Determination of perchlorate by U.S. EPA Method 332.0 using a compact ion chromatography system coupled with mass spectrometry

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Goal

To identify and quantitate trace levels of perchlorate in raw and finished drinking waters according to U.S. EPA Method 332.0 using a compact high-pressure ion chromatography (HPIC) system coupled with a single quadrupole mass spectrometer

Introduction

Perchlorate has been used as an oxidizer in rockets, munitions, and fireworks since the 1950s. It has been found to cause thyroid dysfunction and has been linked to tumors in humans. Perchlorate is regulated under the Safe Drinking Water Act (2011) although the Maximum Contaminant Level Goal (MCLG) and Maximum Contaminant Level (MCL) have not yet been established. Additionally, numerous U.S. states have promulgated enforceable standards for the levels of perchlorate in drinking water. Massachusetts and California have established standards for perchlorate levels in drinking water of 2 µg/L and 6 µg/L, respectively. More than 10 other states have also set advisory levels or health-based goals for perchlorate in drinking water ranging from 1 to 18 µg/L.

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A few methods capable of reliable determination of perchlorate safely below the above limits have been developed by the U.S. EPA, including Methods 314.0, 314.1, 314.2, 331.0, and 332.0. Ion chromatography (IC) with suppressed conductivity detection and electrospray ionization/mass spectrometry (ESI/MS) is one of the most sensitive and robust characterization methods available for perchlorate. EPA Method 332.0 describes the instrumentation and procedures necessary to identify and quantify low levels of perchlorate in drinking water using IC-ESI/MS.[1]

This application note demonstrates the setup and execution of an IC-MS system according to EPA Method 332.0. The described setup includes a Thermo Scientific™ Dionex™ IonPac™ AS20 column set and a recently introduced compact IC system, the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, coupled with a recently introduced Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer. Using a Dionex IonPac AS20 column set, perchlorate is separated from constituent cations and anions in the sample using a potassium hydroxide mobile phase. The Dionex Integrion HPIC system is a robust, easy-to-use system that includes features such as eluent generation, which makes the potassium hydroxide eluent and thereby eliminates eluent preparation errors and ensures high chromatographic reproducibility. Due to the use of a non-volatile mobile phase, the eluate from the column is passed through an electrolytic suppressor to remove the potassium (K^+) ions from the mobile phase and the analyte counter cations prior to the eluate entering the mass spectrometer. An ^{18}O-enriched perchlorate ($^{35}Cl^{18}O_4^-$) internal standard (m/z 107) is used for quantitation to improve accuracy and ruggedness of the method as described in EPA Method 332.

Application Update 72507 demonstrated that the ISQ EC mass spectrometer provides higher sensitivity, more accurate quantitation, and mass confirmation for perchlorate compared to using only conductivity detection.[2] The two predominant perchlorate ions that occur naturally at a ratio of 3.086:1 are $^{35}Cl^{16}O_4^-$, m/z 99, and $^{37}Cl^{16}O_4^-$, m/z 101, respectively.[3] Identification is made by verifying the relative retention time of the two predominant perchlorate ions with respect to the internal standard. Qualitative confirmation of perchlorate is made by confirming that the m/z 99/101

area count ratio is within a specified range. If these conditions are met, along with all other QC requirements defined in EPA Method 332, then the concentration obtained using the m/z 101 quantitation ion is reported. The HESI-II electrospray improves ionization efficiency eliminating the need to add organic solvents to enhance ionization. The ISQ EC MS also offers full integration with the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software that allows novice and advanced users to easily master MS. Here the work in AU72507 has been extended to a more rigorous study that closely follows EPA Method 332. The results show that the Dionex Integrion IC system, in combination with the ISQ-EC MS, delivers the required performance to successfully execute EPA Method 332.

Experimental
Equipment and consumables
- 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 with sample syringe, 250 µL (P/N 074306) and buffer line, 1.2 mL (P/N 074989)

- Thermo Scientific™ Dionex™ 6-port high-pressure valve (P/N 22153-60014)

- ISQ EC single quadrupole mass spectrometer (P/N ISQEC000IC)

- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)

- Peak® Scientific Genius 1022 nitrogen generator (P/N 10-6022 (230v))

- 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™ AERS 500e Anion Electrolytically Regenerated Suppressor for External Water Mode (2 mm), P/N 302662)

- Thermo Scientific™ Dionex™ OnGuard™ II Ba/Ag/H Cartridge (P/N 063955)

- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Tubing Assembly Kits (P/N 088798)

- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)

- Chromeleon Chromatography Data System (CDS) software version 7.2 SR6

- Fisherbrand™ Narrow-Mouth Field Sample Bottles, high density polyethylene (HDPE), 125 mL, 250 mL, and 1000 mL sizes for storage of standards (Fisher Scientific P/N 02-895A, 02-895B, and 02-895D)

- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)

- Air-Tite™ All-Plastic Norm-Ject® Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)

- Thermo Scientific Nalgene 1000 mL, 0.2 µm Nylon Filter Units (P/N 09-740-46)

Reagents and standards
Reagents
- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better filtered through a 0.2 µm filter immediately before use.

Standards
- Sodium perchlorate, Acros Organics™ (Fisher Scientific P/N AC447421000)

- Sodium perchlorate, Sigma-Aldrich® (Sigma-Aldrich P/N 410241-100G)

- Thermo Scientific™ Dionex™ Perchlorate-ISTD (^{18}O-enriched Internal Standard, P/N 062923)

- Sodium chloride, (Crystalline/Certified ACS), Fisher Chemical™ (Fisher Scientific P/N S271-500)

- Sodium sulfate anhydrous, (Granular/Certified ACS), Fisher Chemical (Fisher Scientific P/N S421-500)

- Sodium carbonate anhydrous, (Powder/Certified ACS), Fisher Chemical (Fisher Scientific P/N S263-500)

IC conditions	
IC System:	Dionex Integrion HPIC
Columns:	Dionex IonPac AG20 Guard, 2 × 50 mm Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600
Eluent:	55 mM KOH
Flow Rate:	0.3 mL/min
Injection Volume:	100 µL
Temperature:	30 °C (column compartment), 20 °C (detector compartment)
System Backpressure:	~2350 psi
Detection:	Suppressed Conductivity, Dionex AERS 500e Electrolytically Regenerated Suppressor (2 mm), AutoSuppression, 41 mA, external water mode via Thermo Scientific™ AXP™ Pump, external water flow rate (0.6 mL/min)
Background Conductance:	~ 0.3 µS
Run Time:	14 min

