

Analysis of Perchlorate in Infant Formula by Ion Chromatography-Electrospray-Tandem Mass Spectrometry (IC-ESI-MS/MS)

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Introduction

Ion chromatography-mass spectrometry (IC-MS/MS) can be used to detect, quantify, and confirm a variety of analytes in environmental and food matrices, including haloacetic acids, bromate, and perchlorate. Perchlorate, a naturally occurring and man-made contaminant, is widely found in the environment in surface water, groundwater, and soil. Through environmental contamination, perchlorate has entered the food supply chain and has been detected in a wide variety of foods including eggs, milk, vegetables, and fruits. In humans, perchlorate interferes with the ability of the thyroid gland to take up iodine, which is needed to produce hormones that regulate many body functions after they are released into the blood. Because these thyroid hormones are essential for normal growth and development, infants and children could more likely be affected by perchlorate than adults.

A recent study by the Centers for Disease Control and Prevention examined various types of commercially available powdered infant formulas to determine if perchlorate could be found at measurable levels.¹ All of the powdered infant formulas tested contained perchlorate. Significantly higher concentrations of perchlorate were found in cow's milk-based formulas with lactose than in other types.

A simple and ultra-sensitive IC-MS/MS technique for the quantitation of perchlorate in powdered infant formula, liquid infant formula, and milk is described here. Unlike conventional detection methods that require labor intensive solid phase extraction (SPE) sample enrichment, only a simple sample preparation is necessary.

Goal

To develop a simple and ultra-sensitive IC-MS/MS method to separate and quantitate perchlorate without time-consuming sample preparation.

Experimental Conditions

Sample Preparation

Samples of commercially available liquid infant formula, reconstituted powdered infant formula, and milk were prepared for analysis. A 4-mL sample of formula or milk was mixed with 4 mL of ethanol (pre-cooled at 4 °C) and

0.4 mL of 3% acetic acid. The sample was spiked with 40 µL (100 ng/mL) isotope-labeled internal standard. The mixture was vortexed and then centrifuged at 5000 rpm for 30 minutes under refrigeration (-5 °C). The supernatant was run through a syringe filter that had been pre-conditioned with 5 mL ethanol and 15 mL of deionized water. The filtrate was collected in 10-mL plastic autosampler vials and readied for IC-MS/MS analysis.

Ion Chromatography

IC analysis was performed on a Thermo Scientific Dionex ICS-3000 Reagent-Free ion chromatography (RFIC) system. The IC conditions were as follows:

Column set:	Thermo Scientific Dionex AG16 / AS16 hydroxide selective anion exchange columns
Suppressor:	Thermo Scientific Dionex ASRS 300 self-regenerated suppressor (external water mode)
Column temperature:	30 °C
Injection volume:	100 µL
Eluent:	Isocratic 45 mM hydroxide
Eluent Source:	EGC III KOH
Solvent:	150 µL/min acetonitrile delivered by an AXP-MS pump
Flow rate:	300 µL/min
Detection:	First detector: Suppressed conductivity Second detector: Thermo Scientific TSQ Quantum Access mass spectrometer

Mass Spectrometry

MS analysis was carried out on a TSQ Quantum Access™ triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

Ion source polarity:	Negative ion mode
Spray voltage:	4000 V
Sheath gas pressure:	40 arbitrary units
Ion sweep gas pressure:	15 arbitrary units
Auxiliary gas pressure:	5 arbitrary units
Capillary temperature:	300 °C
Collision gas pressure:	1.8 mTorr
Scan mode:	Selected reaction monitoring (SRM)

Key Words

- TSQ Quantum Access
- Ion chromatography
- Food safety
- Sensitivity

Selected reaction monitoring allowed the following fragmentation patterns to be observed:

m/z 99 ($^{35}\text{ClO}_4^-$) \rightarrow m/z 83 ($^{35}\text{ClO}_3^-$)	primary transition for native perchlorate (quantitative)
m/z 101 ($^{37}\text{ClO}_4^-$) \rightarrow m/z 85 ($^{37}\text{Cl}^{18}\text{O}_3^-$)	secondary transition for native perchlorate (confirmative)
m/z 107 ($^{35}\text{Cl}^{18}\text{O}_4^-$) \rightarrow m/z 89 ($^{35}\text{Cl}^{18}\text{O}_3^-$)	primary transition for labeled IS (quantitative)

Results and Discussion

Figure 1 shows a representative SRM chromatogram for a perchlorate standard of 20 pg/mL at the low end of the calibration range. Even with such a low concentration, perchlorate shows a well-defined peak that can be accurately quantified.

