.



The EFSA is currently reviewing the MRLs for chlorate and perchlorate in food.²

Ion chromatography (IC) with suppressed conductivity detection combined with electrospray ionization/mass spectrometry is one of the most sensitive and robust characterization methods available for chlorate and perchlorate. This Application Note uses a Thermo Scientific™ Dionex™ IonPac™ AS20 column set on a compact IC system (Thermo Scientific™ Dionex™ Integrion™ HPIC system) coupled with a Thermo Scientific™ ISQ EC™ single quadrupole mass spectrometer to determine chlorate and perchlorate in powdered infant formula.

The capacity and selectivity of the Dionex IonPac AS20 column ensures that perchlorate can be separated from cations and anions in the sample using a potassium hydroxide mobile phase, even in the presence of very high concentrations of chloride, carbonate, and sulfate. The ISQ EC mass spectrometer provides peak identification information for chlorate and perchlorate by monitoring the appropriate mass/charge ratio (m/z) and relative isotope abundances (RIAs). The chlorinated compounds were confirmed using naturally occurring chlorine isotopes. The isotopic masses of chlorine masses are 34.969 Da (75.78% abundance) and 36.966 Da (24.22% abundance).³ We demonstrate method linearity, sensitivity, accuracy, and precision.

Experimental

Equipment

- Dionex Integrion HPIC system including:
 - Eluent Generator
 - Pump
 - Degasser
 - Conductivity Detector
 - Column oven temperature control with one injection valve (P/N 22153-60014)
 - Detector-suppressor compartment temperature control
 - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074925) with sample syringe, 250 μ L and buffer line, 1.2 mL
- Thermo Scientific™ Dionex™ 6-port high-pressure valve (P/N 22153-60014)
- ISQ EC single quadrupole mass spectrometer (P/N ISQEC0001C)
- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)
- Peak Scientific™ Genius™ 1022 nitrogen generator (P/N 10-6022 (230v))

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088667)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vials, 10 mL (P/N 074228)
- Amicon™ Ultra-15 Centrifugal Filter Unit with Ultracel™-3 membrane (P/N UFC900396)

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System software version 7.2

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 M Ω -cm resistivity or better
- Thermo Scientific™ Dionex™ Chlorate Standard, 1000 mg/L (P/N 303170)
- Potassium chlorate (90–95% chemical purity) ($^{18}\text{O}_3$, 98%) 100 μ g/mL in ^{18}O -water, Cambridge Isotope Laboratories (Cambridge Isotope Laboratories, P/N OLM-10485-1.2)
- Sodium perchlorate, Acros Organics™ (Fisher Scientific™ P/N AC447421000)
- Thermo Scientific™ Dionex™ Perchlorate-ISTD (^{18}O -enriched Internal Standard, P/N 062923)
- Sodium Hydroxide Solution (50% w/w/Certified), Fisher Chemical™ (Fisher Scientific P/N SS254-500)

Samples

- Five infant formula samples were provided by the National Institute of Standards and Technology (NIST™). These were labeled, B, C, D, E, and F.
- Infant formula T from a local store

Conditions	
IC system	Dionex Integrion HPIC system
MS detector	ISQ EC single quadrupole mass spectrometer
Columns	Dionex IonPac AG20 Guard, 2 × 50 mm (P/N 063066) Dionex IonPac AS20 Analytical, 2 × 250 mm (P/N 063065)
Eluent source	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600
Gradient	<i>Time (min)</i> <i>KOH (mM)</i> <i>Divert valve</i>
	-3.0 5.0 Eluent to waste
	0.0 5.0
	11.0 5.0 Eluent to MS
	18.0 5.0
	18.0 22.0
	40.0 22.0 Eluent to waste
	45.0 80.0
	48.0 80.0
48.0 5.0	
50.0 Stop run	
Flow rate	0.25 mL/min
Injection volume	100 µL
Temperature	4 °C (Autosampler tray temperature), 30 °C (column compartment), 20 °C (detector compartment)
System backpressure	~2350 psi (with backpressure coils) (100 psi = 0.6894 MPa)
Detection	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm), AutoSuppression, suppressor current 50 mA, external water mode via AXP Pump, external water flow rate (0.5 mL/min)
Background conductance	~0.3 µS/cm
Run time	50 min

System configuration

Figure 1 shows the schematic of the setup used for this study. In this method, an auxiliary six-port valve is placed between the conductivity detector and the mass spectrometer (MS) to divert suppressed eluent either to the MS or to the suppressor as regenerant, then to the CR-ATC, and then to waste. Diverting ions to waste can minimize MS ion source contamination from the sample.

