Determination of chlorate and perchlorate in milk using ion chromatography-mass spectrometry

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Goal

To develop an IC-MS method for the determination of chlorate and perchlorate in milk

Introduction

Chlorate in milk and dairy products can arise from chlorinated water and chlorine-contaiing disinfectants for cleaning and sanitation of process equipment at both the farm and food processor stages. Perchlorate is an environmental contaminant that occurs naturally, primarily near potash deposits and in arid regions. It can also originate from perchlorate salts in military and industrial products such as solid rocket fuels, explosives, fireworks, and some fertilizers.[1]

Chlorate and other oxychlorine species have been associated with inhibition of iodine uptake in humans and the formation of methemoglobin. Infants and young children are at high-risk due to their greater dependency on milk products and higher consumption/body weight ratio.[2]

Chlorate and perchlorate have traditionally been analyzed by ion chromatography (IC). More recently, IC coupled

to a mass spectrometer (IC-MS) has become a highly preferred method for determining chlorate and perchlorate in complex sample matrices. MS detection provides greater data confidence while minimizing labor-intensive sample preparation.

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 MS. With a simple sample preparation, this method offers an easy approach to quantifying chlorate and perchlorate in milk. The Dionex IonPac AS20 column is a high capacity, hydroxide selective anion exchange column designed to separate perchlorate from cations

and anions in a variety of samples. The ISQ EC mass spectrometer provides better sensitivity and selectivity for chlorate and perchlorate over suppressed conductivity detection. In addition to using retention times and the nominal mass of the pseudo molecular ions ($[M-H]^-$), the chlorinated compounds were further confirmed using isotope ratio.[3] 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
  - Detector-suppressor compartment temperature control
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074926) with sample syringe, 250 µL, and buffer line, 1.2 mL
- Thermo Scientific™ Dionex™ 6-port high-pressure valve (P/N 22153-60014)
- Thermo Scientific™ ISQ EC™ single quadrupole mass spectrometer (P/N ISQEC–IC)
- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)
- Peak Scientific™ Genius™ 1022 nitrogen generator (P/N 1R77606-3230)

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)
- Dionex IC PEEK Viper Fitting Tubing Assembly Kits (P/N 088798)
- Dionex AS-AP Autosampler Vials 10 mL (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 resistivity or better
- Thermo Scientific™ Dionex™ Chlorate Standard, 1000 mg/L (P/N 303170)
- Potassium chlorate (90-95% chemical purity) ($^{18}O_3$, 98%) 100 µg/mL in ^{18}O-water, Cambridge Isotope Laboratories (Cambridge Isotope Laboratories, P/N OLM-10485-1.2)
- Sodium perchlorate, ($NaClO_4$, anhydrous, 99% pure grade, or better, F.W. = 122.4, CASRN 7601-89-0) Acros Organics™ (Fisher Scientific™ P/N AC447421000)
- Thermo Scientific™ Dionex™ Perchlorate-ISTD (^{18}O-enriched Internal Standard at 1 mg/L, P/N 062923)
- Sodium hydroxide solution (50% w/w/Certified), Fisher Chemical™ (Fisher Scientific P/N SS254-500)

Samples

- Vitamin D milk
- Organic whole milk
- 2% reduced-fat milk
- Fat-free milk

Note: Samples were purchased 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	*Time (min)* / *KOH (mM)* / *Divert valve* -3.0 / 5.0 / Eluent to waste 0.0 / 5.0 12.0 / 5.0 / Eluent to MS 18.0 / 5.0 18.0 / 15.0 42.0 / 15.0 45.0 / 80.0 / Eluent to waste 52.0 / 80.0 52.0 / 5.0 55.0 / 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	58 min

