Application Note

Food Testing & Agriculture

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Trace-Level Analysis of Chlorates in Milk and Infant Formula

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Abstract

This application note highlights an 8-minute analytical method for the quantification of chlorate and perchlorate in milk and infant formula. The method uses an Agilent 1290 Infinity II LC and Ultivo triple quadrupole LC/MS (LC/TQ) with an AJS source. The Ultivo LC/TQ was designed to save laboratory space, while maintaining the performance and robustness required for high-throughput analyses. Chlorate and perchlorate have maximum residue limits (MRLs) of 10 µg/kg in milk and infant formula, as defined by the European Commission. This method achieved accurate quantitation at one tenth the level of the MRL. The method precision was <10% (%RSD) at the lowest level of quantitation with n = 10.

Introduction

Chlorate and perchlorate residues have become a growing concern with agriculturally produced foods, such as dairy products, fruits, and vegetables. The presence of chlorate and perchlorate in dairy products can occur when chlorinated water and chlorine-containing detergents are used for cleaning and sanitizing process equipment at both farm and processor level. Perchlorate can be found in agricultural-based foods as it is heavily used in the production of nitrate-based fertilizers. Disinfection of milk processing equipment is important for the prevention of food-borne diseases and the use of nitrite-based fertilizers is important in crop yields, therefore, widespread use of chlorate and perchlorate for sanitation in industrial food production processes have been observed. The maximum residue level (MRL) of chlorate and perchlorate is set at 0.01 mg/kg in milk and infant formula, and can accurately quantify down to 1/10 MRL. The Ultivo triple quadrupole LC/MS is an appropriate system for these measurements.

Note that chlorate and perchlorate contamination from consumables in the analytical system should be checked before tests begin. Nylon or regenerated cellulose (RC) filters and plastic tubes can be significant sources of perchlorate.

Experimental

Reagents and chemicals

All reagents used were LC/MS grade. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ultrapure water was sourced from an Elga PURELAB classic system. Formic acid and acetic acid were purchased from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Honeywell, Riedel-de Haen (Morristown, USA). Perchlorate and chlorate were purchased from CPAchem (Stara Zagora, Bulgaria). Isotopically labeled standards of perchloric acid, sodium salt ($^{18}O_4$), and potassium chlorate ($^{18}O_3$) were purchased from Cambridge Isotope Laboratories (Andover, USA).

Sample preparation

Fresh UHT milk and infant formula were obtained from local grocery stores. Homogenized infant formula sample (5 g) was weighted into a 50 mL centrifuge tube and mixed with 30 mL of Millipore water, as described in the instructions on the product label. 10 mL of prepared infant formula or milk was transferred into a clean 50 mL centrifuge tube and spiked with 100 µL of isotopically labeled internal standard mix. The samples were extracted using 10 mL of methanol containing 1% formic acid and shaken using a multiple shaker (Heidolph Hei-MIX) for 15 minutes. All samples were centrifuged at 4,000 rpm for 10 minutes at 10 °C. 2 mL of supernatant was transferred into a tube containing 100 mg of C18 sorbent and 2 mL of acetonitrile. This was shaken for 1 minute and centrifuged at 4,000 rpm for 5 minutes. The supernatant was filtered using a PTFE 0.45 µm filter into the HPLC vial. Figure 1 represents the flowchart of sample preparation steps.

Instrumentation

Agilent LC system

- 1290 Infinity II High Speed Pump (G7120A)
- 1260 Infinity II Vial Sampler with cooler (G7129A)
- 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent Ultivo triple quadrupole LC/MS system (G6465B)
- Agilent Jet Stream (AJS) electrospray ion source (G1958B)

Method

Table 1 summarizes the UHPLC conditions. Table 2 summarizes the Ultivo triple quadrupole parameters and Agilent Jet Stream source parameters. Analysis was carried out using multiple reaction monitoring (MRM) with negative ionization. Data were evaluated using the Agilent MassHunter Quantitative Analysis software B.09 with the Quant-My-Way feature.