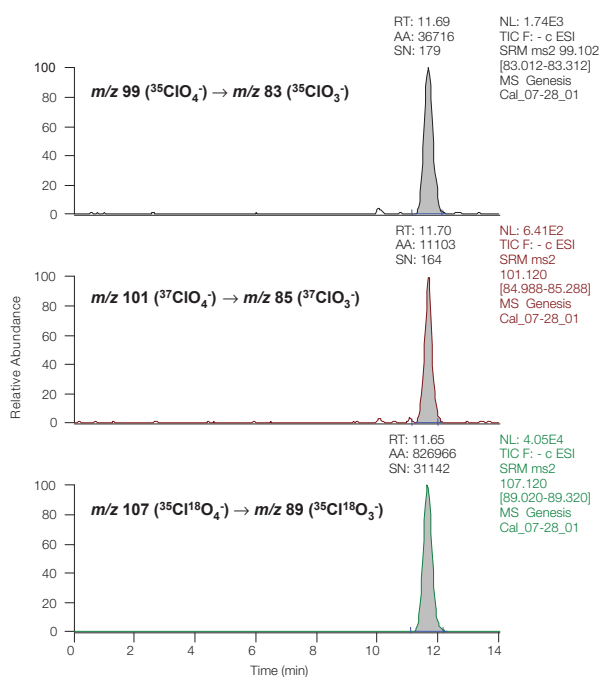


Figure 1: SRM chromatograms of a perchlorate standard at 20 pg/mL

Calibration curves generated on the TSQ Quantum Access mass spectrometer show excellent linearity (Figure 2). For quantitative analysis, the 99 \rightarrow 83 SRM transition of perchlorate was used (ClO_4^- Quan), and for qualitative analysis, the 101 \rightarrow 85 SRM transition of perchlorate was used (ClO_4^- Qual). The labeled IS was used with the quantitative and qualitative ions to calculate the squared correlation coefficients (r^2) of the 99 \rightarrow 83 and 101 \rightarrow 85 SRM transitions of perchlorate, which were 0.9996 and 0.9998, respectively. The data was weighted by $1/X$ to ensure better quantification accuracy for low level samples. The calibration range was 20–10,000 pg/mL.

The instrument lower detection limit was determined to be 5 pg/mL with $S/N > 10$, which is significantly below the lowest reported value (30 pg/mL)¹. The upper calibration limit was set at 10 ng/mL, which covered the highest reported value (5.05 ng/mL)¹. The

practical limit of detection (LOD) was determined by $\text{Amt}_{\text{Blank}} + 3 \times S_0 = 28.9$ pg/mL. The systematic factor $\text{Amt}_{\text{Blank}}$ was calculated by running deionized water instead of real sample through all sample preparation and instrument analysis procedures.

The IC-MS/MS system provides excellent chromatographic performance and allows separation of perchlorate from complex matrix components. Figure 3 displays the SRM chromatograms for perchlorate in unspiked infant formula and milk samples.

The recovery was evaluated by comparing the differences between unspiked and spiked (5 ng/mL) samples ($n=2$, $n=3$). Excellent recoveries were achieved as shown in Table 1.

Table 1. Recovery of perchlorate in infant formula and milk

	Unspiked	Spiked	Recovery
Liquid Infant Formula	1.74 (2.68)	6.91 (4.95)	103%
Milk	2.21 (2.34)	7.93 (2.71)	114%†

†Reported amounts are in the units of ng/mL with %RSD included in parenthesis.

The instrument accuracy and precision were evaluated by repeat injections of standards at three levels, as shown in Table 2.

Table 2. Method performance – Accuracy and precision

Specified Amount	Quantified Amount	%RSD	%Deviation	%RSD _{RT}
100 pg/mL	98.18 pg/mL	3.05	1.82	0.13
1 ng/mL	1.04 ng/mL	1.62	3.52	0.08
10 ng/mL	10.74 ng/mL	1.45	7.36	0.11

Perchlorate was detected in every sample tested in this study. The method precision, evaluated by repeat assays of each unknown sample, was excellent as shown in Table 3. The powdered infant formula sample was quantified at 2.44 ng/mL, and the milk sample was quantified at 4.64 ng/mL. No interference was detected for any of the samples analyzed.

Table 3. Method performance – Real samples

Sample	Quantified Amount (ng/mL)	%RSD
LIF-1	1.74	2.68 (n=3)
LIF-2	2.21	2.34 (n=3)
LIF-3	1.05	3.63 (n=7)
PIF-1	2.44	1.74 (n=3)
MLK	4.64	1.96 (n=7)