Mass spectrometric detection							
Ionization interface	Electrospray ionization (ESI), negative mode						
Divert valve time to the MS	12.0–45.0 min						
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						
Scan mode	*Time (min)*	*Name*	*Mass list or range (amu)*	*Dwell or scan times (s)*	*SIM widths (amu)*	*Ion polarity*	*Source CID voltage*
	12–20	Chlorate 83	83	1.2	0.3	Negative	40
		Chlorate 85	85	1.2	0.3	Negative	40
		Chlorate ISTD	89	1.2	0.3	Negative	40
	35–45	Perchlorate 99	99	1.2	0.3	Negative	40
		Perchlorate 101	101	1.2	0.3	Negative	40
		Perchlorate ISTD	107	1.2	0.3	Negative	40
Groups:	Min. baseline peak width: 25 s						

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 MS to divert suppressed eluent either to the MS or 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-MS Auxiliary Pump flowing to the ISQ EC MS. The connection to the MS is not made until the background 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. Simultaneously, the DI water delivered by the Dionex AXP-MS 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 and the DI water delivered by the Dionex AXP-MS pump is sent to the MS. More information on setting up the ISQ EC MS with an IC system can be found in Application Update 72507.[4]

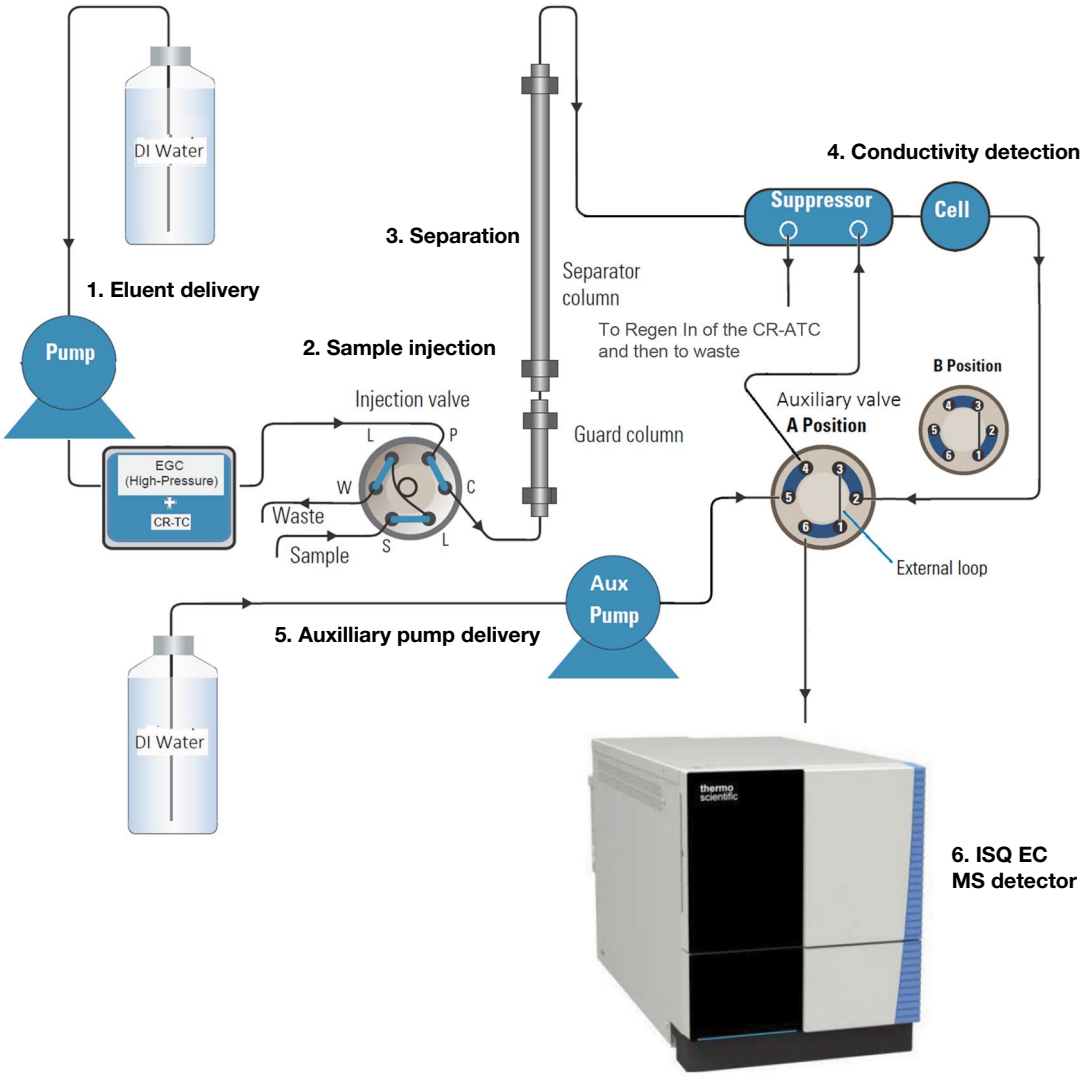

Figure 1. IC-MS configuration with matrix diversion

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 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 internal standard (ISTD) solution of ^{18}O chlorate at 1 mg/L is prepared by adding 143.7 µL of 100 µg/mL potassium chlorate ^{18}O standard into 9.856 mL DI water. The recommended internal standard concentration is 1 µg/L in the final sample solution.

Working standard solutions

Prepare the standard solutions by diluting the 1000 mg/L stock standard with 0.02% NaOH. 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. Next, a 100 µg/L solution of chlorate/perchlorate mix is prepared by adding 1 mL of 10 mg/L chlorate/perchlorate mix into 99 mL 0.02% NaOH.

