Determination of Ultratrace Level Perchlorate in Liquid and Powdered Baby Formula

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ABSTRACT

Perchlorate is a widespread contaminant in the United States.[1] Research has demonstrated its presence in various media, including water, soil, various foods, and also human biological fluids, such as urine and breast milk.[2-6] Perchlorate causes an adverse health effect by competitively inhibiting iodide uptake by the thyroid gland because it exhibits a much higher selectivity factor than iodide.[7] The reduced uptake of iodide inhibits thyroid hormone production, which is essential for proper protein expression, neuronal differentiation, and other functions.[6] Certain populations, for example, infants and children, may be at higher risk due to their greater dependency on milk products and higher consumption/body weight ratio. *Health Implications of Perchlorate Ingestion*, a report issued by NAS, has strongly encouraged continued research on the potential exposure routes and adverse effects of perchlorate contamination in sensitive populations.[8]

Analytical methods for perchlorate quantification include ion chromatography (IC), equipped with various anion-exchange columns, and conductivity and/or mass spectrometric (MS) detections.[5,6,9,10] The high sensitivity and selectivity of MS detection makes it a highly preferred method for complex sample matrices. Thus, MS detection provides greater data confidence while minimizing labor-intensive sample preparation.

This study describes an IC-MS/MS method for ultratrace-level perchlorate analysis in both liquid and powdered baby formula. Without sample enrichment, perchlorate in baby formula matrix can be quantified at low parts-per-trillion (ppt) levels with simple sample preparation. Linear calibration was established in the range from 20 ppt to 10 parts-per-billion (ppb), with a coefficient of determination (r^2) greater than 0.999. Recovery was achieved at 103% and 114% by spiking 5 ppb perchlorate in two samples. Repeated injections of standards showed %RSDs from 1.82% (100 ppt) to 7.36% (10 ppb); repeated injections of one prepared baby formula sample showed %RSD of 3.63%; and repeated assays (n=3) of samples showed %RSD from 1.63% to 3.55%. Perchlorate was found in every tested sample of commercially available infant formula obtained from local markets, ranging from 1.02 to 2.43 ppb.

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EXPERIMENTAL SECTION

Ion Chromatography Conditions

System:	ICS-3000 RFIC™ system
Column:	IonPac® AS16 and AG16 hydroxide-selective anion exchange columns (2 mm) ASRS® 300 self-regenerated suppressor (external water mode)
Column Temp.:	30 °C
Flow Rate:	300 µL/min
Mobile Phase:	Isocratic 45 mM hydroxide generated from EGC-II KOH cartridge
Solvent:	150 µL/min acetonitrile delivered by AXP-MS pump
Detection:	1st detector: Suppressed Conductivity 2nd detector: TSQ Quantum Access™ Mass Spectrometer

Mass Spectrometric Conditions:

System:	TSQ Quantum Access Triple Quadrupole Mass Spectrometer
Interface:	Electrospray Ionization (ESI) Source
Spray Voltage:	4000 V
Sheath Gas:	40 arbitrary units
Auxiliary Gas:	5 arbitrary units
Ion Sweep Gas:	15 arbitrary units
Capillary Temp:	300 °C
Collision Gas:	Argon at 1.8 mTorr
Operating Mode:	Selected Reaction Monitoring (SRM)

Analyte	*Parent Ion*	*Product Ion*	*Collision Energy*
Perchlorate	99.1	83.2	24
Perchlorate	101.1	85.1	24
Perchlorate-$^{18}O_4$	107.1	89.2	24

Standard Solutions

A perchlorate standard solution was purchased from Ultra Scientific (P/N: ICC-013), and a stable-labeled ($NaCl^{18}O_4$) perchlorate internal standard solution was obtained from Dionex (P/N: 062923). The perchlorate standard solution was diluted to 100 ppb, 10 ppb, and 1 ppb, as working standards to prepare calibration standards. The perchlorate internal standard (IStd) was diluted to 100 ppb for preparing calibration standards and spiking unknown samples. Calibration standards were prepared at 0 ppt (non-native perchlorate-spiked), 20 ppt, 50 ppt, 100 ppt, 200 ppt, 500 pt, 1 ppb, 2 ppb, 5 ppb, and 10 ppb, with all standards containing IStd at 1 ppb at each level.