LIF: Liquid infant formula; PIF: Powdered infant formula; MLK: Milk

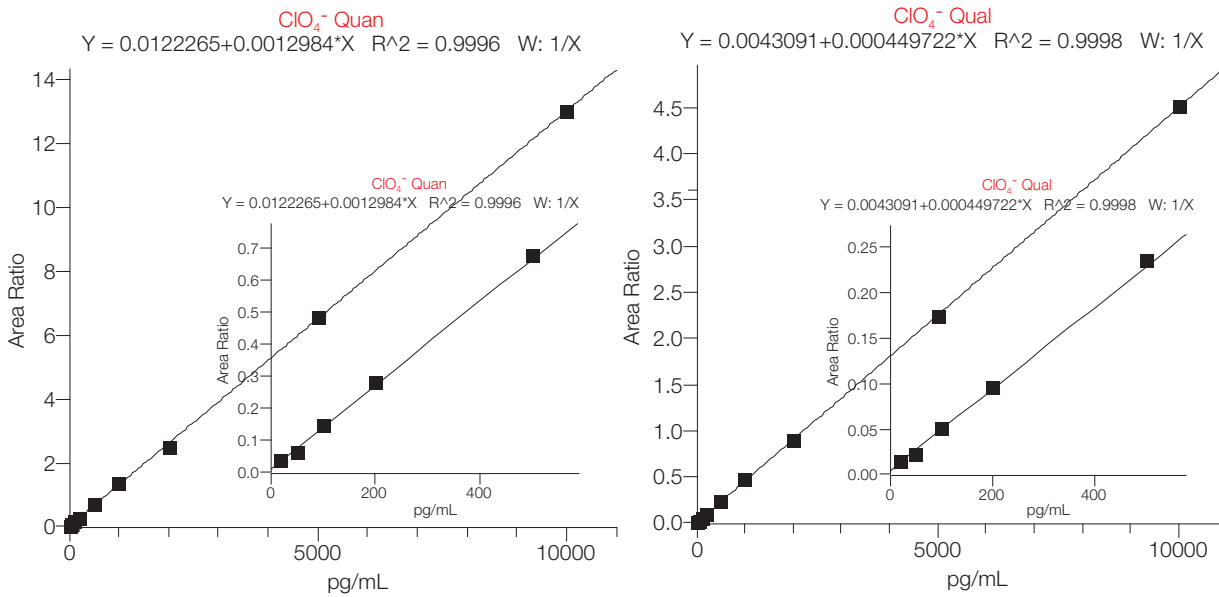


Figure 2. Calibration curves for quantitative and qualitative analysis of perchlorate

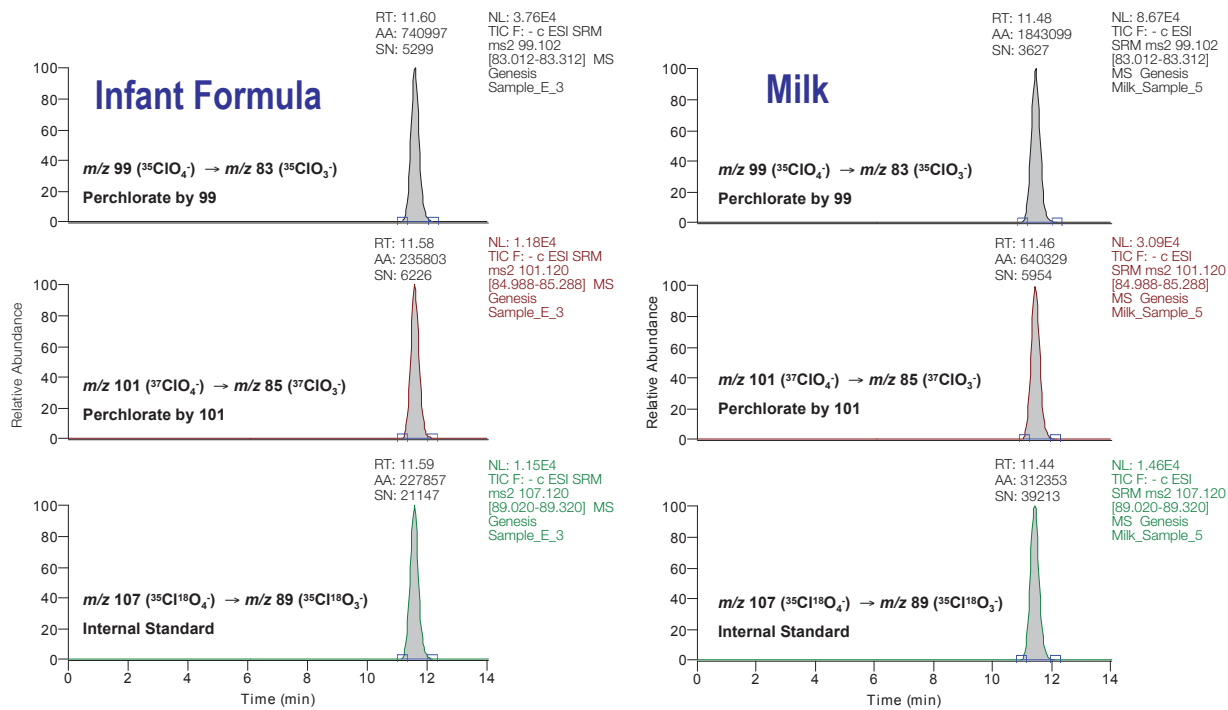


Figure 3. Perchlorate in unspiked infant formula and milk samples. The calculated concentrations are shown in Table 3.

Conclusion

A selective and ultra-sensitive IC-MS/MS method has been successfully applied for the quantification of perchlorate in infant formula and milk. Because the simple sample preparation was deployed and not the long SPE enrichment method, several hours of sample preparation time was saved. The wide linear range covers the reported perchlorate levels in infant formula. Excellent reproducibility (%RSD=3.63, n=7), accuracy, and precision were achieved.

References

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