Calibration standard solutions

Calibration standards were prepared by diluting the 100 µg/L working standard to 0.1, 0.25, 0.5, 1, 2, 5, and 10 µg/L with 0.02% NaOH. For example, a 10 µg/L solution of chlorate/perchlorate mix is prepared by adding 1 mL of 100 µg/L chlorate/perchlorate mix into 9 mL 0.02% NaOH. Add 10 µL 1 mg/L chlorate ISTD and 10 µL 1 mg/L perchlorate ISTD to each 10 mL standard.

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

- Dilute 1 mL milk with 49 mL 0.02% NaOH solution.
- Add 50 µL 1 mg/L chlorate ISTD and 50 µL 1 mg/L perchlorate ISTD into diluted milk samples. This is a recommended starting point and is good for most samples. Shake for 30 min.
- Transfer 5 mL to a 50 mL Amicon Ultra-15 centrifugal filter device and cap. Centrifuge for 30 min at 5000 rpm at 20 °C.
- The ultrafiltrate in the centrifuge tube is used for IC-MS analysis.

Sensitivity study

- Add 2 µL of 1 mg/L chlorate ISTD and 1 µL 1 mg/L perchlorate ISTD into 2 mL vitamin D milk (final con. = 1 µg/L chlorate ISTD, and 0.5 µg perchlorate ISTD in milk).
- Dilute 2 mL vitamin D milk with 18 mL 0.02% NaOH solution. Shake for 30 min.
- Finish the preparation using the same final two steps as in the step-by-step procedure.

Precision study

- Dilute 1 mL vitamin D milk with 49 mL 0.02% NaOH solution.
- Add 50 µL 1 mg/L chlorate ISTD and 50 µL 1 mg/L perchlorate ISTD into the sample solution that has been spiked with 50 µL 1 mg/L chlorate/perchlorate working solution (final con. = 1 µg/L). Shake for 30 min.
- Finish the preparation using the same final two steps as in the step-by-step procedure.

Recovery study

- Dilute 1 mL vitamin D milk with 49 mL 0.02% NaOH solution.
- Add 50 µL of 1 mg/L chlorate ISTD and 50 µL of 1 mg/L perchlorate ISTD into the sample solution spiked with a 50 µL aliquot of chlorate and perchlorate at three levels (1, 2, and 4 mg/L, equivalent to 1, 2, and 4 µg/L in the sample solution). Shake for 30 min.
- Finish the preparation using the same final two steps as in the step-by-step procedure.