MS conditions

MS Detector:	ISQ EC single quadrupole mass spectrometer
Ionization Interface:	Electrospray ionization (ESI), negative mode
Divert Valve Switch Time:	6.0–14.0 min (0.0–6.0 min to waste, 6.0–14.0 min to ISQ EC MS)
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 Temp.:	200 °C

Advanced Scan Mode:

Scan Name	Mass List (amu)	Dwell or Scan: Times (s)	SIM Widths (amu)	Ion Polarity	Source CID Voltage
Perchlorate 99	99	1.2	0.3	Negative	0
Perchlorate 101	101	1.2	0.3	Negative	0
Perchlorate ISTD	107	1.2	0.3	Negative	0

Groups:	Chrom. Filter Peak Width: 25 s

System configuration

The information in this section is provided as a general discussion of the main connections required for this method. See Figure 1 for a schematic of the setup used for this study. In this method, the auxiliary six-port valve was placed between the conductivity detector and the mass spectrometer.

Follow the flow diagram shown in Figure 1 to plumb the consumables and modules of the Dionex Integrion HPIC system in external water mode. Install and hydrate the Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 Continuously Regenerating Anion Trap Column, and Dionex AERS 500e suppressor according to the product manual instructions.[4–6] The ISQ EC mass spectrometer is installed according to the ISQ EC Operating Manual.[7] Note: The system pressure needs to be above 2000 psi for effective degassing of the KOH eluent produced by the eluent generator. The Dionex AERS 500e suppressor is recommended for all applications where external water mode is employed, in particular IC-MS applications.

The auxiliary six-port valve is plumbed as shown in Figure 1, with the flow from the Dionex AXP-MS Auxiliary Pump flowing to the ISQ EC mass spectrometer until the background conductivity is below 1.5 μS/cm. The auxiliary valve can be configured as A position and B position. Figure 1 shows the liquid flow paths through the valve ports at each valve position. When it is in B position, the suppressor runs in recycle mode, which uses the suppressed conductivity cell effluent as the source of water for the regenerant. In EPA Method 332, this valve may be placed between the exit of the column and the entrance of the suppressor, or alternatively, it may be placed between the conductivity detector and the MS. In the latter configuration, a 50/50 water/acetonitrile mixture is mixed with the eluate before it enters the MS using a static mixing tee. This study used the latter configuration, but without the need for the acetonitrile (i.e. 100% DI water is diverted to the MS when the flow from the cell is sent to the suppressor regenerant channel). Ionization improvements to the ISQ EC mass spectrometer's electrospray source eliminate the need for addition of organic solvents, and thus simplify operation.

The connection to the mass spectrometer is not made until the background conductivity is below 1.5 μS/cm.

Preparation of reagents and standards
High purity reagent water (RW)
Purified water does not contain any measurable quantity of the target analyte or interfering compounds at concentrations > 1/3 the minimum reporting level (MRL) for the target analyte. Water purity is very important for the successful execution of this method. For this work, DI water was further purified using a bench model Millipore® water purification system (Millipore Corp, Billerica, MA, Model No. Milli-Q® Gradient A10 or equivalent).

Stock solution
A 1000 mg/L solution of perchlorate is prepared by dissolving 0.1231 g of sodium perchlorate ($NaClO_4$, anhydrous, 99% pure grade, or better, M = 122.4 g/mol, CASRN 7601-89-0) in 100 mL of RW. 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.
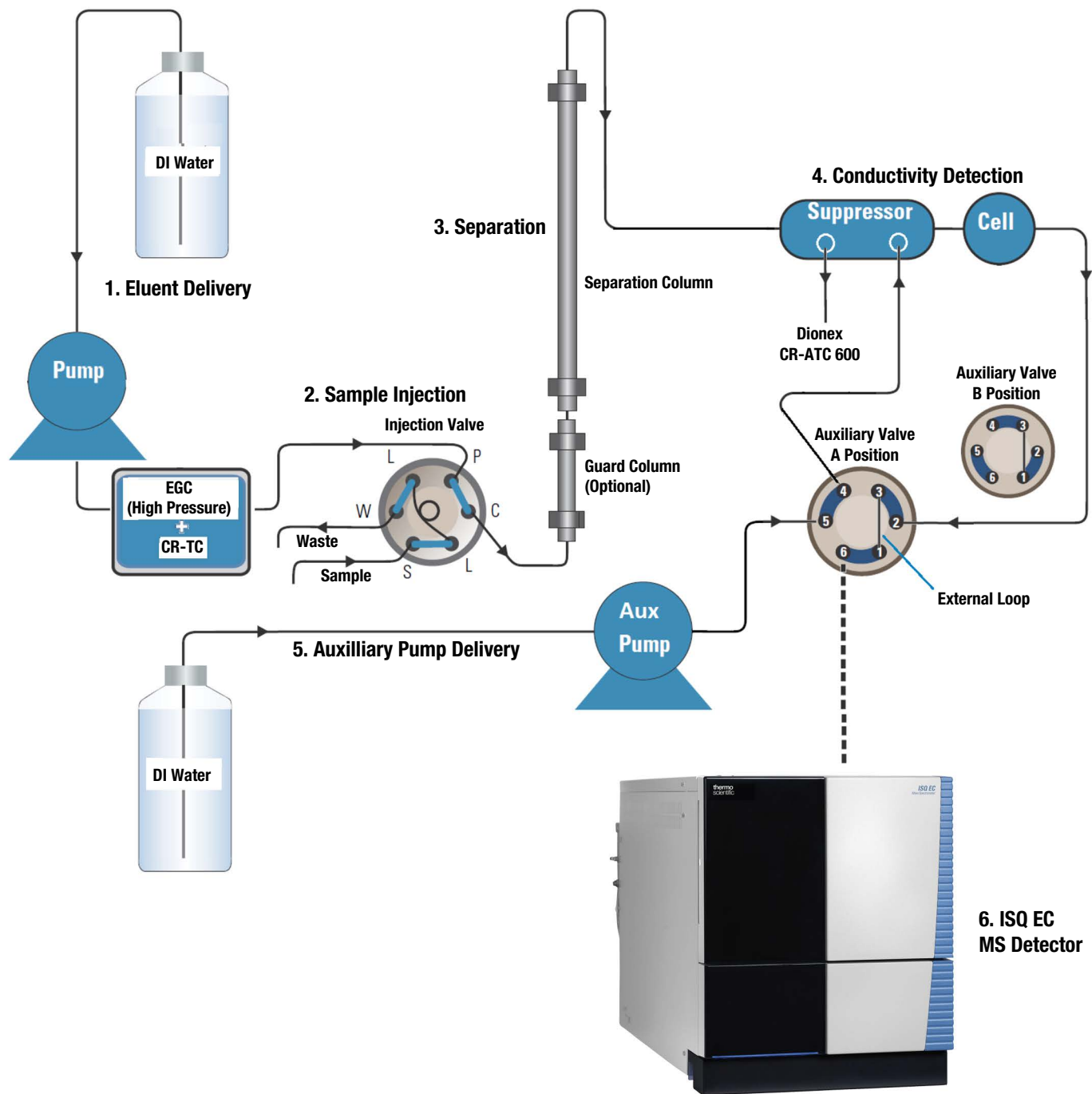
Figure 1. IC-MS configuration with matrix diversion.