The auxiliary six-port valve is plumbed as shown in Figure 1, with DI water flow from the Dionex AXP Auxiliary Pump flowing to the ISQ EC mass spectrometer. The connection to the MS is not made until the background

Mass spectrometric detection	
Ionization interface	Electrospray ionization (ESI), negative mode
Divert valve switch time	11.0–40.0 min (-3.0–11.0 min to waste, 11.0–40.0 min to ISQ EC MS, 40.0–50.0 min to waste)
Gas control	Sheath gas pressure: 50 psi Aux gas pressure: 5 psi Sweep gas pressure: 0.0 psi
Source voltage	-2500 V
Vaporizer temperature	450 °C
Ion transfer tube temperature	200 °C
Component mode	See Table 1
Groups	Min. baseline peak width: 25 s Desired scans per peak: 6 SIM scan width: 0.7 amu

Table 1. Component mode parameters

Name	Start time (min)	End time (min)	Mass	Ion polarity	Source CID voltage
Chlorate 83	11	20	83	Negative	40
Chlorate 85	11	20	85	Negative	40
Chlorate ISTD	11	20	89	Negative	40
Perchlorate 99	28	40	99	Negative	40
Perchlorate 101	28	40	101	Negative	40
Perchlorate ISTD	28	40	107	Negative	40

conductivity is below 1.5 µS/cm. The auxiliary valve can be configured as A position or B position. Figure 1 shows the liquid flow paths through the valve ports at each valve position. In the A position, eluent from the conductivity detector is sent to the MS. At the same time, the DI water delivered by the AXP pump is sent to the suppressor. In the B position, the suppressor runs in recycle mode that uses the suppressed conductivity cell effluent as the source of water for the regenerant. Instead, the DI water delivered by the AXP pump is sent to the MS. More information on setting up the ISQ EC with an IC system can be found in Application Update 72507.⁴