Sample Preparation for Infant Formula and Milk Samples

Powdered infant formula (PIF) samples were prepared to liquid form with deionized (DI) water according to the manufacturer-provided instructions. Liquid infant formula (LIF) samples were used as is from their containers. Deionized water (obtained from Millipore water station with 18.2 MΩ-cm resistivity) was observed to contain no integrable perchlorate peak (detection limit < 5 ppt). Any materials coming into contact with the sample during sample preparation procedures were prerinsed with this DI water. Each sample (4 mL) was pipetted into a 50 mL centrifuge tube and spiked with 40 µL of IStd (4 ng). For protein precipitation, 4 mL of precooled ethanol (stored under refrigeration at 4 °C; reagent-grade, EM Science) and 0.4 mL of 3% acetic acid (HPLC grade, J.T. Baker; diluted in DI water to 3% for sample preparation) were added to each sample. Each sample was then mixed in a vortex mixer for 10 s and centrifuged at 5000 rpm for 30 min at –5 °C. The supernatant (~6 mL; volume is not critical due to the use of IStd) was passed through a 0.25 µm preconditioned syringe filter (PALL, IC-certified; preconditioned by passing 5 mL of ethanol and 15 mL of DI water). The filtrate was collected in a 10 mL autosampler vial (polystyrene, Dionex) and analyzed by IC-MS/MS.

A second sample preparation was also evaluated to improve method ruggedness. A Dionex OnGuard® RP II reversed-phase cartridge was added onto the syringe filter in series (using the same preconditioning procedure as stated previously) to remove the fat and organic content from samples. The latter sample preparation technique facilitated the successful removal of fat and organic contents, thus substantially improving analytical column longevity and reducing the requirements for column regeneration.

Laboratory Analysis and Report

Each batch of samples was processed with a *Laboratory Blank* (DI water processed as a real sample) to monitor perchlorate background level and reported as a *Method Blank*. Perchlorate was quantified on the peak area ratios of analyte to stable isotope-labeled IStd against the calibration curve. The average of the amounts calculated by two SRMs was used to report the perchlorate level. The peak area ratios of two SRMs were also monitored for standards and unknown samples to prevent potential, invisible interferences. In this study, the peak area ratio ($PeakArea_{99\rightarrow83}$ / $PeakArea_{101\rightarrow85}$) ranges from 2.46 to 3.86 with %RSD at 9.39%. A significant deviation from this range (peak area ratio) may indicate interference occurred for one or both of the SRMs.

RESULTS AND DISCUSSION

Analytical Results

Five samples were purchased locally and analyzed in this study, including three liquid baby formulas, one powdered baby formula, and one reduced fat bovine milk. The intention was not to conduct a complete survey but to check perchlorate levels in randomly selected samples and to evaluate method performance.

Perchlorate was found in every sample tested in this study, with concentrations varying from 1.02 to 2.44 ppb in baby formula, and 4.65 ppb in the milk sample. Results are seen in Table 1. Observed amounts of perchlorate present in this study agree with earlier reported values this year from the Centers for Disease Control and Prevention.[1]

Table 1. Perchlorate in Infant Formula and Milk

	Quantified Amount (ppb)	%RSD
LIF-1	1.742	2.68 (n=3)
LIF-2	2.213	2.34 (n=3)
LIF-3	1.053	3.63 (n=7)
PIF-1	2.439	1.74 (n=3)
MLK	4.636	1.33 (n=5)

LIF: liquid infant formula samples
PIF: powdered infant formula sample
MLK: milk sample

Ion Chromatography Conditions

System:	ICS-3000 RFIC system
Column:	AS16 & AG16 Hydroxide Selective anion exchange columns
	ASRS 300 self regenerated suppressor (external water mode)
Column Temp:	30 °C
Flow Rate:	300 µL/min
Inj. Volume:	100 µL
Mobile Phase:	Isocratic 45 mM hydroxide from EG-II KOH catridge
Solvent:	150 µL/min acetonitrile delivered by AXP-MS pump
Detection:	1st detector: Suppressed Conductivity
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Iom Sweep Gas:	15 arbitary units
Capillary Temp:	300 °C
Collision Gas:	Argon at 1.8 m Torr
Operating Mode:	Selected Reaction Monitoring (SRM)