Software

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

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. The elution method started at 5 mM KOH for 18 min to elute chlorate, then to 15 mM KOH at 18 min, held at 15 mM KOH until 42 min to resolve perchlorate from interfering peaks, gradually increased up to 80 mM KOH at 45 min, held at 80 mM KOH for 7 min, and returned to 5 mM KOH at 52 min to re-equilibrate the column for 6 min prior to the next injection. The total run time was 58 min. The KOH eluent was neutralized using a Dionex ADRS 600 2 mm dynamically regenerated suppressor before suppressed conductivity and MS detections.

Chlorate and perchlorate were confirmed with authentic standards through matching retention times, *m/z*, and isotope ion ratios. The isotope ion ratio, defined as the ratio between the monoisotopic ion M as a quantifier ion and the M+2 ion corresponding to the natural isotope ^{37}Cl, has been calculated as a confirmation criterion. The chlorinated compounds have naturally occurring chlorine isotopes, ^{35}Cl (75.78%) and ^{37}Cl (24.22%). The theoretical isotope ion ratio is 3.13. Milk samples are complex and present a challenge to accurate quantification of chlorate and perchlorate.

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 µg/L in 0.02% NaOH solution. For chlorate, the isotope ion ratio is 2.88, and for perchlorate it is 3.00. Figure 3 shows conductivity and SIM chromatograms of a vitamin D milk 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 µg/L in a final solution before ultrafiltration. For chlorate, the isotope ion ratio is 2.96, and for perchlorate is 2.61. These figures show that IC-MS provides superior selectivity and sensitivity for chlorate and perchlorate than suppressed conductivity detection does, especially in complex matrices. The tolerance of the ion ratios must not exceed those from Decision 2002/657/EC [5]: A measured isotope ion ratio should be between 2.31 and 3.95, ±25% of the natural ion abundance (note that we are using the values for LC-MS as IC-MS is not specified). This is also the range specified in the United States Environmental Protection Agency's Method 332.0 for the determination of perchlorate in drinking water by IC-MS.[6] The measured isotope ion ratio in the spiked milk sample met expected values, with differences <25%.

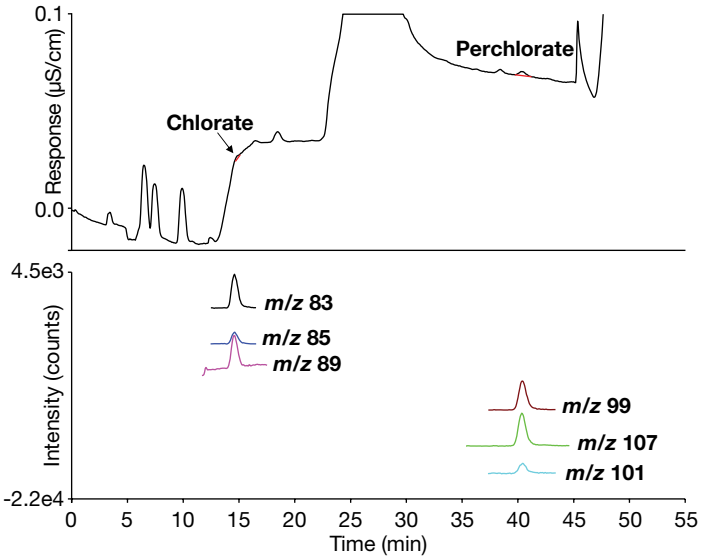

Figure 2. Conductivity and SIM chromatograms of a standard containing chlorate, perchlorate, and their internal standards (each at 1.0 µg/L in 0.02% NaOH solution)