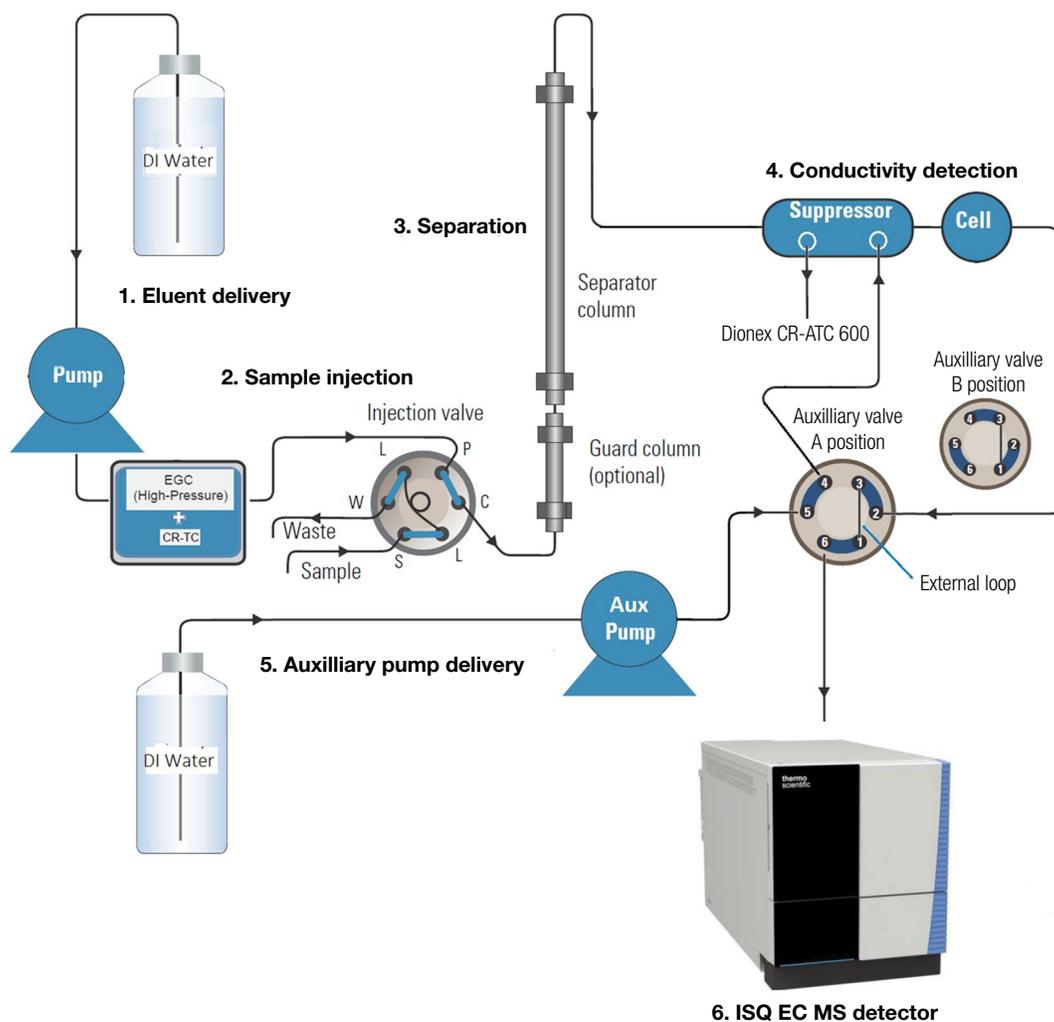


Figure 1. IC-MS configuration with matrix diversion

Preparation of solutions and reagents

Note: Plasticware was used to avoid adsorption of the analytes onto glass surfaces.

Stock solution

A 1,000 mg/L solution of perchlorate is prepared by dissolving 0.1231 g of sodium perchlorate (NaClO_4 , anhydrous, 99% pure grade, or better, CASRN 7601-89-0) in 100 mL DI water. The solution may be stored in a HDPE screw-cap bottle. The anhydrous sodium perchlorate salt should be stored in a desiccator to minimize absorption of water from the atmosphere. The recommended holding time is one year.

The 1 mg/L internal standard (ISTD) solution of ^{18}O chlorate is prepared by adding 143.7 μL of 100 $\mu\text{g}/\text{mL}$ potassium chlorate into 9.856 mL DI water. The recommended internal standard concentration in sample solution is 1 $\mu\text{g}/\text{L}$ (equivalent to 0.2 mg/kg).

Working standard solutions

Prepare the standard solutions by diluting the 1000 mg/L stock standard with 0.02% NaOH. The standard solutions for matrix-extracted calibration standards (MES) were 0.25, 0.5, 1, 2, 5, 10 mg/L for chlorate and perchlorate. For example, a 10 mg/L solution of chlorate/perchlorate mix is prepared by adding 1 mL of 1,000 mg/L chlorate and 1 mL of 1,000 mg/L perchlorate into 98 mL 0.02% NaOH.

Sample preparation

Preparation of 0.02% (w/w) sodium hydroxide solution

Add 0.4 g of 50% w/w NaOH into 999.6 g DI water.

Step-by-step procedure

- Weigh 0.1 g infant formula powder
- Add 20 μ L 1 mg/L chlorate ISTD and 20 μ L 1 mg/L perchlorate ISTD into Samples B, C, F, and T. Then, dissolve Samples B, C, F, and T in 20 mL 0.02% NaOH solution, which is recommended as a starting point and is good for most samples. However, due to the matrix effect and concentration of chlorate and perchlorate in some samples, volumes need to be adjusted. For example, to make the concentration fall within the calibration range, add 10 μ L 1 mg/L chlorate ISTD and 10 μ L 1 mg/L perchlorate ISTD into Sample D then add 10 mL 0.02% NaOH solution. To minimize the matrix effect, dissolve Sample E in 50 mL 0.02% NaOH solution with 50 μ L 1 mg/L chlorate ISTD and 50 μ L 1 mg/L perchlorate ISTD. Shake for 30 min.
- Transfer 5 mL to a 50 mL Amicon Ultra-15 centrifugal filter device and cap. Centrifuge for 30 min at 5,000 rpm at 20 °C.
- The ultrafiltrate in the centrifuge tube is used for IC-MS analysis.