Calibration standard solutions
A serial dilution is used to prepare the highest concentration working standard solutions (10 µg/L) by pipetting the appropriate amount of 1000 mg/L stock into a 100 mL volumetric flask and diluting to the mark with RW. Prepare the 0, 125, 250, 400, 800, 1000, 2000, and 5000 ng/L perchlorate standard solution mixes by diluting the 10 µg/L working standard with RW. Add 10 µL 1 mg/L perchlorate ISTD to each 10 mL standard. The holding time for the calibration standard solution is one month.

Laboratory synthetic sample matrix (LSSM)
To prepare a solution of 1000 mg/L each of chloride, carbonate, and sulfate, add 1.48 g of sodium sulfate, 1.65 g of sodium chloride, and 1.77 g of sodium carbonate to a 1 L volumetric flask and dilute to volume with RW. The EPA Method 332 recommended holding time is one year.

Laboratory fortified synthetic sample matrix (LFSSM)

Prepare an LFSSM at the mid-level concentration of the calibration curve using the LSSM. In this method, the LFSSM contains 1 µg/L perchlorate and 1 µg/L ISTD. The holding time is one month.

Laboratory reagent blank (LRB)

The LRB is an aliquot of RW or other blank matrix that is treated exactly as a sample including exposure to all filtration equipment, storage containers, and internal standards. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, the reagents, or apparatus.

Laboratory synthetic sample matrix blank (LSSMB)

The LSSMB is a solution of 1000 mg/L each of chloride, sulfate, and carbonate (Cl^-, SO_4^{2-}, and CO_3^{2-}) that is processed like a field sample. The LSSMB is a reagent blank that must be analyzed with each LFSSM.

Laboratory fortified blank (LFB)

The LFB is an aliquot of RW or other blank matrix to which known quantities of the method analyte and internal standard are added in the laboratory. The LFB is analyzed exactly like a sample, including preservation procedures, and its purpose is to determine whether the method, inclusive of sample processing, is in control, and whether the laboratory is capable of making accurate and precise measurements.

Procedure

Sample collection, preservation, and storage

Follow the instructions as described in Section 8 of EPA Method 332.1

MS mass calibration and instrument optimization

An ISQ EC mass spectrometer ships with two bottles of calibration solution. Each bottle contains over 250 mL of liquid and is stable at room temperature for up to one year. Ideally, the spare bottle should be stored at 4 °C. After the mass calibration has been performed, the analyst must check mass accuracy for perchlorate. Inject a calibration standard solution containing 1 µg/L perchlorate and 1 µg/L ISTD, scan over the range of 95–115 amu, and verify that the perchlorate peaks are symmetric about m/z 99, 101, and 107. If the peaks are not symmetric about the mass assignments

(i.e., 99 ± 0.3, 101 ± 0.3, and 107 ± 0.3), then a new mass calibration of the MS, or other instrument maintenance according to the manufacturer's recommendations, should be performed.

MS conditions need to be optimized for enhanced sensitivity and improved qualitative detection of perchlorate. For a quick analysis, an optional syringe drive pump can be paired with your ISQ EC and directly infused or teed into your mobile phase. As the sample is delivered, it can be monitored in real time using the Real Time Scan feature on the instrument ePanel. This allows the quick adjustment of scan and source settings without waiting on injection cycles or chromatography. Suggested operating conditions are listed in the section "Conditions". Optimal conditions may vary depending on instrumentation.

Initial calibration

Construct the calibration curve using the internal standard calibration technique. The lowest concentration of the calibration standard must be at or below the MRL. Prepare the 125, 250, 400, 800, 1000, 2000, and 5000 ng/L perchlorate standard solutions containing 1 µg/L ISTD. Inject 100 µL of each standard into the IC-MS, and inject a RW blank after the 5000 ng/L perchlorate standard to check for carryover. This type of blank is not filtered or processed as a sample. Use a quality control sample (QCS) to verify initial calibration. The QCS sample is fortified with 1000 ng/L perchlorate near the midpoint of the calibration range. The QCS sample was sourced from Sigma-Aldrich so that it is different than that from the Fisher Scientific product used for the calibration standards. The carryover result should demonstrate that perchlorate is < 1/3 the MRL (at both m/z 99 and m/z 101 at the retention time for perchlorate), and that possible interferences do not prevent the identification or quantification of perchlorate.

Minimum reporting level (MRL) confirmation

Select a target concentration for the MRL based on the intended use of the method. Establish an *Initial Calibration* in which the lowest calibration standard used to establish the *Initial Calibration* must be at or below the concentration of the target MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements.

Confirm the targeted MRL following the procedure outlined below.

1. Prepare and analyze seven replicate laboratory fortified blanks (LFBs) containing 150 ng/L perchlorate. All samples must be processed using the sample collection devices described in Section 8.1.4.1 of EPA Method 332.[1] Calculate the mean (*Mean*) and standard deviation for these replicates using the *m/z* 101 ion. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below:

$$HR_{PIR} = 3.963 \times S$$

Where *S* is the standard deviation, and 3.963 is a constant value for seven replicates.