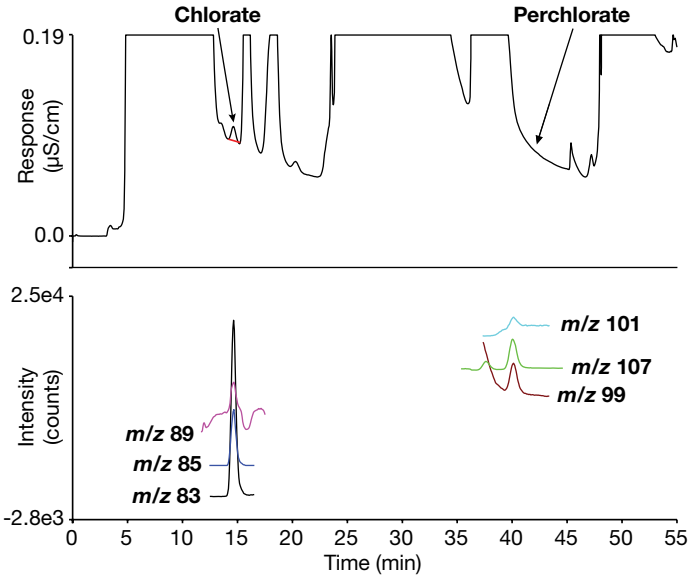

Figure 3. Conductivity and SIM chromatograms of a vitamin D milk sample spiked with chlorate, perchlorate, and their internal standards (each at 1.0 µg/L in a final solution before ultrafiltration)

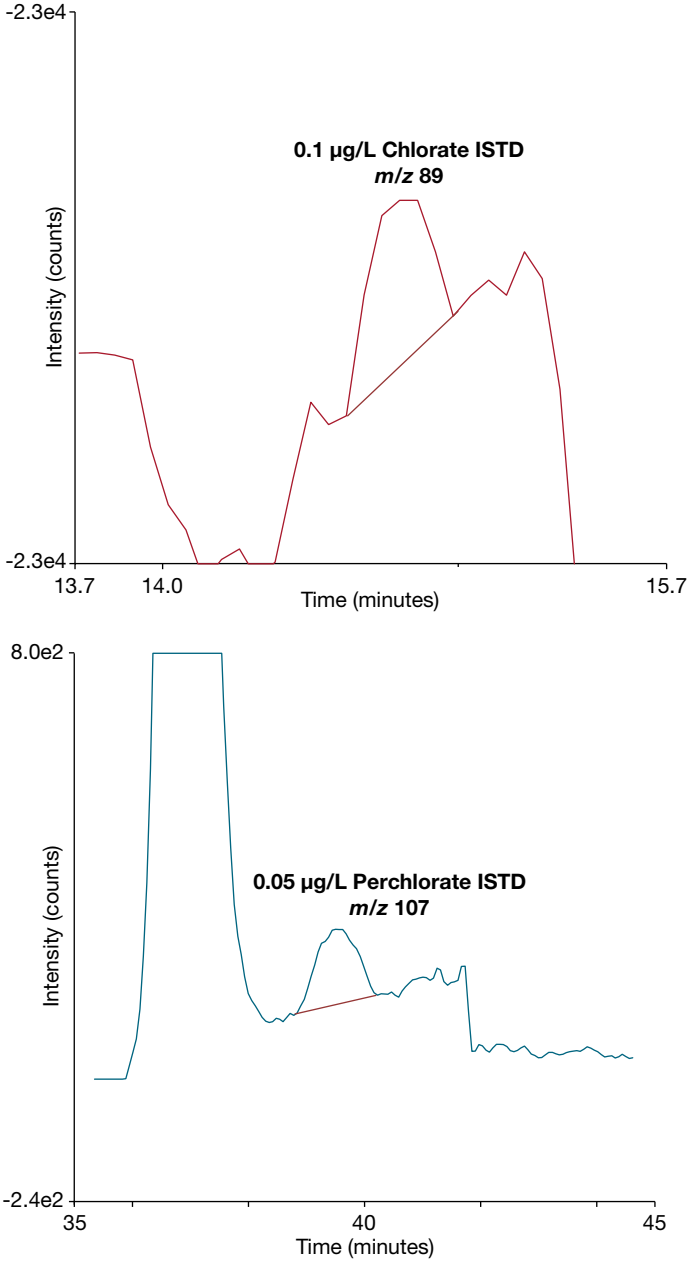

Figure 4. SIM chromatograms of 0.1 µg/L chlorate ISTD and 0.05 µg/L perchlorate ISTD in a vitamin D milk sample

Sensitivity and calibration

The sensitivity of the method was assessed using the chlorate ISTD at 0.1 µg/L, and the perchlorate ISTD at 0.05 µg/L in a vitamin D milk sample. As each unspiked sample contains chlorate and perchlorate, we use isotopically labeled chlorate and perchlorate to evaluate chlorate and perchlorate sensitivity. Figure 4 shows good sensitivity for chlorate at 0.1 µg/L and perchlorate at 0.05 µg/L in a vitamin D milk sample.

