Tomorrow's quantitation: robust, reproducible quantitation workflows of perchlorate in water with IC-MS/MS

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Goal

Development and easy implementation of a robust, reliable, and reproducible workflow solution for the analysis and quantitation of perchlorate in water using a triple-stage quadrupole mass spectrometer (MS).

Application benefits

- Development of a robust workflow for the analysis and quantitation of perchlorate in water with ion chromatography (IC) and the Thermo Scientific™ TSQ Fortis™ triple-stage quadrupole mass spectrometer (QqQ)

- Leveraging enhanced performance of a robust, sensitive QqQ to reduce cost per sample for every analytical environment

Introduction

Clean drinking water in today's world is becoming scarce, and any contamination can result in long-lasting damage to human health. Besides purifying water by means of mechanical measures, disinfection also plays an essential role in ensuring the supply of clean drinking water. Drinking water goes through an extensive disinfection process to ensure high quality;

however, contaminants continue to cause some severe health risks. In 1997, low-level perchlorate contamination (<50 ng/mL or parts per billion) was discovered in the Western U.S. Since then, perchlorate has been found in sites scattered around the nation. Although the U.S. Environmental Protection Agency has not established a regulation for perchlorate in drinking water, it has placed perchlorate on the contaminant candidate list (CCL) and the unregulated contaminants monitoring rule (UCMR).[1]

The need for techniques and methods for determining perchlorate is not constrained to environmental chemistry. Perchlorate salts are used pharmaceutically in Europe to treat Graves' disease and amiodarone-induced thyrotoxicosis. Ammonium perchlorate is also used as a solid oxidant in fireworks, emergency flares, rockets, rocket boosters, and intercontinental ballistic missiles. Thus, methods and techniques are necessary for quality control and quality assurance monitoring of perchlorate in water sources. Moreover, analysis of explosives and post-explosion residues has made quantitation of perchlorate important in forensic chemistry. As the breadth of matrices undergoing analysis expands from potable water to agricultural runoff, fertilizers, fruit juices, or physiological and botanical fluids, the risk for interference becomes greater.

The analysis of contaminants, especially polar molecules in drinking water, can be achieved by several techniques. EPA Method 314 for perchlorate has high concentrations of salts such as chloride and sulfate salts that interfere with analysis. Using LC-MS and LC-MS/MS analysis will eliminate this false positive perchlorate quantification. While liquid chromatography (LC) coupled to triple-stage quadrupole mass spectrometry (MS/MS) offers very specific, selective, and quality information, analysis of polar molecules utilizing LC is challenging, as LC typically work best for non-polar molecules. This calls for derivatization of samples, which can be time consuming and adds challenges towards achieving the result, faster and with confidence. Fortunately, ion chromatography (IC) offers significant benefits owing to its capability

to address polar molecules. In this study, we report a robust, reliable, reproducible quantitation assay for the determination of perchlorate in drinking water with IC-MS/MS using a Thermo Scientific™ Dionex™ ICS-5000⁺ Hybrid HPIC™ system, a Thermo Scientific™ TSQ Fortis™ triple-stage quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ 4.1 software.

Experimental
Sample preparation
Calibration curve standards of 4, 10, 20, and 50 µg/L and ERA QC samples were prepared by Santa Clara Valley Water District (SCVWD). All samples were prepared in Milli-Q® water (Millipore Sigma). NH_4Cl was added as a preservative at 100 mg/L to all water samples. No further sample preparation or pretreatment was performed prior to injection.

Ion chromatography
IC analysis was performed on a Dionex ICS-5000⁺ Hybrid HPIC system. Samples were directly injected; no sample pretreatment was required. A 100 µL sample was injected onto a 2 × 250 mm Thermo Scientific™ Dionex™ IonPac™ AS20 column, which is specifically designed to separate method analytes from the following common anions (matrix components) in drinking water: carbonate, bicarbonate, hydroxide, tetraborate, etc. A guard column (Dionex IonPac AG20, 2 × 50 mm) and a Thermo Scientific™ Dionex™ ASRS 500 conductivity compressor electrolytically regenerated suppressor, 2 mm, were used. The mobile phase was 300 µL/min KOH, which was automatically prepared by the eluent generator of the ICS-5000⁺. The concentration of the KOH was maintained at 75 mM during the method (isocratic). Isopropyl alcohol was added to the eluent post column via a T at a rate of 200 µL/min to assist in nebulization of the eluent in the MS ion source. The Thermo Scientific™ Dionex™ AXP auxiliary pump water for suppressor regeneration was maintained at 600 µL/min. The column temperature was maintained at 15 °C. Each assay had a run time of 15 min. Seven injections were performed at each concentration level.