2. Confirm that the upper and lower limits for the Prediction Interval of Results ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below:

The Upper PIR Limit must be ≤ 150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100 \leq 150\%$$

The Lower PIP Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100 \leq 50\%$$

3. The target MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above. If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration

Initial demonstration of capability (IDC)
The IDC must be successfully performed prior to analyzing any field samples. Prior to conducting the IDC, the analyst must first meet the calibration requirements described above. Requirements for the initial demonstration of laboratory capability are described in the following sections and are summarized in Table 1, which is a duplication of the far three right hand columns of Table 7 in EPA Method 332.

Continuing calibration checks (CCCs)
At the beginning of the *Analysis Batch*, the *Initial Calibration* must be verified by analyzing a mid-level (1000 ng/L perchlorate) and MRL level (150 ng/L) CCC. Throughout an *Analysis Batch* the calibration is verified after every ten field samples by the analysis of a CCC that is rotated between low (≤ MRL), medium (mid-level calibration concentration) and high concentration (upper-level calibration concentration). CCCs are not counted as samples. Analyze CCCs under the same conditions used during the *Initial Calibration*. Acceptance criteria for the mid-level CCC is 80–120% recovery, and for MRL CCC is 50–150% recovery. Note: If the IS response drifts below 50% of the average IS response of the calibration standards from the *Initial Calibration,* instrument maintenance or ESI/MS detector inlet cleaning may be required.

Table 1. Initial Demonstration of Capability (IDC) Requirements.

Requirement	Specification and Frequency	Acceptance Criteria
Demonstration of Low System Background	Analyze an LRB and LSSMB prior to any other IDC steps and when modifications are made.	< 1/3 MRL
Concentration Dependent Carryover	During IDC and when modifications are made. Analyze a RW blank after the high calibration standard during *Initial Calibration.*	Demonstrate that carryover from one injection to the next is < 1/3 the MRL.
Demonstration of Precision in LFBs and LFSSMs	Analyze seven replicates fortified near mid-range of calibration.	≤ 20% R.S.D.
Demonstration of Accuracy in LFBs and LFSSMs	Calculate the average recovery for replicates	80–120% recovery at the mid-level concentration
Minimum Reporting Level (MRL) Confirmation	Analyze seven replicate LFBs at the target MRL. Use the equation provided to verify the MRL. Repeat after major instrument or operational changes.	MRL acceptance based on project objects or regulatory requirements. Upper PIR ≤ 150% Lower PIR ≥ 50%

Detection limit determination

Prepare and analyze at least seven replicate LFBs at a concentration of 50 ng/L perchlorate over at least 3 days. This fortification level may be estimated by selecting a concentration with a signal of 2–5 times the noise level.

Calculate the DL using the equation:

$$DL = S * t_{(n-1,\ 1-alpha=0.99)}$$

Where $t_{(n-1,\ 1-alpha=0.99)}$ = Student's t for the 99% confidence level with n-1 degrees of freedom. Student's t=3.143 for n=7

n= number of replicates

S= standard deviation of replicate analyses

Note: Do not subtract blank values when performing MRL or DL calculations.

Sample preparation

Collect and store field samples as described in Section 8.1 of EPA Method 332.1. For refrigerated samples or field samples arriving at the laboratory cold, ensure the samples are at room temperature prior to analysis by allowing the samples to sit on the bench for at least 30 min. Add 5 or 10 µL 1 mg/L perchlorate ISTD to each 5 mL or 10 mL sample, respectively.

Sample analysis

1. Establish optimal operating conditions for the IC-MS. Operating conditions may vary depending on instrumentation. The analyst is responsible for determining optimal conditions for their instrumentation. The configuration and the operation conditions used here are shown in Figure 1 and "Conditions".

2. Establish a valid *Initial Calibration* or confirm that the calibration is still valid by analyzing the required CCCs.

3. Inject field samples and QC samples under the same conditions used for the *Initial Calibration.* A sample *Analysis Batch* is presented in Table 2, which is a duplication of Table 3 in EPA Method 332 with a few edits shown in bold font.

4. At the conclusion of data acquisition, use the same data acquisition method that was used for the *Initial Calibration* to identify peaks in the chromatogram. Use the data acquisition method to determine the relative retention times and integrate the peak areas of the monitored ions (*m/z* 99, 101, and 107).

Compound identification

Identification/confirmation of perchlorate in a sample is made by detecting perchlorate at *m/z* 101 and *m/z* 99 at the retention time of the internal standard and by passing the QC criteria established for the *m/z* 99/101 area count ratio.

Because the perchlorate ISTD has the same retention time as naturally occurring perchlorate, the retention time ratio of *m/z* 99/107 and *m/z* 101/107 in samples must be within 0.98–1.02 (± 2% of ideal ratio of 1) for confirmation of perchlorate in a sample. All calibration standards, QC samples, and field samples must meet the *m/z* 99/101 area count ratio requirement for confirmation of perchlorate. The measured ratio must fall within ± 25% (2.31–3.85). If this area count ratio requirement is not met for a CCC or LFB, then all samples in the *Analysis Batch* are considered invalid and must be reanalyzed after reestablishing acceptable instrument performance. When area count ratios fall outside this range due to sulfate interference and/or a high-ionic strength matrix, the sample should be treated with Dionex OnGuard II Ba/Ag/H cartridges to reduce high background to a level that allows better integration of the perchlorate peak at *m/z* 99, and thus, better *m/z* 99/101 area count ratios for confirmation. Note: Add the IS to the sample prior to sample pretreatment using the cartridges.

Table 2. Sample *Analysis Batch*.