To determine the content of chlorate and perchlorate in milk, calibration curves with seven concentration levels were constructed for chlorate and perchlorate, ranging from 0.1 to 10 µg/L using the internal standard method (Figures 5 and 6). Each of the standards was injected in triplicate. The results yielded a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.9998 for chlorate and 0.9999 for perchlorate (Table 1).

Sample analysis

Four samples were purchased locally and analyzed in this study, including vitamin D milk, organic whole milk, 2% reduced-fat milk, and fat-free milk. Due to the matrix complexity, isotope ion ratios (chlorate, *m/z* 83/85 and perchlorate, *m/z* 99/101) at the retention time of the internal standard were monitored to confirm the chlorate and perchlorate peaks in a sample.

Table 1. Method calibration

Compound	Range (µg/L)	Quantifier ions	ISTD ions	Curve fit	Coefficient of determination (r^2)
Chlorate	0.1–10	*m/z* 83	*m/z* 89	Linear, WithOffset	0.9998
Perchlorate	0.1–10	*m/z* 99	*m/z* 107	Linear, WithOffset	0.9999

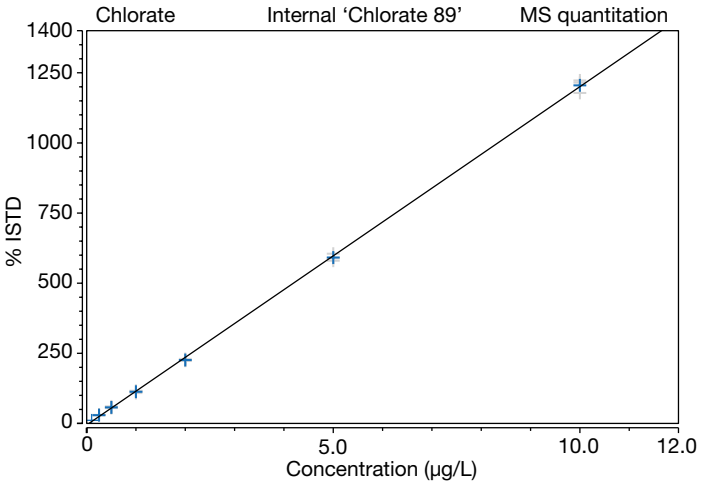

Figure 5. The calibration curve for chlorate ranging from 0.1 to 10 µg/L using the internal standard

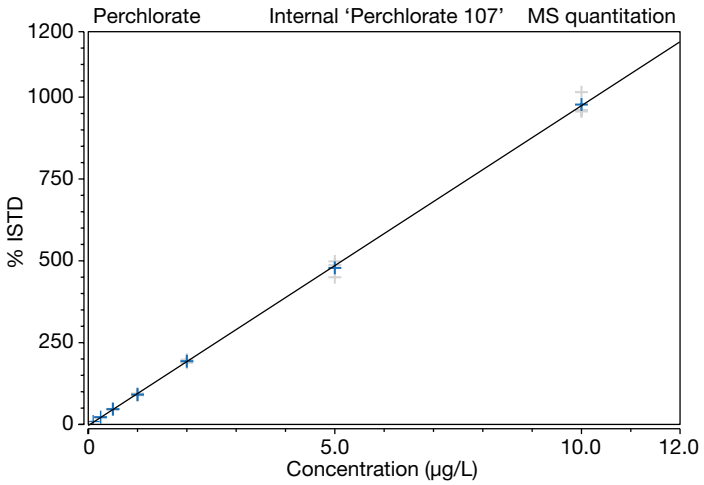

Figure 6. The calibration curve for perchlorate ranging from 0.1 to 10 µg/L using the internal standard