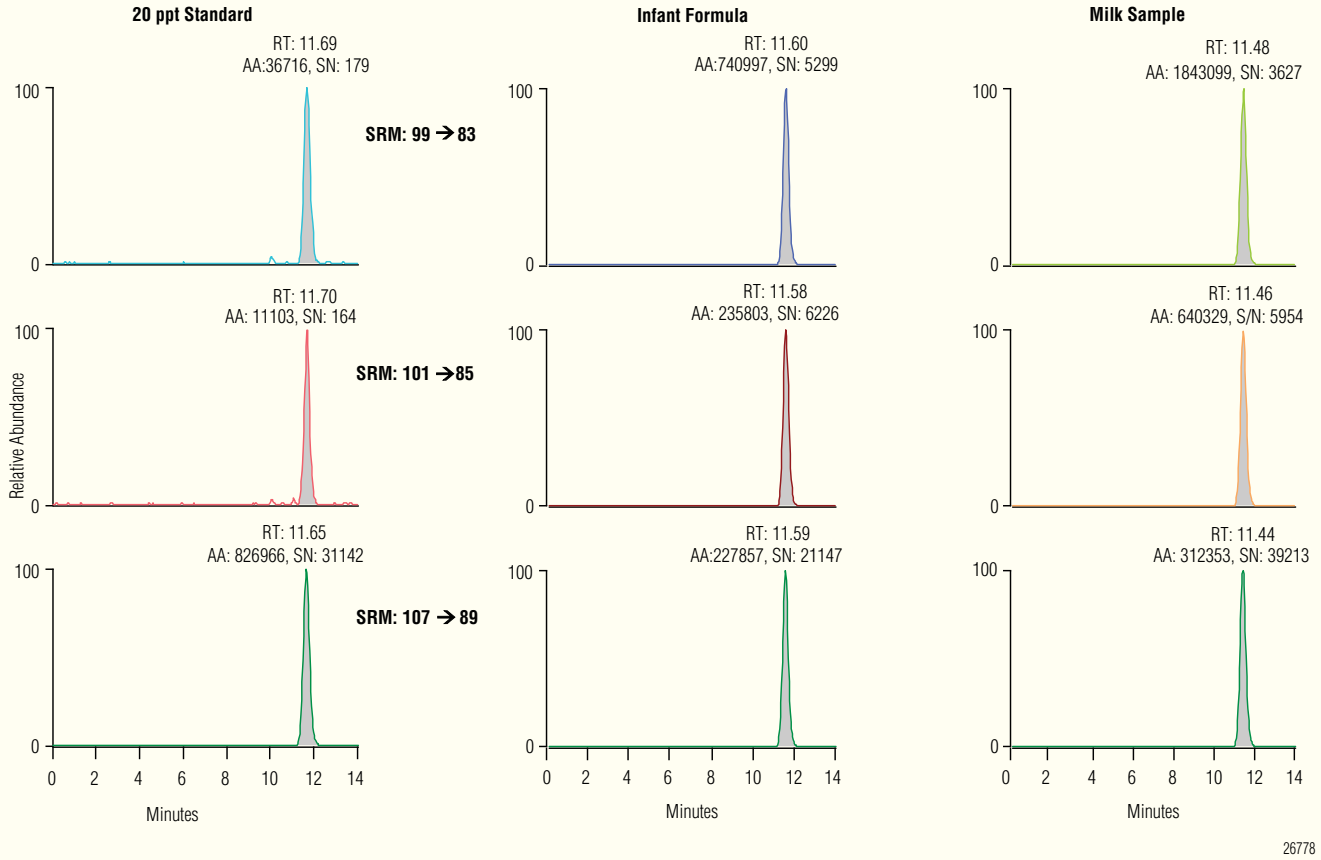

Figure 1. SRM chromatograms for A) a standard prepared at 20 ppt; B) an infant formula sample quantified at 2.44 ppb; and C) a milk sample quantified at 4.65 ppb.

METHOD PERFORMANCE

Specificity

Method specificity was evaluated by injecting the IC-MS/MS system with DI water and monitoring the perchlorate level. No peak was observed in the elution time window for both SRM channels. However, due to the widespread existence of perchlorate, 20.09 ppt perchlorate was found as the background level for the whole analysis process (*Method Blank*), which is the sum of perchlorate from all potential sources, including the ethanol and acetic acid used in sample preparation.

An experiment was also performed to evaluate the impurity level (unlabeled perchlorate) in IStd. DI water was spiked with IStd at 1 ppb and analyzed by this method. No unlabeled perchlorate peak was observed, thus indicating that the use of IStd did not artificially elevate perchlorate levels.

Linearity and Calibration Range

Linearity was achieved for both SRMs in the range of 20 ppt to 10 ppb. Although the lower calibration range can be extended to 5 ppt, earlier reports have demonstrated the lowest observed level of perchlorate of 30 ppt in infant formula samples.[1] Subsequently, background perchlorate level (*Method Blank*) in this study was observed at 20.09 ppt, and so the lower calibration limit was set at 20 ppt. The upper limit of the calibration range was set at 10 ppb, which was determined according to the highest perchlorate level found in infant formula samples (5.05 ppb). A coefficient of determination (r^2) at 0.9996 was achieved for SRM 99 → 83 and 0.9998 for SRM 101 → 85, indicating excellent linearity within the calibration range. The calibration curve was weighted by the reciprocal of concentration (1/X) to produce the best quantification accuracy for low-level samples.