Injection #	Sample Description	Acceptance Criteria	Remedial Action
1	Mid-Level CCC	80–120 % recovery using *Initial Calibration*	Instrument maintenance and recalibration.
2	MRL CCC	50–150% recovery	Instrument maintenance to recover sensitivity and recalibration.
3	LRB	< 1/3 MRL concentration	Find and correct source of contamination.
4	LFB ≤ MRL concentration or >MRL to highest calibration standard	50–150% recovery (≤ MRL) 80–120% recovery (> MRL)	Identify and correct source of problem.
5–14	Field Samples 1–10	Pass RT, *m/z* 99/101 area count ratio, and IS area count QC criteria at concentrations ≥ MRL concentration.	If problem is due to **high-ionic-strength matrix,** clean up sample using **Dionex OnGuard II Ba/Ag/H cartridge,** otherwise report.
15	CCC (rotating concentrations)	80–120% recovery using *Initial Calibration* for concentrations > MRL 50–150% recovery for concentrations ≤ MRL	Instrument maintenance and recalibration.
16	LFSM of a field sample previously analyzed	At fortification concentrations > MRL concentration, 80–120% recovery. At fortification concentrations ≤ MRL, 50–150% of true value.	If problem is due to **high-ionic-strength matrix**, clean up sample using **Dionex OnGuard II Ba/Ag/H cartridge,** otherwise report.
17	Laboratory Duplicate or a LFSMD of field sample previously analyzed. Choose LFSMD if samples are low in perchlorate.	Relative percent difference (RPD) <20% for concentrations > 2× MRL RPD <50% for samples ≤ 2× MRL	If RPD out of the designated range, but the laboratory performance is acceptable in LFB, the recovery problem is judged to be matrix related. Label sample "suspect matrix".
18–27	Field Samples 11–20	Pass RT, *m/z* 99/101 area count ratio, and IS area count QC criteria.	If problem is due to **high-ionic-strength matrix,** clean up sample using **Dionex OnGuard II Ba/Ag/H cartridge,** otherwise report.
28	Final CCC (rotating concentrations)	80–120 % recovery using *Initial Calibration* for concentration > MRL	Instrument maintenance and recalibration.

Data analysis

Calculate sample concentration on the calibration curve using the *m/z* 101 ion. If the measured concentration of a field sample exceeds the calibration range, a fresh aliquot of the sample must be diluted and re-analyzed and pass the confirmation criteria. Frequently check all the on-going QC requirements as the samples continue to be analyzed. If a CCC failed at any point during an *Analysis Batch,* it will be necessary to re-analyze all samples after the last successful CCC.

Results and discussion

IC-MS conditions

The conditions used in this method are shown in the "IC conditions" and "MS conditions" tables.

Figure 2 shows chromatograms of a 125 ng/L calibration standard with retention times for the ions monitored in this method (*m/z* 99, 101, and 107). Figure 3 shows chromatograms of a 1.0 µg/L perchlorate LFSSM solution containing 1000 mg/L of chloride, sulfate, and carbonate. Figure 3 also illustrates the effect of a high background at *m/z* 99 due to $H^{34}SO_4^-$.
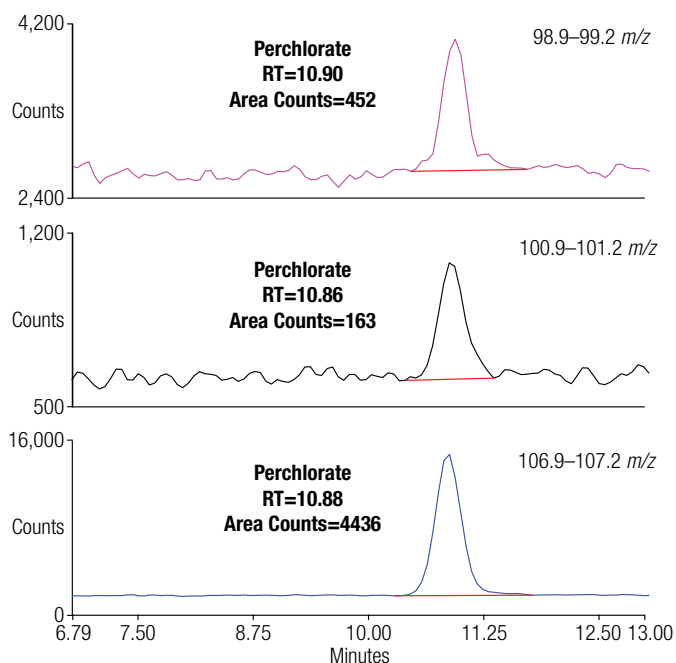

Figure 2. Mass chromatogram of a standard containing 125 ng/L perchlorate and 1.0 µg/L internal standard.
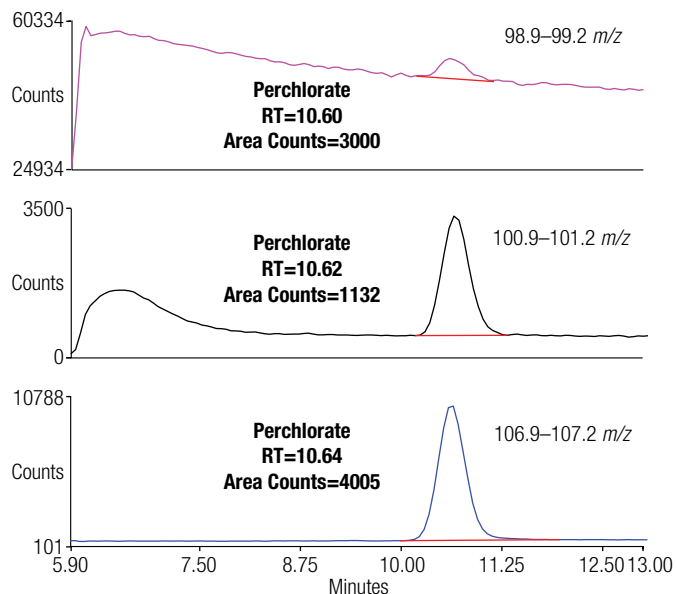
Figure 3. Mass chromatogram of an LFSSM containing 1.0 µg/L perchlorate and 1.0 µg/L internal standard.