Mass spectrometry

The TSQ Fortis triple-stage quadrupole mass spectrometer was used for this detection of perchlorate. All compounds for this study were analyzed in negative ion heated electrospray ionization (HESI) mode. The experimental conditions were optimized with a static spray voltage, a cycle time of 2 s, and Q1 resolution maintained at 0.7 Da FWHM. During method development, individual standards were infused into the mass spectrometer to determine optimum tube lens settings and collision energies for the product ions. The SRM table along with other critical MS features for all target analytes are listed in Table 1.

Software

Data acquisition and processing were conducted using Thermo Scientific™ TraceFinder™ software version 4.1.

Results and discussion

The calibration curve standards 4 µg/L and 10 µg/L from SCVWD were used to dilute further as indicated in Table 2.

Table 1. Optimized mass spectrometer transitions for perchlorate analyzed in this experiment with retention time of 9.55 min for every sample. The parameters were optimized following the EPA method.

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Tube Lens (V)	Source Fragmentation (V)
Perchlorate_99	99	66.9	36	78	8
Perchlorate_99	99	82.9	25	78	8
Perchlorate_101	101	68.9	36	78	8
Perchlorate_101	101	84.9	25	78	8

Table 2. Extended calibration curve prepared by dilution of standards 4 µg/L and 10 µg/L with Milli-Q water

Calibration Std	Prepared From (µg/L)	Volume Taken (mL)	Final Volume (mL)	Concentration (µg/L)
Cal 1	Cal 2	5.0	10.0	0.002
Cal 2	Cal 3	1.0	10.0	0.004
Cal 3	Cal 4	1.0	10.0	0.01
Cal 4	Cal 5	1.0	10.0	0.04
Cal 5	Cal 6	1.0	10.0	0.1
Cal 6	4.0	1.0	10.0	0.4
Cal 7	10.0	1.0	10.0	1.0

The perchlorate calibration curve from the calibration standards provided by SCVWD was obtained for the perchlorate parent ion with m/z, 99 (Figure 1).

The calculated concentrations (Table 3) for all the calibration standards and the QC sample were well within permissible variability.

Perchlorate_99-3
Y = 2.195e5X + 2.082e5; R^2: 0.9967; Origin: Ignore; W: 1/X; Area