Sensitivity study

- Weigh 1 g infant formula Sample B.
- Add 50 μ L 1 mg/L chlorate ISTD and 50 μ L 1 mg/L perchlorate ISTD into Sample B spiked with a 50 μ L 1 mg/L perchlorate (final conc. = 0.05 mg/kg).
- Dissolve Sample B in 200 mL 0.02% NaOH solution. Shake for 30 min.
- Finish the preparation using the same last two steps as in the step-by-step procedure.

Matrix-extracted calibration standards (MES)

All unspiked samples were found to contain chlorate. To determine the amount of chlorate in the infant formula, a standard addition curve for chlorate was constructed using

MES and an internal standard, and the calibration curve for perchlorate was constructed using MES and an internal standard.

- Weigh 0.1 g infant formula Sample B.
- Add 20 μ L 1 mg/L chlorate ISTD and 20 μ L 1 mg/L perchlorate ISTD into Sample B spiked with a 20 μ L aliquot of chlorate and perchlorate (0.25, 0.5, 1, 2, 5, and 10 mg/L, respectively). The concentrations of MES calibration standards were 0.05, 0.1, 0.2, 0.4, 1, and 2 mg/kg.
- Dissolve Sample B in 20 mL 0.02% NaOH solution. Shake for 30 min.
- Finish the preparation using the same last two steps as in the step-by-step procedure.

Precision study

- Weigh 0.1 g infant formula Sample B.
- Add 20 μ L 1 mg/L chlorate ISTD and 20 μ L 1 mg/L perchlorate ISTD into Sample B spiked with 20 μ L 1 mg/L chlorate/perchlorate working solution (final conc. = 1 μ g/L).
- Dissolve Sample B in 20 mL 0.02% NaOH solution. Shake for 30 min.
- Finish the preparation using the same last two steps as in the step-by-step procedure.

Recovery study

- Weigh 0.1 g infant formula Sample C.
- Add 20 μ L 1 mg/L chlorate ISTD and 20 μ L 1 mg/L perchlorate ISTD into Sample C spiked with a 20 μ L aliquot of chlorate and perchlorate at three levels (0.5, 1, and 2.5 mg/L, equivalent to 0.1, 0.2, and 0.5 mg/kg in the sample).
- Dissolve Sample C in 20 mL 0.02% NaOH solution. Shake for 30 min.
- Finish the preparation using the same last two steps as in the step-by-step procedure.

Results and discussion

IC-MS separation and peak identification

The separation of chlorate and perchlorate was performed on a Dionex IonPac AS20 column set with a KOH gradient at 0.25 mL/min flow rate. The elution method started at 5 mM KOH for 18 min to elute chlorate, then to 22 mM KOH at 18 min, held at 22 mM KOH until 40 min to elute perchlorate, gradually increased up to 80 mM KOH at 45 min, held at 80 mM KOH for 3 min, and returned to 5 mM KOH at 48 min to re-equilibrate the column for 2 min prior to the next injection. The total run time was 50 min. The KOH eluent was neutralized using a Dionex ADRS 600 anion dynamically regenerated suppressor (2 mm) prior to suppressed conductivity and MS detections.

Assay specificity was assessed by comparing the chromatograms of standards with samples. Infant formula samples contain complex matrix components that can interfere with the compounds of interest. For chlorate and perchlorate, RIA is considered as an identification criterion. It was confirmed using naturally occurring chlorine isotopes, with chlorine masses of 34.969 Da (75.78% abundance) and 36.966 Da (24.22% abundance).