Initial calibration

All the calibration standards are evaluated if their *m/z* 99/101 area count ratios are within the acceptance limits of 2.31–3.85 and the relative retention time ratios for *m/z* 99/107 and *m/z* 101/107 are between 0.98 and 1.02 (Table 3). Table 3 is based on Table 4 in EPA Method 332. The Sample ID column has been replaced with Concentration. Using the data obtained in the "Initial calibration" subsection of the "Procedure" section on page 6, perform a regression (e.g., linear, weighted linear, quadratic) of the *m/z* 101/107 area count ratio vs. concentration of perchlorate. To evaluate if the chosen regression model yields accurate results across the range, reprocess (do not re-analyze) calibration standards as unknown and determine the calculated concentrations. Determine the percent recoveries of the reprocessed calibration standards based on the known concentrations. Recoveries at all the tested concentrations must be within 80–120% for

concentrations > the MRL. For concentrations ≤ the MRL, the minimum acceptance criterion is 50–150% recovery. In this study, both linear and quadratic regression were performed. Both recoveries were acceptable, and quadratic regression was used for the following evaluation (Table 4). If the recoveries are not within the acceptable ranges, a different regression model such as a weighted linear, quadratic, or weighted quadratic

should be tested. An acceptable calibration has been obtained when recoveries of reprocessed standards are within the acceptance criteria stated above. A QCS sample fortified with 1000 ng/L perchlorate was analyzed to verify initial calibration (Table 4). The QCS should be prepared and analyzed just like a CCC. The recovery of QCS sample fortified near the midpoint of the calibration range should fall within 80–120%.

Table 3. Tabulation of standard data for QC requirement.

Concentration (ng/L)	Ion (m/z)	Area Counts	Retention Time (min)	Relative Retention Time Ratio (m/z 99/107, m/z 101/107)*	Area Count Ratio (m/z 99/101)**
125	99	452	10.9	1.00	
	101	160	10.9	1.00	2.85
	107	4334	10.9		97.0%***
250	99	959	10.9	1.00	
	101	325	10.9	1.00	2.95
	107	4347	10.9		97.3%***
400	99	1560	10.9	1.00	
	101	526	10.9	1.00	2.97
	107	4484	10.9		100%***
800	99	3283	10.9	1.00	
	101	1067	10.9	1.00	3.08
	107	4464	10.9		100%***
1000	99	3900	10.9	1.00	
	101	1275	10.9	1.00	3.06
	107	4310	10.9		96.5%***
2000	99	8055	10.9	1.00	
	101	2621	10.9	1.00	3.07
	107	4327	10.9		96.8%***
5000	99	20215	10.9	1.00	
	101	6406	10.9	1.00	3.16
	107	4037	10.9		90.4%***

* Acceptance Criteria (0.98–1.02)
** Acceptance Criteria (2.31–3.85)
*** The IS should be ±30% of the first CCC.

Table 4. Method calibration and recoveries at *m/z* 101/107.

Range (ng/L)	Regression	Coefficient of Determination (r²)	Regression	Coefficient of Determination (r²)
125–5000	Linear	0.9997	Quadratic	1.0000
Concentration (ng/L)	**Recovery**		**Recovery**	
125	125%		99.9%	
250	110%		101%	
400	102%		99.3%	
800	98.9%		101%	
1000	96.3%		99.3%	
2000	97.2%		100%	
5000	101%		100%	
QCS sample at 1000 ng/L	101%		102%	

IDC validation

System background, precision, and accuracy have been demonstrated and are reported in Table 5, which was adapted from Table 7 in EPA Method 332.

Table 5. System background, precision, and accuracy.

Requirement	Specification and Frequency	Results	Acceptance Criteria
Demonstration of Low System Background	Analyze an LRB and LSSMB prior to any other IDC steps and when modifications are made.	Free of any contaminants that would prevent the determination of perchlorate	< 1/3 MRL
Concentration Dependent Carryover	During IDC and when modifications are made. Analyze a RW blank after the high calibration standard during *Initial Calibration.*	Free of any contaminants that would prevent the determination of perchlorate	Demonstrate that carryover from one injection to the next is < 1/3 the MRL.
Demonstration of Precision in LFBs and LFSSMs	Analyze seven replicates fortified near mid-range of calibration.	2.28% R.S.D. for LFBs at 1000 ng/L 2.36% R.S.D. for LFSSMs at 1000 ng/L	≤ 20% R.S.D.
Demonstration of Accuracy in LFBs and LFSSMs	Calculate the average recovery for replicates	98% recovery for LFBs at 1000 ng/L 103% recovery for LFSSMs at 1000 ng/L	80–120% recovery at the mid-level concentration

MRL confirmation

Seven replicate 150 ng/L LFBs results are shown in Table 6. The upper PIR is below 150%, and lower PIR is above 50%, so the MRL of 150 ng/L level was validated.

Table 6. MRL confirmation.

Concentration (ng/L)	Measured Concentration (ng/L)	Mean (ng/L)	Standard Deviation	HR_{PIR}	Upper PIR	Lower PIR
150	151 164 170 145 150 156 152	155	8.57	34.0	126%	80.7%

Sample analysis

The sample *Analysis Batch* was set up following the "Sample analysis" subsection of the "Procedure" section. The results are shown in Tables 7 and 8 (Adaptations of Tables 3 and 4 in EPA Method 332, as previously discussed).

Table 7. Sample Analysis Batch.

Injection #	Sample Description	Certified Concentration (ng/L)	Measured Concentration (ng/L)	Recovery (%)	Acceptance Criteria
1	Mid-Level CCC	1000	1033	103	80–120% recovery using *Initial Calibration*
2	MRL CCC	150	131	87.3	50–150% recovery
3	LRB	0	0	n.a.	< 1/3 MRL concentration
4	LFB ≤ MRL concentration or > MRL to highest calibration standard	125 1000 5000	151 1034 5018	121 103 100	50–150% recovery (≤ MRL) 80–120% recovery (> MRL)
5–14	Field Samples 1–10		See Table 8		Pass RT, *m/z* 99/101 area count ratio, and IS area count QC criteria at concentrations ≥ MRL concentration.
15	CCC (rotating concentrations)	125 1000 5000	144 967 4892	115 96.7 97.8	80–120% recovery using *Initial Calibration* for concentrations > MRL 50–150% recovery for concentrations ≤ MRL
16	LFSM of a field sample previously analyzed	500	458	91.6	At fortification concentrations > MRL concentration, 80–120% recovery. At fortification concentrations ≤ MRL, 50–150% of true value.
17	Laboratory Duplicate or a LFSMD of field sample previously analyzed. Choose LFSMD if samples are low in perchlorate.	500	478	95.6 (RPD = 4.2% < 20% for concentrations > 2× MRL)	Relative percent difference (RPD) < 20% for concentrations > 2× MRL RPD < 50% for samples ≤ 2× MRL
18–27	Field Samples 11–20		See Table 8		Pass RT, *m/z* 99/101 area count ratio, and IS area count QC criteria.
28	Final CCC (rotating concentrations)	125 1000 5000	126 1003 4902	101 100 98.0	80–120% recovery using *Initial Calibration* for concentration > MRL

Table 8A. Tabulation of sample data for QC requirement (samples 1–10).