The concentrations (µg/L) of chlorate and perchlorate were calculated using their calibration curves. The contents (µg/L) of chlorate and perchlorate in the samples were calculated as below:

$$\text{Content (µg/L)} = \text{Calculated concentration (µg/L)} \times \frac{50\ \text{mL}}{1\ \text{mL}}$$

Chlorate and perchlorate were found in every sample in this study, with the content of chlorate varying from 152 to 404 µg/L and the content of perchlorate ranging from <LOQ to 7.53 µg/L (Table 2). The isotope ion ratios for chlorate are between 3.04 and 3.12, all within ±25% of the natural ion abundance (Table 2). For perchlorate, the value was not possible to calculate in all samples due to the low concentration levels of some samples.

Table 2. The contents of chlorate and perchlorate in milk samples

Sample	Chlorate (µg/L)	Isotope ion ratio (*m/z* 83/85)	Perchlorate (µg/L)	Isotope ion ratio (*m/z* 99/101)
Vitamin D 1	217	3.04	<LOQ	-
Vitamin D 2	220	3.04	<LOQ	-
Organic whole milk 1	403	3.12	6.10	*m/z* 101 <LOQ
Organic whole milk 2	405	3.18	6.70	*m/z* 101 <LOQ
2% reduced fat 1	152	3.03	<LOQ	-
2% reduced fat 2	153	3.06	<LOQ	-
Fat-free 1	385	3.15	7.45	3.73
Fat-free 2	389	3.10	7.60	3.08

Precision

The precision of the method was evaluated by duplicate injections of vitamin D milk spiked with 1 µg/L chlorate and perchlorate (final solution before ultrafiltration), respectively, and running over four consecutive days. The calculation of the relative standard deviation (RSD) was performed using all eight injections. The content RSDs and the retention time RSDs for chlorate are 1.22% and 0.60%, respectively, and for perchlorate are 3.34% and 0.17% (Table 3).

Table 3. Retention time and content precisions (n=8) for spiked vitamin D milk analysis

Analyte	RT (min)	Content RSD	Retention time RSD
Chlorate	14.6	1.22	0.60
Perchlorate	40.1	3.34	0.17

Accuracy

Method accuracy was validated by determining the recovery of chlorate and perchlorate in spiked vitamin D milk over three levels (1, 2, 4 µg/L), with five replicates of each concentration. Recoveries were calculated as the amount of spiked analyte found as a percentage to the theoretically spiked amount added, shown as below:

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

Table 4 shows the recovery ranged from 88.9 to 109%, indicating that this method can be applied to the determination of chlorate and perchlorate in milk.

Robustness

The current sample preparation substantially improved the robustness of the IC-MS system. From the first to the 958th injection of standards and samples, there was 0.24 min loss of retention time for chlorate. Additionally, we evaluated the retention time stability over the last 200 injections. Over that period, the retention time of perchlorate did not change. After over 900 injections of standards, DI water, and matrix extracts, peak shapes remained stable. The column and the MS source remained clean with no required maintenance.

Conclusion

This study describes an IC-MS method for the determination of chlorate and perchlorate in milk. This method applies a simple and fast sample preparation method without labor-intensive SPE cleanup and highly selective detection using MS detection. The method uses a Dionex IonPac AS20 column on an Integrion IC system, which electrolytically generated the eluent for separation, coupled to an ISQ EC single quadrupole MS. The method showed good sensitivity for the detection, identification, and quantification of chlorate and perchlorate. Excellent linearities ($r^2 > 0.999$) were achieved through the calibration range from 0.1 to 10 µg/L. The accuracy and precision of the method determined at different concentrations gave excellent results.

Table 4. Method accuracy (n=5) for vitamin D milk

Analyte	Endogenous (µg/L)	Mean observed (µg/L)	Spike level (µg/L)	Mean recovery (%)	Mean RSD
Chlorate	4.37	5.46	1	109	2.67
		6.40	2	96.4	2.64
		8.35	4	99.5	1.60
Perchlorate	<LOQ	0.960	1	96.0	1.04
		1.78	2	88.9	2.28
		3.81	4	95.3	2.97

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