Figure 2 shows the conductivity and SIM chromatograms of a standard containing chlorate (m/z 83 and 85), perchlorate (m/z 99 and 101), and their internal standards (m/z 89 and 107, respectively) each at 1.0 $\mu\text{g/L}$ in 0.02% NaOH solution. Figure 3 shows conductivity and SIM chromatograms of an infant formula sample spiked with chlorate (m/z 83 and 85), perchlorate (m/z 99 and 101), and their internal standards (m/z 89 and 107, respectively) each at 1.0 $\mu\text{g/L}$. These figures show that compared to conductivity detection, IC-MS selectivity for chlorate and perchlorate was excellent with sharp peaks detected for their quantifier ions. The selectivity IC-MS in the SIM mode also allows chlorate and perchlorate to be quantified from interfering matrix ions in the infant formula samples. Measured RIAs in the sample met expected values, with differences usually <20%.

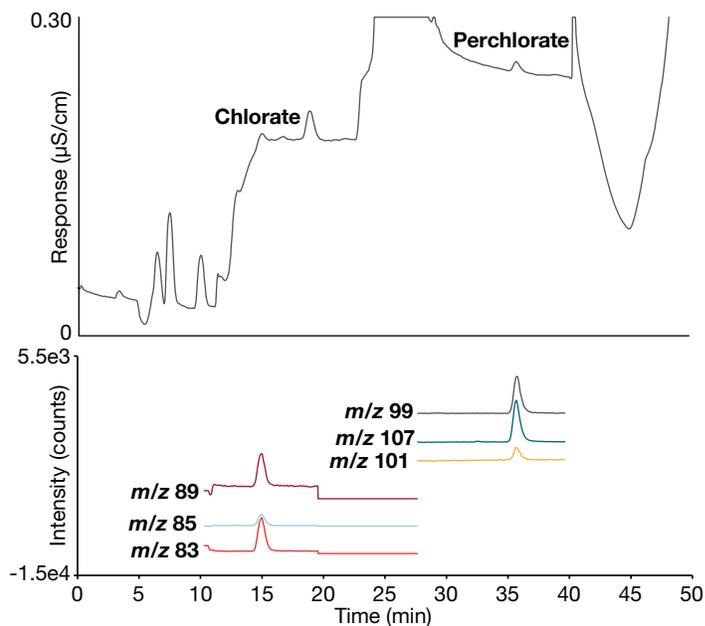


Figure 2. Conductivity and SIM chromatograms of a standard containing chlorate, perchlorate, and their internal standards each at 1.0 $\mu\text{g/L}$ in 0.02% NaOH solution

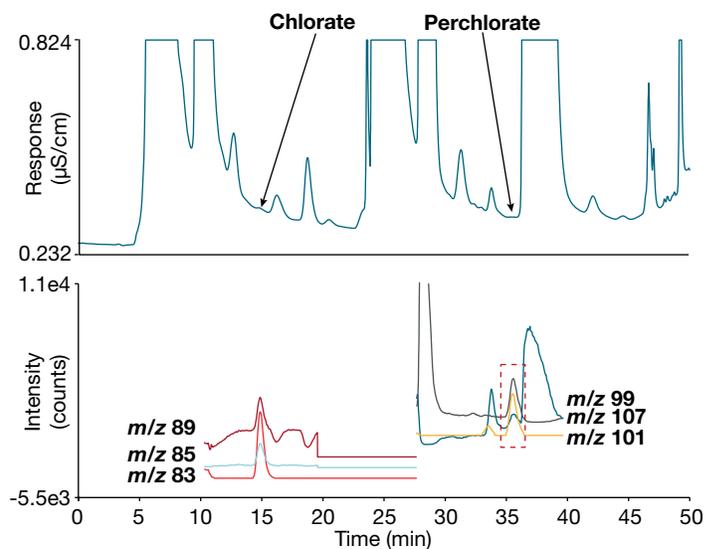


Figure 3. Conductivity and SIM chromatograms of an infant formula sample spiked with chlorate, perchlorate, and their internal standards each at 1.0 $\mu\text{g/L}$

Sensitivity and calibration

Method sensitivity was assessed at 0.05 mg/kg of chlorate ISTD, perchlorate, and perchlorate ISTD in infant formula. As each unspiked sample contains chlorate, we used isotopically labeled chlorate to evaluate the sensitivity of chlorate. Figure 4 shows good sensitivity for chlorate and perchlorate at 0.05 mg/kg in infant formula (equivalent to 0.25 µg/L).