Concentration (ng/L)	Ion (*m/z*)	Area Counts	Retention Time (min)	Relative Retention Time Ratio (*m/z* 99/107, *m/z* 101/107)*		Area Count Ratio (*m/z* 99/101)**
Sample 1	99	2466	10.2	1.00		2.80
	101	882	10.3	1.01		
	107	4868	10.2		90.7%***	
Sample 2	99	2561	10.2	1.00		2.86
	101	895	10.3	1.01		
	107	4773	10.2		88.9%***	
Sample 3	99	2524	10.2	1.00		2.81
	101	897	10.3	1.01		
	107	4749	10.2		88.5%***	
Sample 4	99	2420	10.2	1.00		2.69
	101	900	10.3	1.01		
	107	4682	10.2		87.2%***	
Sample 5	99	2585	10.2	1.00		3.00
	101	863	10.3	1.01		
	107	4762	10.2		88.7%***	
Sample 6	99	2559	10.2	1.00		3.07
	101	831	10.3	1.01		
	107	4622	10.2		86.1%***	
Sample 7	99	2499	10.2	1.00		3.00
	101	833	10.3	1.01		
	107	4765	10.2		88.8%***	
Sample 8	99	2319	10.2	1.00		2.88
	101	804	10.3	1.01		
	107	4427	10.2		82.5%***	
Sample 9	99	2514	10.2	1.00		2.94
	101	854	10.3	1.01		
	107	4523	10.2		84.3%***	
Sample 10	99	2368	10.2	1.00		2.88
	101	823	10.3	1.01		
	107	4528	10.2		84.4%***	

* Acceptance Criteria (0.98–1.02)
** Acceptance Criteria (2.31–3.85)
*** The IS should be ±30% of the first CCC.

Table 8B. Tabulation of sample data for QC requirement (samples 11–20).

Concentration (ng/L)	Ion (*m/z*)	Area Counts	Retention Time (min)	Relative Retention Time Ratio (*m/z* 99/107, *m/z* 101/107)*		Area Count Ratio (*m/z* 99/101)**
Sample 11	99	4033	10.2	1.00		2.86
	101	1408	10.3	1.01		
	107	4700	10.2		87.6%***	
Sample 12	99	4103	10.2	1.00		2.80
	101	1466	10.3	1.01		
	107	4973	10.2		92.7%***	
Sample 13	99	3945	10.2	1.00		2.82
	101	1397	10.3	1.01		
	107	4728	10.2		88.1%***	
Sample 14	99	3950	10.2	1.00		2.78
	101	1422	10.3	1.01		
	107	4681	10.2		87.2%***	
Sample 15	99	4121	10.2	1.00		2.74
	101	1502	10.3	1.01		
	107	4871	10.2		90.8%***	
Sample 16	99	4051	10.2	1.00		2.88
	101	1409	10.3	1.01		
	107	4862	10.2		90.6%***	
Sample 17	99	3996	10.2	1.00		2.89
	101	1383	10.3	1.01		
	107	4851	10.2		90.4%***	
Sample 18	99	4106	10.2	1.00		2.74
	101	1497	10.3	1.01		
	107	4721	10.2		88.0%***	
Sample 19	99	3823	10.2	1.00		2.74
	101	1394	10.3	1.01		
	107	4804	10.2		89.5%***	
Sample 20	99	3960	10.2	1.00		2.83
	101	1397	10.3	1.01		
	107	4821	10.2		89.8%***	

* Acceptance Criteria (0.98–1.02)
** Acceptance Criteria (2.31–3.85)
*** The IS should be ±30% of the first CCC.

Method performance

Single laboratory precision in drinking waters, as measured by percent relative standard deviation (R.S.D.) of replicate analyses (n=7), was < 5% at concentrations > MRL perchlorate (Table 9). Accuracy, as measured by percent recoveries of fortified drinking water samples and external Quality Control samples, was 95.6–102% for concentrations > MRL perchlorate, and 111% for concentrations < MRL perchlorate (Table 10).

Table 9. Single laboratory precision in drinking waters.

Drinking Water 1 (ng/L, n=7)	Mean (ng/L)	S.D.	R.S.D.
635			
639			
651			
614	622	20.3	3.26%
609			
592			
615			
Drinking Water 2 (ng/L, n=7)	**Mean (ng/L)**	**S.D.**	**R.S.D.**
992			
994			
1022			
1037	1007	36.9	3.66%
976			
960			
1066			

Table 10. Single laboratory accuracy in drinking waters and external quality control samples.

Sample	Certified Concentration (ng/L)	Measured Concentration (ng/L)	Recovery (%)
Fortified Drinking Water 1	125	139	111
Fortified Drinking Water 2	1000	980	98.0
External Quality Control Sample 1	500	478	95.6
External Quality Control Sample 2	1000	1019	101.9

Single laboratory precision in fortified synthetic waters containing up to 1000 mg/L of each of the common anions (LFSSM), as measured by R.S.D. of replicate analyses (n=7), was < 5% at concentrations > MRL perchlorate (Table 11). Accuracy, as measured by percent recovery of fortified synthetic high ionic waters containing up to 1000 mg/L of each of the common anions (LFSSM), was 100–104% for concentrations > MRL perchlorate (Table 12).

Table 11. Single laboratory precision in LFSSM.

LFSSM (ng/L, n=7)	Mean (ng/L)	S.D.	R.S.D.
1005			
1046			
1013			
1056	1035	24.4	2.36
1072			
1035			
1019			

Table 12. Single laboratory accuracy in LFSSM.

Fortified LFSSM (ng/L)	Measured Concentration (ng/L)	Recovery %
200	200	100
400	400	100
800	814	102
1000	1035	104

Table 13 lists single laboratory DL and single-laboratory lowest concentration minimum reporting level (LCMRL) data in RW.