SCVW_Cal-1_01 Perchlorate_99-3 m/z: 82.90

(b)
Calibration level, 4 µg/L

RT: 9.58
AA: 1013766
SN: 59448.23

SCVW_ERA_QC_05 Perchlorate_99-3 m/z: 82.90

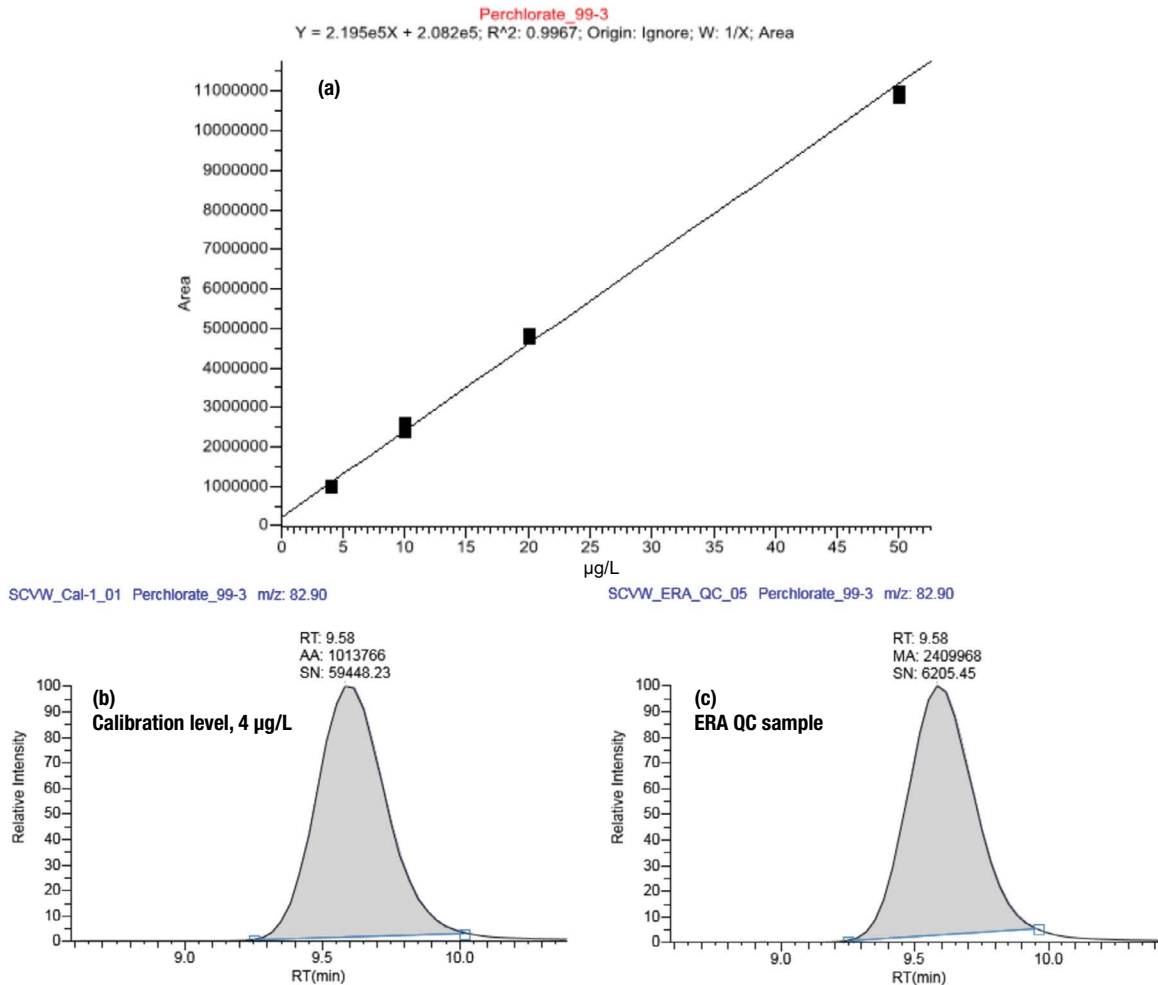(c)
ERA QC sample

RT: 9.58
MA: 2409968
SN: 6205.45

Figure 1. (a) Calibration curve for the calibration standards prepared by SCVWD ranging from 4 to 50 µg/L, (b) chromatogram for the lowest calibration level, 4 µg/L, and (c) chromatogram for the ERA QC sample with parent ion of m/z = 99

Table 3. Calculated concentrations and variability (%RSD) of calibration and QC samples for perchlorate with the parent ion at m/z = 99

Sample	Calculated Concentration (µg/L)	%RSD
Cal 1	3.7	1.22
Cal 2	10.6	1.73
Cal 3	20.8	0.60
Cal 4	48.9	0.84
QC	10.5	1.19

Seven injections were performed at each concentration level.

Chromatograms for further diluted calibration levels 0.002, 0.004, and 0.01 µg/L are shown in Figure 2.

The calibration curve plotted with the data should satisfy the following criteria, R^2 value of ≥ 0.98 and variability (%CV) < 15%. The % accuracy and ion ratio for each sample must fall between ± 20%, and the required signal-to-noise ratio had a minimum threshold of 3:1 for it to be acceptable. An extended calibration curve for the parent ion (m/z = 99) was created with a larger array of samples shown in Table 4. Each sample in the extended calibration curve showed %RSD of <5% while the signal-to-noise ratio was higher than 3. The %RSD for calibration levels 3–11 were calculated from seven injections; whereas for calibration levels 1 and 2, the number of injections used was 4.
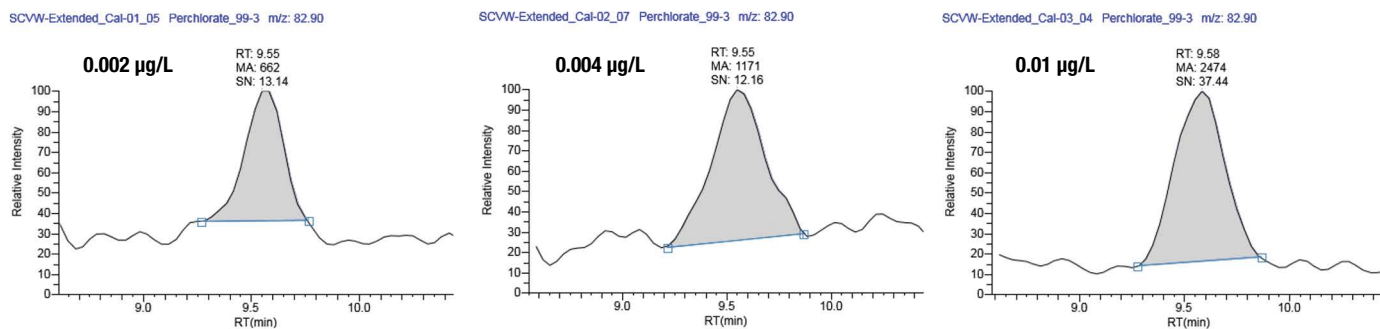