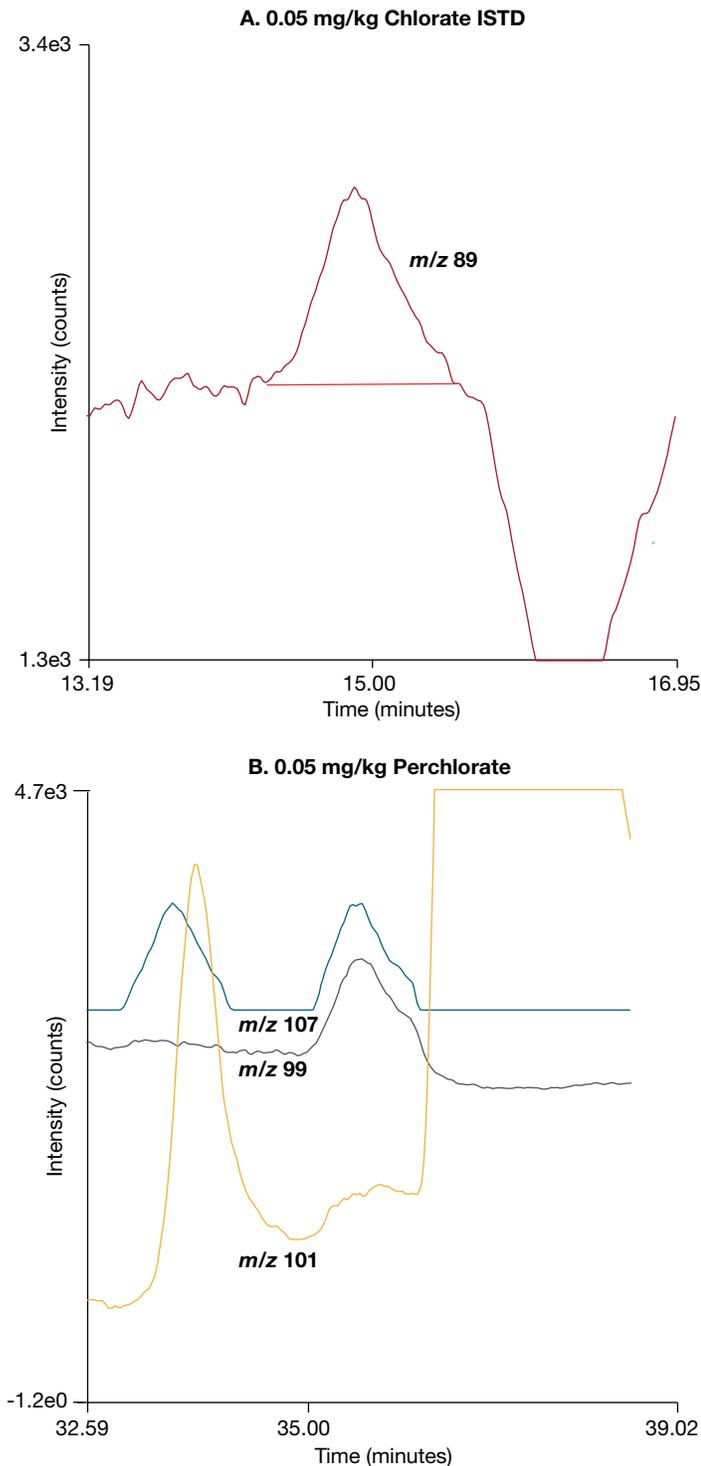


Figure 4. SIM chromatograms of 0.05 mg/kg chlorate ISTD (A), and perchlorate and perchlorate ISTD (B) in an infant formula sample

To determine the content of chlorate and perchlorate in infant formula, a standard addition curve for chlorate was constructed using MES and an internal standard ($^{35}\text{Cl}^{18}\text{O}_3^-$, m/z 89), and the calibration curve for perchlorate was constructed using MES and an internal standard ($^{35}\text{Cl}^{18}\text{O}_4^-$, m/z 107). This method of standard addition is used in situations where the sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach. Two conditions must be fulfilled for successful application of the method of standard additions:

- The calibration graph must be linear.
- The calibration curve of the analyte passes through the origin.