Detection Limits

The IC-MS/MS system is able to routinely detect perchlorate at 5 ppt with S/N greater than 10. However, as mentioned earlier, perchlorate was observed at 20.09 ppt in the method blank sample, and the *Lower Reportable Limit* was determined as $Amount_{MethodBlank} + 3 \times S_0$, where the $Amount_{MethodBlank}$ is the mean observed perchlorate in processed blank samples and S_0 is the standard deviation of calculated perchlorate in method blank samples ($S_0 = 2.94$, n = 6). The lowest reportable limit in this study is 28.9 ppt.

Carryover

Instrument carryover was evaluated by analyzing the most concentrated standard (10 ppb) followed by two DI water blank samples spiked with IStd at 1 ppb. No carryover peak was observed in any of the DI water blank samples, thus indicating no detectable carryover for the IC-MS/MS system.

Instrument Precision and Accuracy

Instrument precision and accuracy were evaluated by running standard solutions at three known levels: 100 ppt, 1 ppb, and 10 ppb (n=7). Results are seen in Table 2. Instrument precision was within 3.05% for quantified amount and within 0.2% for retention time.

The IC-MS/MS system was also evaluated for precision with prepared real samples. The sample LIF-3 was prepared by the procedures with fat removal and the MLK sample was prepared by the procedure without fat removal. Each sample was injected seven times for analysis. The %RSDs were observed at 1.83% and 1.96% respectively.

Table 2. Instrument Precision and Accuracy

Specified Amount (ppt)	Quantified Amount (ppt)	$\%RSD_{Amount}$	%Accuracy	$\%RSD_{R.T.}$
100	98.18	3.05	1.82	0.13
1000	1035	1.62	3.52	0.08
10000	10736	1.45	7.36	0.11

Instrument precision is measured by $\%RSD_{Amount}$ which shows the relative standard deviation of observed amounts.

Instrument accuracy is measured by %Accuracy which was calculated by (*Quantified Amount – Specified Amount)/ Specified Amount ×100%.*

$\%RSD_{R.T.}$ shows the relative standard deviation of retention time in percentage.

Recovery and Reproducibility

Recovery was evaluated by spiking a known amount of perchlorate (5 ppb) in two infant formula samples (LIF-1 and LIF-3, n=3) and calculated by the difference between unspiked and spiked samples. Recovery was observed as 103% for LIF-1 and 114% for LIF-3.

Reproducibility was evaluated by replicate assays of LIF-3 (n=7) and MLK (n=5) and shown as %RSD in Table 1. Excellent reproducibility was achieved: 3.63 %RSD for LIF-3 and 1.33 %RSD for MLK.

Sample Preparation and Method Ruggedness

After a number of multiple injections (actual number depending on the age of analytical column and the nature of injected samples), the analytical column may require a regeneration process to remove the accumulated fat and organic content. This reduces column efficiency and capacity (known symptoms include decreased retention time and asymmetric peaks). To regenerate, the analytical column (with guard column) was conditioned with a mobile phase consisting of 50/50 (v/v) acetonitrile/60mM hydroxide at 0.3 mL/min overnight, and then equilibrated with 45 mM hydroxide mobile phase. After regeneration, the total conductivity observed should be less than 3 µS, or the column will need to be replaced.

The alternate sample preparation process is highly recommended and includes the removal of fat and organic contents by an OnGuard RP cartridge.

Both sample preparation procedures were evaluated for a statistically significant difference. The two procedures yielded no statistically distinguishable difference in either standard deviation (evaluated by F-test) or reported value (t-test).

Method ruggedness was significantly improved by using the sample preparation procedure with fat removal. Under equilibrated conditions, retention time was monitored by repeated injections (n = 10) of samples prepared following the two sample preparation procedures respectively. Minimum retention time decrease (0.12 minute, $\%RSD_{R.T.} = 0.33$) was observed for the sample prepared with the fat removal procedure while a 0.85 min decrease ($\%RSD_{R.T.} = 2.44$) was observed for the sample prepared without fat removal.

CONCLUSION

This poster describes an IC-MS/MS method for a simple, fast, and ultra-sensitive quantitative determination of perchlorate in infant formula and milk products. This method applies a simple and fast sample preparation method without labor-intensive SPE cleanup and concentration, and highly selective and sensitive detection using MS/MS detection operating in SRM mode. This instrument system is capable of detecting as low as 5 ppt perchlorate; however, due to the widespread existence of trace-level perchlorate as the analytical background, the practical lower reportable level of this method is set at 28.9 ppt. Excellent linearity ($r^2 > 0.999$) was achieved through the calibration range from 20 ppt to 10 ppb. This method also demonstrated excellent instrument precision, accuracy, and good recovery and reproducibility.

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