Table 13. Detection limit and LCMRL for perchlorate in reagent water.

Replicate	Amount *m/z* 101 (ppt)
1	34.9
2	35.3
3	54.6
4	40.6
5	44.6
6	40.2
7	56.0
Average	43.7
S.D.	8.58
R.S.D.	19.6
Detection Limit * (3.14 × S.D.)	26.9
LCMRL	125

* Fortification concentration - 50 ng/L

Table 14 lists precision and recovery data for perchlorate in various drinking water and synthetic water samples at low and high fortification concentrations. Note: When it was unable to detect the *m/z* 99 ion in the LSSM fortified with 200 ng/L perchlorate due to the high-ionic-strength matrix, the remedial action was employed that consisted of using a Dionex OnGuard II Ba/Ag/H cartridge to reduce high background to a level that allows better integration of the perchlorate peak at *m/z* 99, and thus, better *m/z* 99/101 area count ratios for confirmation (Figure 4).

Table 14. Precision and recovery data for perchlorate in various matrices (n=7).

Matrix	Background Conc. (ng/L)	Fortification Conc. (ng/L)	*m/z* 99/101 Area Ratio	Avg. Recovery (%)	R.S.D.
Reagent water	ND	50	3.54	87.5	19.6
		500	3.17	96.0	3.35
LSSM	ND	200	2.97 (after using Ba/Ag/H cartridge)	100	7.07
		1000	2.78	104	1.89
Tap water	ND	1000	2.85	101	3.66
Bottled water	270	200	2.96	100	3.24
		1000	3.07	98.8	2.47
Bottled water	< LCMRL	200	3.08	103	3.56
		1000	3.07	98.7	1.25

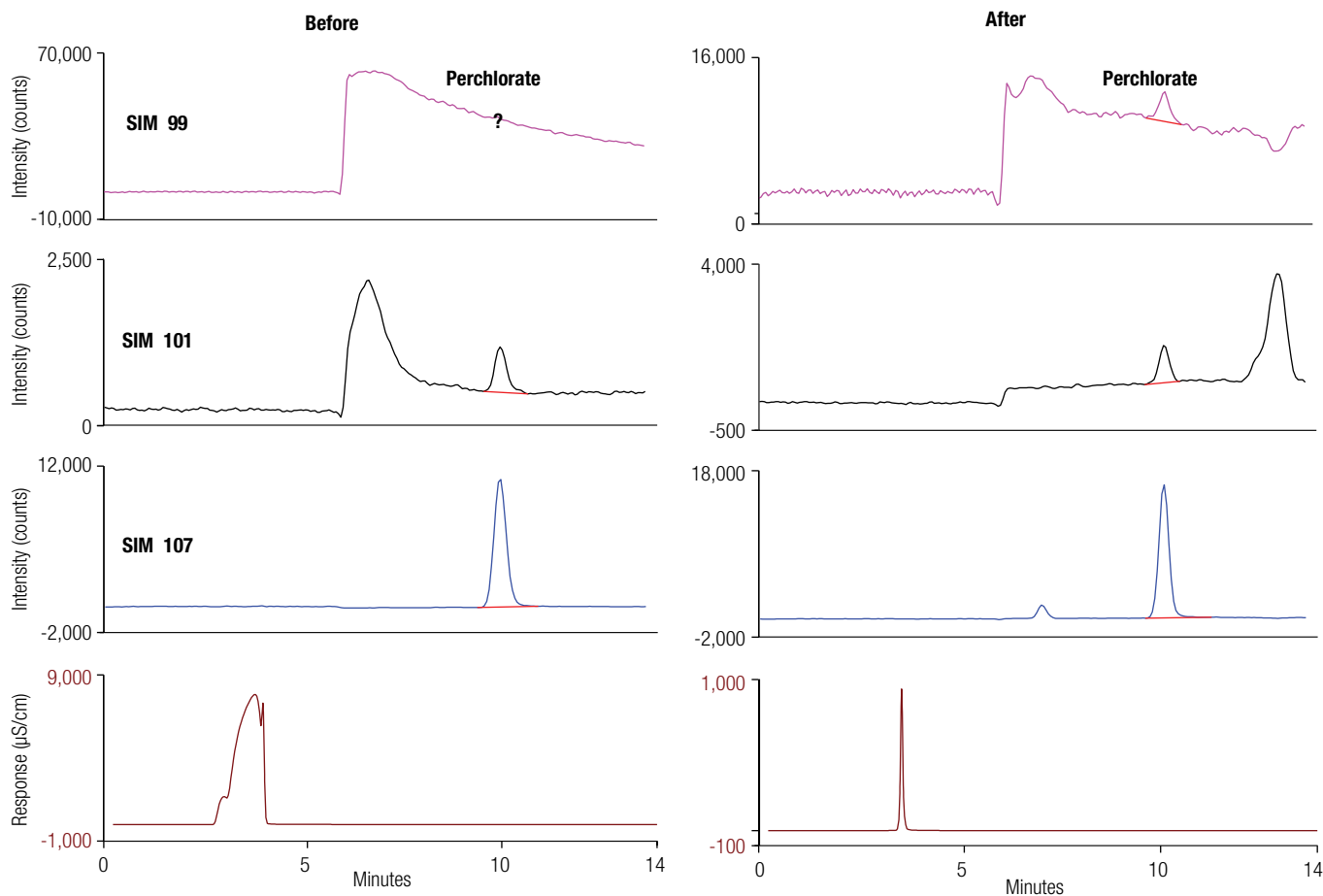ND means "not detected"
LCMRL=125 ng/L

Figure 4. Conductivity and SIM chromatograms of 200 ng/L perchlorate in LSSM before and after using a Dionex OnGuard II Ba/Ag/H cartridge.

Conclusion

This application note evaluated the performance of a Dionex Integrion HPIC system coupled with an ISQ EC MS to fulfill the requirements of EPA Method 332. The results showed this new IC-MS platform can successfully implement EPA Method 332 for the determination of perchlorate in drinking water. This study demonstrated great sensitivity, good precision and accuracy, and ruggedness while all conditions were met, along with passing all other QC requirements defined in EPA Method 332.

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