Table 1. UHPLC conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 HILIC-OH5 2.1 × 150 mm, 2.7 µm (p/n 683775-601)
Column Temperature	40 °C
Observed Column Backpressure Range	130 to 190 bar
Injection Volume	2 µL
Mobile Phase	A: 2 mM ammonium acetate in water, 1% formic acid B: Acetonitrile
Flow Rate	0.4 mL/min
Gradient	Time (min) B% 0 90 1.0 90 2.0 70 5.0 70 5.1 90 6.0 90
Stop Time	7.0 min
Post Time	1.0 min

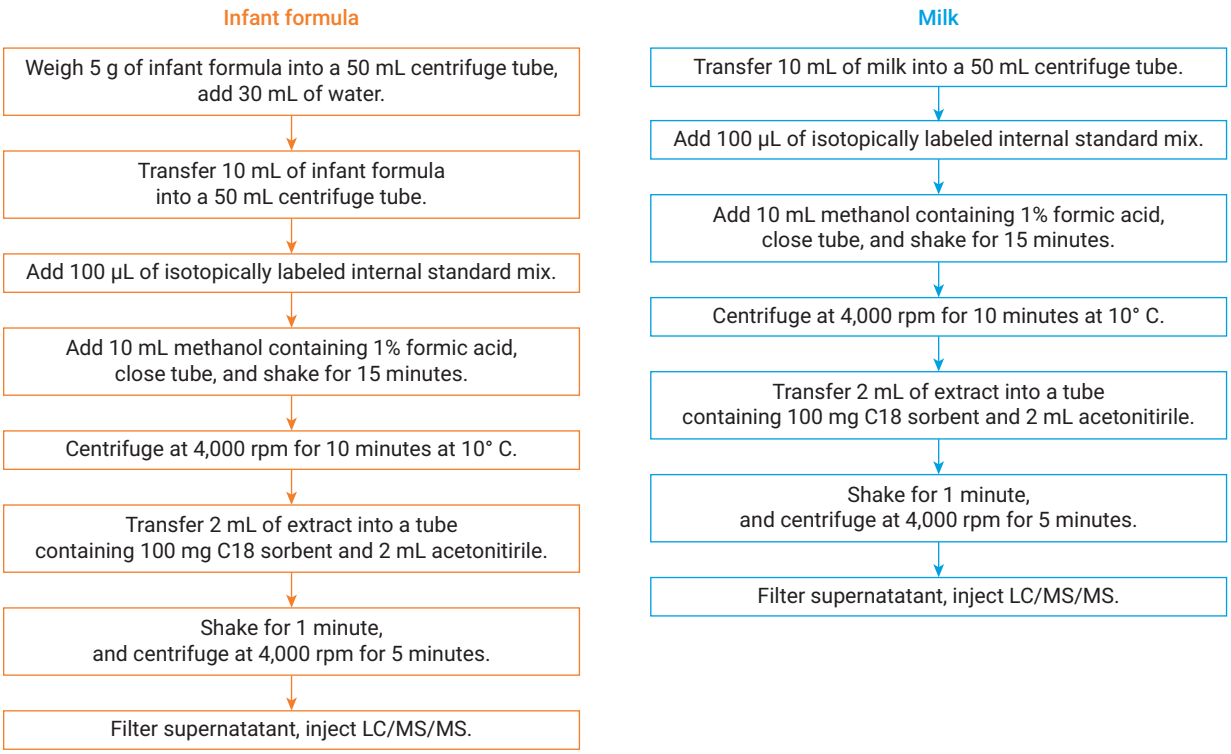


Figure 1. Sample preparation procedure for milk and infant formula.

Table 2. Agilent Ultivo triple quadrupole LC/MS source parameters.

Parameter	Value
Drying Gas Temperature	200 °C
Drying Gas Flow	10 L/min
Sheath Gas Temperature	400 °C
Sheath Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Capillary Voltage	2,500 V(–)
Nozzle Voltage	0 V(+)
Cycle Time	350 ms

Table 3. Optimized transitions.