Figure 2. Ion chromatograms for perchlorate in water samples at very low concentrations with parent ion of m/z = 99. The concentration ranges varied from 0.002 µg/L (left), 0.004 µg/L (middle), and 0.01 µg/L (right).

Table 4. Extended calibration curve of perchlorate ion in water with parent ion m/z = 99

Sample	Calculated Concentration (µg/L)	%RSD	S/N
Cal 1	0.002	4.6	>3
Cal 2	0.004	3.5	>3
Cal 3	0.01	3.4	>3
Cal 4	0.04	2.0	>3
Cal 5	0.10	1.8	>3
Cal 6	0.41	2.5	>3
Cal 7	1.0	1.3	>3
Cal 8	4.1	1.2	>3
Cal 9	10.2	1.1	>3
Cal 10	19.6	1.0	>3
Cal 11	50.2	0.7	>3
QC	10.1	0.7	>3

Similar to the parent ion $m/z = 99$, the extended calibration curve with parent ion $m/z = 101$ also shows linearity of ≥ 0.98. Calibration levels 3–11 show %RSD < 4% and signal-to-noise ratios greater than 3 for seven injections (Table 5). The %RSD for the lowest calibration levels 0.002 and 0.004 µg/L were calculated with three and four injections, respectively, while they maintained a signal-to-noise ratio of greater than 3 (Figure 3).

Table 5. Extended calibration curve of perchlorate ion in water with parent ion $m/z = 101$