A standard addition curve for chlorate is shown in Figure 5 from 0.05 to 2 mg/kg with a coefficient of determination (r^2) of 0.9999 (Table 2).

Figure 6 demonstrates the good linearity of the calibration plot for perchlorate over the range of 0.05 to 2 mg/kg in the matrix. The calibration curve for perchlorate was linear with a coefficient of determination (r^2) of 0.9998 (Table 2).

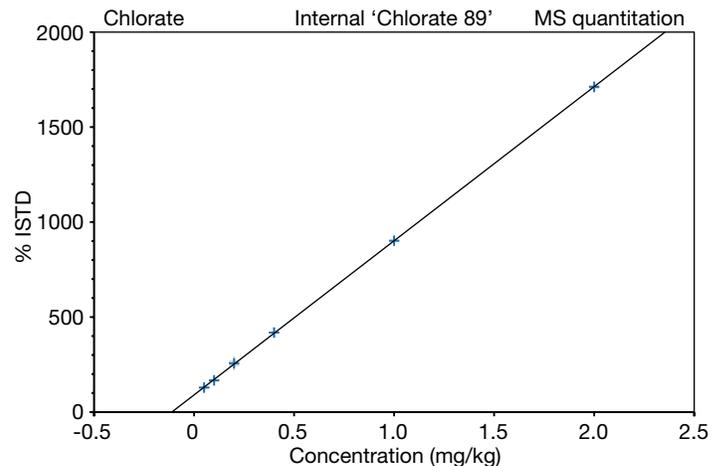


Figure 5. The standard addition curve for chlorate ranging from 0.05 to 2 mg/kg using MES and the internal standard

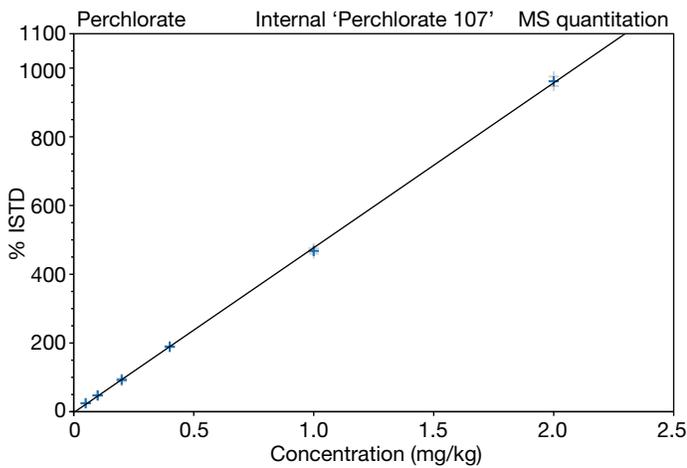


Figure 6. The calibration curve for perchlorate ranging from 0.05 to 2 mg/kg using MES and the internal standard

Table 2. Method calibration

Compound	Range (mg/kg)	Quantifier ions	Curve fit	Coefficient of determination (r^2)
Chlorate	0.05–2	m/z 83	Linear	0.9999
Perchlorate	0.05–2	m/z 99	Linear, WithOffset	0.9998

Sample analysis

Chlorate and perchlorate concentrations were determined in a broad range of powdered infant formula products, including both finished products (Samples B, C, F, and T) and ingredients (Samples D and E). Eighteen samples, three from each infant formula, were analyzed for chlorate and perchlorate contents. Due to the complexity of the matrix, RIAs (chlorate, m/z 83/85 and perchlorate, m/z 99/101) at the retention time of the internal standard were monitored to confirm the peaks of chlorate and perchlorate in a sample.

The concentration (mg/kg) of chlorate and perchlorate was calculated using their calibration curves. The content (mg/kg) of chlorate and perchlorate in the samples was calculated as below:

$$\text{Content (mg/kg)} = \text{Calculated concentration (mg/kg)} \times \frac{\text{Extraction volume (mL)}}{20 \text{ mL}}$$

With the sample preparation used, Samples B, C, F, and T have an extraction volume of 20 mL, Sample D has an extraction volume of 10 mL, and Sample E has an extraction volume of 50 mL.