Compound Name	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
Chlorate	83	66.9	1.02	130	18	Negative
Chlorate	85	68.9	1.02	130	20	Negative
Perchlorate	98.9	82.8	1.72	130	24	Negative
Perchlorate	101	84.8	1.72	130	26	Negative
Perchlorate-$^{18}O_4$	108.9	91	1.72	130	26	Negative
Perchlorate-$^{18}O_4$	107	88.9	1.72	130	26	Negative
Chlorate-$^{18}O_3$	89	71	1.02	130	20	Negative
Chlorate-$^{18}O_3$	91	73	1.02	130	20	Negative

Results and discussion

Method sensitivity and precision

Chlorate and perchlorate could be accurately quantified at 1/10 MRL (the lowest level tested in this study). Figure 2 shows excellent signal response for all analytes at the testing level in milk and infant formula. Chlorate and perchlorate also showed excellent precision with %RSD below 5% for all compounds tested, as well as each compound's lowest testing level, as shown in Table 4. All compounds had strong signal response at 1/10 MRL, indicating that the true quantitation limit is considerably lower than 1/10 MRL (examples are included in Figure 3).

Table 4. Recovery and precision of perchlorate and chlorate in milk and infant formula at the low and high testing levels.

Milk					
Compound	LOQ (µg/L)	%RSD (n=10) (Low level 10 µg/L)	%RSD (n=10) (High level 25 µg/L)	Rec.% (Low level 10 µg/L)	Rec.% (High level 25 µg/L)
Chlorate	0.30	1.53	1.49	103	99
Perchlorate	0.23	1.53	1.20	102	100
Infant Formula					
Compound	LOQ (µg/L)	%RSD (n=10) (Low level 10 µg/L)	%RSD (n=10) (High level 25 µg/L)	Rec.% (Low level 10 µg/L)	Rec.% (High level 25 µg/L)
Chlorate	0.37	1.17	1.54	108	98
Perchlorate	0.11	1.26	1.40	107	98