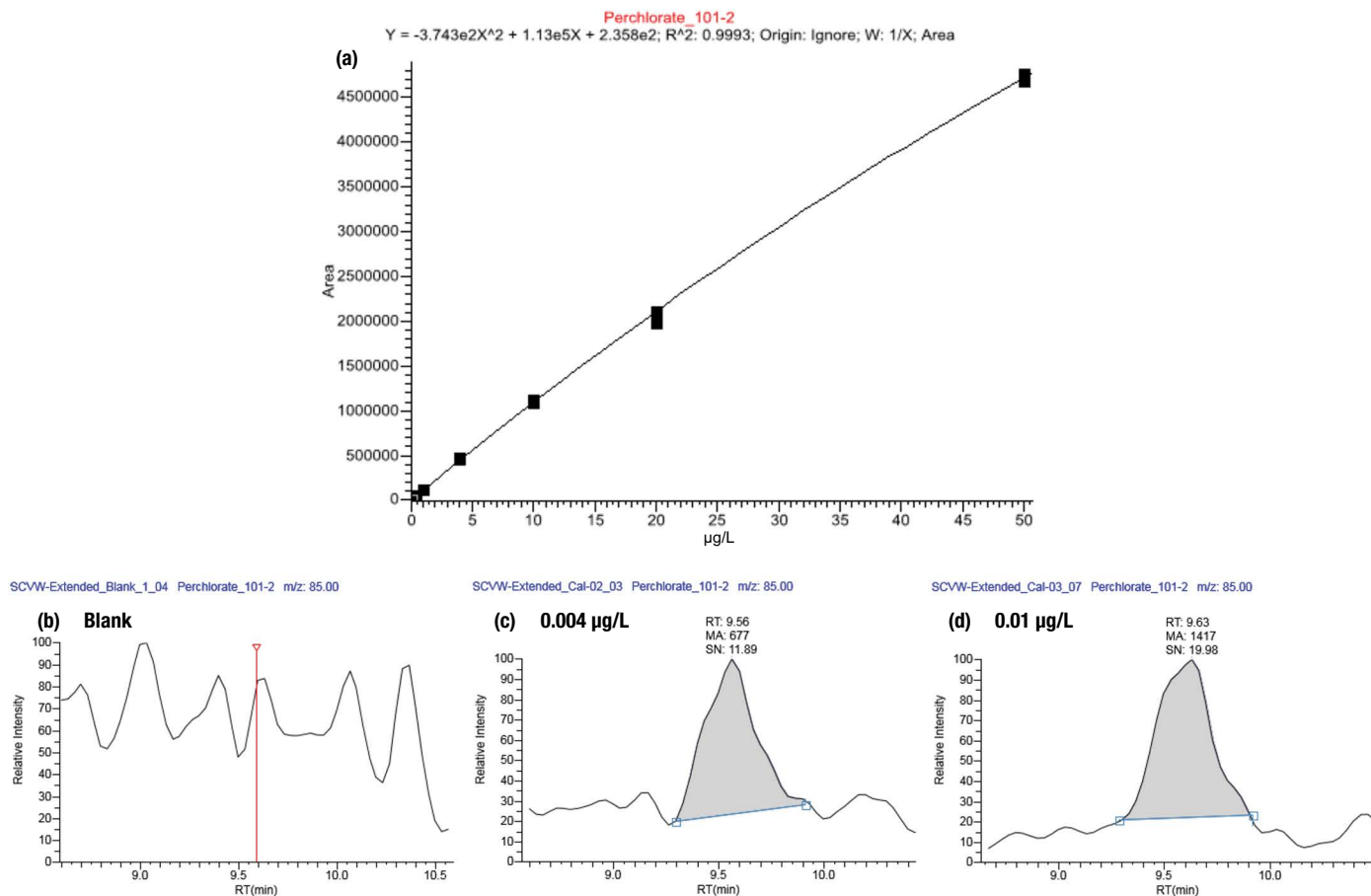| Sample	Calculated Concentration (µg/L)	%RSD	S/N
Cal 1	0.002	1.8	>3
Cal 2	0.004	2.3	>3
Cal 3	0.01	3.7	>3
Cal 4	0.04	4.0	>3
Cal 5	0.10	3.5	>3
Cal 6	0.42	2.9	>3
Cal 7	1.0	1.5	>3
Cal 8	4.1	1.4	>3
Cal 9	10.2	1.1	>3
Cal 10	19.3	2.8	>3
Cal 11	50.3	0.5	>3
ERA-QC	9.94	1.5	>3

Figure 3. (a) Calibration curve for the calibration standards ranging from 0.002 to 50 µg/L, (b) ion chromatogram for blank, (c) ion chromatogram for 0.004 µg/L, and (d) ion chromatogram for 0.01 µg/L with parent ion of $m/z = 99$

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Conclusions

The presence of perchlorate can cause significant health issues, especially in drinking water. Analysis and confident quantitation of perchlorate in water can pose several challenges, especially in view of their increasing complexity. A reagent-free IC system coupled with the TSQ Fortis MS is a powerful platform solution that can enable development of robust, reproducible, fast, and sensitive quantitation assays for perchlorate in water.

The sensitivity observed for the calibration standard provided by SCVWD was very high, hence, the standards were further diluted with Milli-Q water. All the analytes in this assay were detected to the lowest calibration level and the accuracy is within the criteria. The limit of detection was less than 0.002 µg/L based on the criterion of signal-to-noise ratio of greater than 3. The prepared quality control (QC) samples from ERA were found to be within the vendor acceptance limits of 8.88 to 12.3 µg/L and with % RSDs of 0.7 and 1.5 for parent ions of 99 and 101, respectively. Last, but not least, the optimal performance of the Dionex ICS-5000$^+$ HPIC system and TSQ Fortis MS platform solution exhibited excellent reproducibility and quantitation of perchlorate in water samples.

Reference
1. Urbansky, E. T. Quantitation of Perchlorate Ion: Practices and Advances Applied to the Analysis of Common Matrices, *Critical Reviews in Analytical Chemistry* **2007,** *30*(4), 32.

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