As shown in Table 3, variability was found in the chlorate content of infant formula among individuals. The chlorate range for all samples is 0.0656–2.16 mg/kg. The measured RIA of chlorate is between 2.84 and 3.17 within 3.13 (naturally) \pm 20% (Table 3). No perchlorate was detected in the samples.

Table 3. The contents of chlorate and perchlorate in infant formula

	Chlorate (mg/kg)	RIA (m/z 83/85)	Perchlorate (mg/kg)
Sample B1	0.110	3.31	n.d.
Sample B2	0.104	2.75	n.d.
Sample B3	0.0982	2.80	n.d.
Mean	0.104	2.95	-
RSD (n=3)	5.67	10.5	-
Sample C1	0.294	3.12	n.d.
Sample C2	0.290	2.87	n.d.
Sample C3	0.302	2.90	n.d.
Mean	0.295	2.96	-
RSD (n=3)	2.07	4.61	-
Sample D1	0.0663	3.23	n.d.
Sample D2	0.0660	3.03	n.d.
Sample D3	0.0645	3.25	n.d.
Mean	0.0656	3.17	-
RSD (n=3)	1.47	3.84	-
Sample E1	2.08	3.04	n.d.
Sample E2	2.20	3.26	n.d.
Sample E3	2.20	3.04	n.d.
Mean	2.16	3.11	-
RSD (n=3)	3.21	4.08	-
Sample F1	0.308	2.91	n.d.
Sample F2	0.317	2.81	n.d.
Sample F3	0.309	2.79	n.d.
Mean	0.311	2.84	-
RSD (n=3)	1.58	2.27	-
Sample T1	0.348	2.86	n.d.
Sample T2	0.348	2.94	n.d.
Sample T3	0.370	2.99	n.d.
Mean	0.355	2.93	-
RSD (n=3)	3.57	2.24	-

n.d.= not detectable

Precision

The precision of the method was evaluated by triplicate injections of sample B spiked with 1 µg/L chlorate and perchlorate (equivalent to 0.2 mg/kg), respectively, with injections made over four consecutive days. The calculation of the relative standard deviation (RSD) was performed using all twelve injections. The content RSDs and the retention time RSDs for chlorate were 2.80% and 0.19%, respectively, and for perchlorate were 3.13% and 0.10% (Table 4).

Table 4. Retention time and content precisions (n=12) for spiked infant formula B analysis

Analyte	Content RSD	Retention time RSD
Chlorate	2.80	0.19
Perchlorate	3.13	0.10

Accuracy

Method accuracy was validated by determining the recovery of chlorate and perchlorate in spiked infant formula Sample C over three levels (0.1, 0.2, 0.5 mg/kg), with five replicates of each concentration. Sample B was chosen to make MES and evaluate the precision. Therefore, Sample C was chosen for the recovery study to evaluate method accuracy. Recoveries were calculated as the amount of spiked analyte found as a percentage to the theoretically spiked amount added, as shown below:

$$\text{Recovery \%} = \frac{\text{Observed value} - \text{Original sample value}}{\text{Added value}} \times 100$$

Table 5. Method accuracy (n=5) for infant formula Sample C

Analyte	Original sample (mg/kg)	Mean observed (mg/kg)	Spike level (mg/kg)	Mean recovery (%)	Mean RSD
Chlorate	0.285	0.386	0.1	101	10.4
		0.488	0.2	102	8.48
		0.786	0.5	100	6.69
Perchlorate	n.d.	0.105	0.1	105	3.01
		0.200	0.2	100	4.08
		0.498	0.5	99.6	1.73

Table 5 shows the recovery ranged from 99.6 to 105%, indicating that this method can be applied to the determination of chlorate and perchlorate in infant formula.

Conclusion

This study describes an IC-MS method for the determination of chlorate and perchlorate in powdered infant formula. The sample preparation was optimized for effective extraction and cleanup and can be applied to various powdered dairy samples. The method uses a Dionex IonPac AS20 column on an Integriion IC system, which electrolytically generated the eluent for separation, coupled to an ISQ EC single quadrupole mass spectrometer. The method showed good sensitivity for the detection, identification, and quantification of chlorate and perchlorate. The accuracy and precision of the method determined at different concentrations gave excellent results.

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