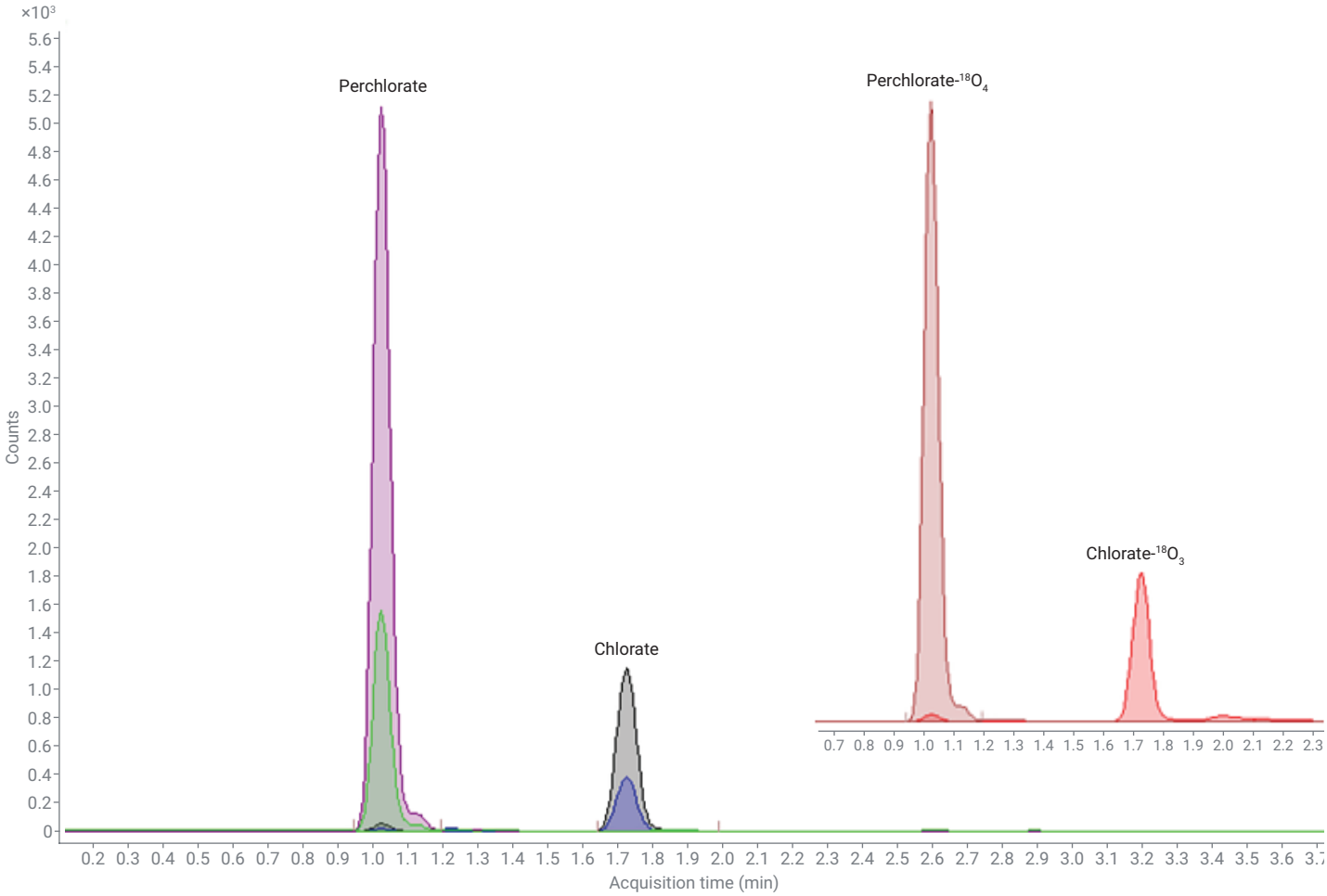
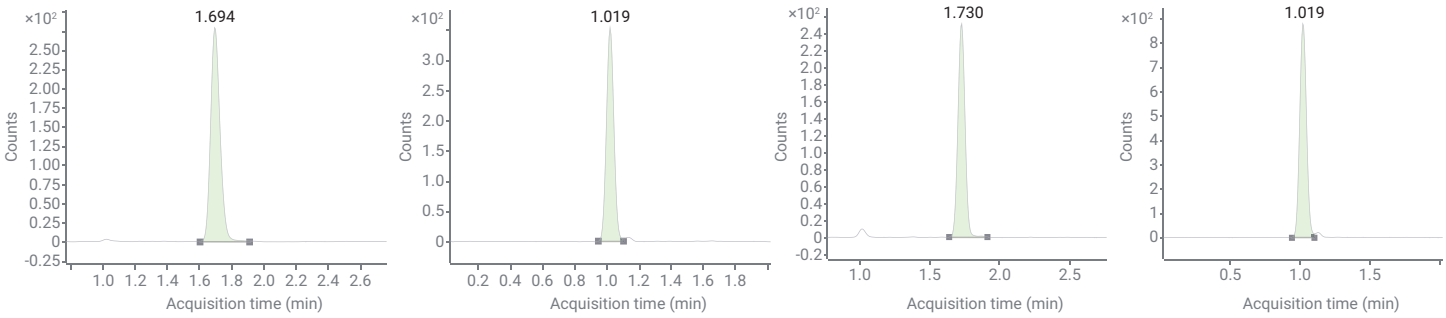


Figure 2. Chromatogram of chlorate and perchlorate spiked into milk at the level of 25 ppb.



Chlorate in infant formula
1/10 MRL → 1 ppb
Peak area: 6131
S/N: 621

Perchlorate in infant formula
1/10 MRL → 1 ppb
Peak area: 15901
S/N: 1150

Chlorate in milk
1/10 MRL → 1 ppb
Peak area: 4996
S/N: 1047

Perchlorate in milk
1/10 MRL → 1 ppb
Peak area: 15606
S/N: 1655

Figure 3. All compounds with strong signal response at 1/10 MRL.

Method linearity

Chlorate and perchlorate showed good linearity with 1/x weighting and blank offset. All calibration curves have R^2 values greater than 0.99. Calibration levels ranged from 0.5 to 100 ppb for all analytes, shown in Figure 5.

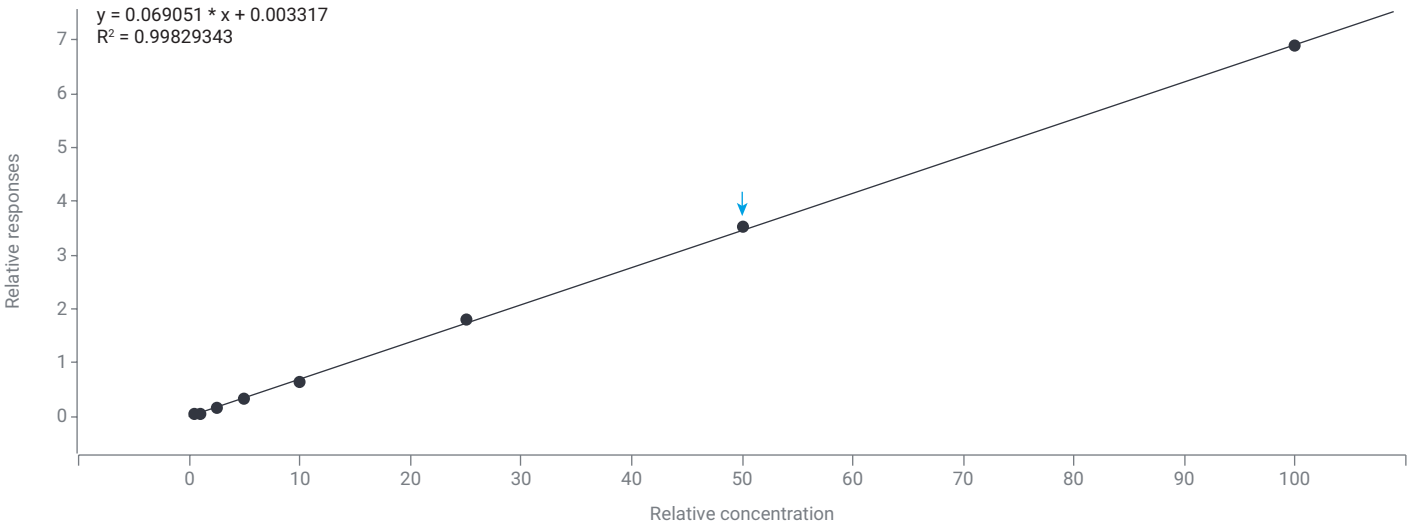


Figure 4. Calibration curve of perchlorate in infant formula at concentrations ranging from 0.5 to 100 µg/L.

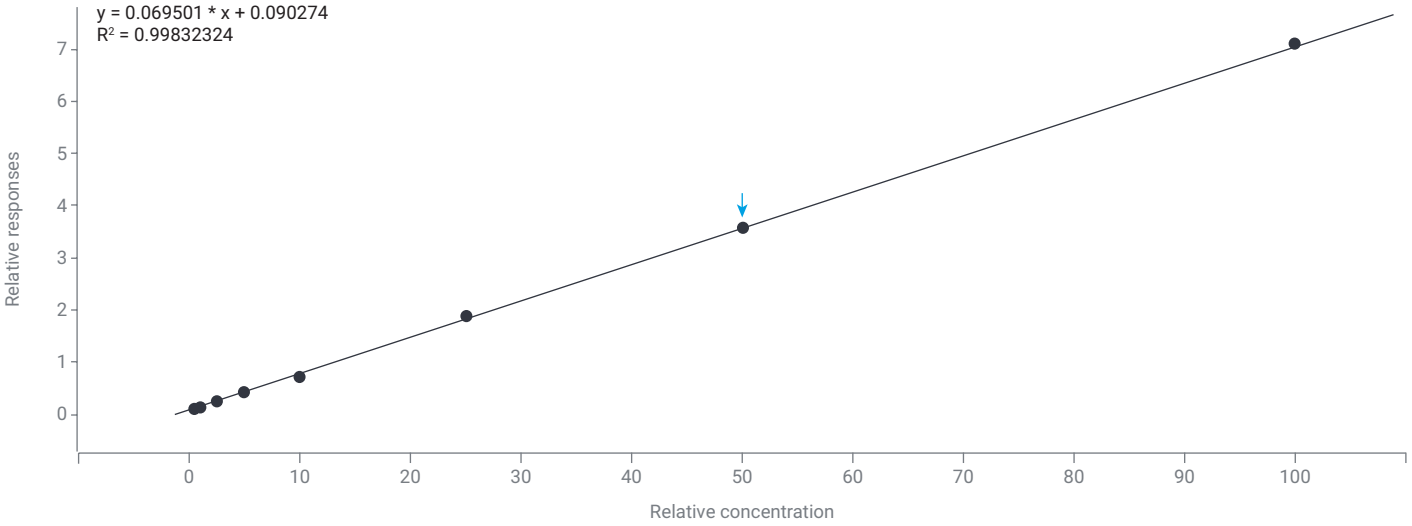


Figure 5. Calibration curve of chlorate in infant formula at concentrations ranging from 0.5 to 100 µg/L.

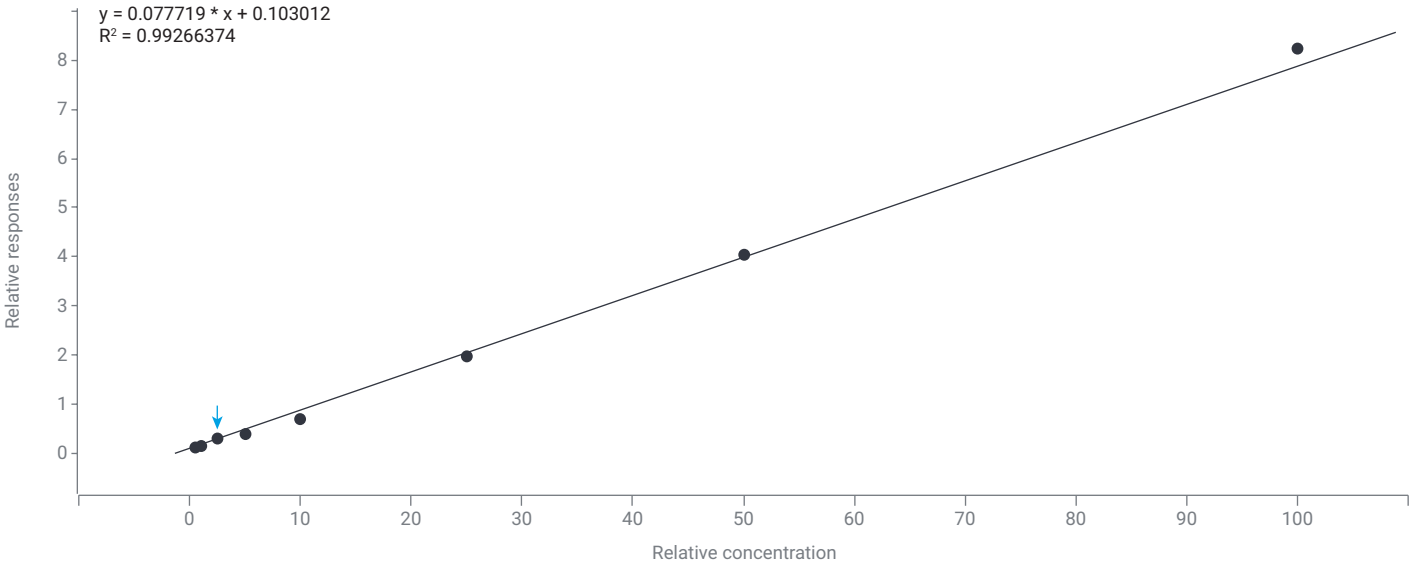


Figure 6. Calibration curve of chlorate in milk at concentrations ranging from 0.5 to 100 µg/L.

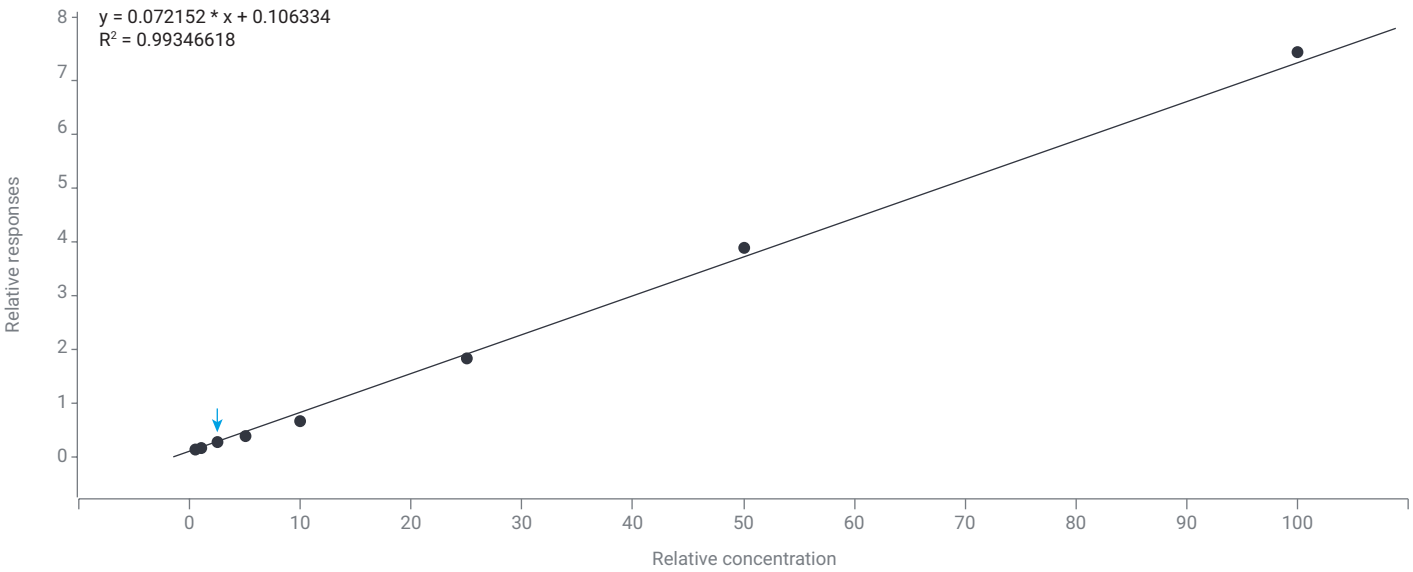


Figure 7. Calibration curve of perchlorate in milk at concentrations ranging from 0.5 to 100 µg/L.

Method recovery

The recovery of chlorate and perchlorate was evaluated in both milk and infant formula at two levels (10 and 25 ppb). Ten replicates of each spiking level were evaluated in the recovery study. The recovery was between 98 and 108% at all levels in both matrices (Figure 8).

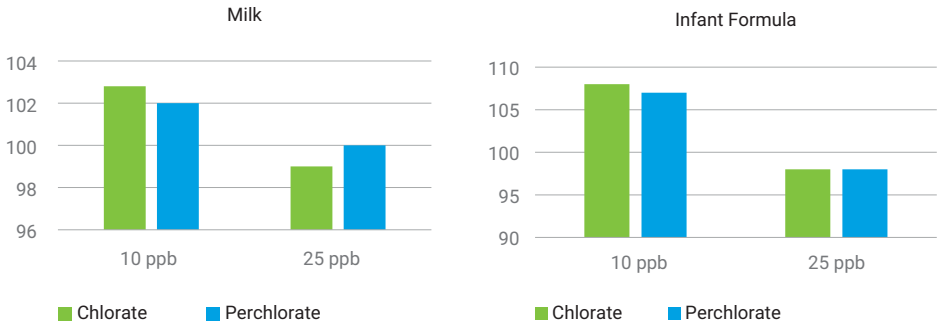


Figure 8. The recovery of chlorate and perchlorate in milk and infant formula.

Conclusion

The MRL requirements for chlorate and perchlorate set by European Commission for milk and infant formula were exceeded with excellent precision when an Ultivo triple quadrupole LC/MS equipped with Jet Stream ESI source was used for analysis. The Agilent Infinity II LC was an ideal system for separation of perchlorate and chlorate, respectively, with a HILIC column. This configuration of Ultivo with Jet Stream ESI source is an excellent fit-for-purpose choice.

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