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METHOD 331.0 DETERMINATION OF PERCHLORATE IN DRINKING WATER BY LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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METHOD 331.0

DETERMINATION OF PERCHLORATE IN DRINKING WATER BY LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION MASS SPECTROMETRY (LC/ESI/MS)

1. SCOPE AND APPLICATION

1.1 This is a liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS) method for the determination of perchlorate in raw and finished drinking waters. This method can be used to acquire data using either Selected Ion Monitoring (SIM) or Multiple Reaction Monitoring (MRM) detection. Based on known interferences, MRM detection is recommended, however, SIM detection may be used if all of the criteria outlined in Section 9.1 are met. Precision and accuracy data have been generated for both SIM and MRM detection of perchlorate in reagent water, finished groundwater, finished surface water and a synthetic high ionic strength matrix. The single laboratory Lowest Concentration Minimum Reporting Level (LCMRL) has also been determined for both detection modes in reagent water.[1]

Analyte	Chemical Abstract Services Registry Number (CASRN)
Perchlorate	14797-73-0

1.2 The SIM and MRM mass spectrometry conditions described in this method were developed using a conventional LC system. Analysts interested in using an ion chromatography system with MS detection should consult EPA Method 332.0.

1.3 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets the Data Quality Objectives (DQOs) which are based on the intended use of this method. The single laboratory LCMRL is the lowest true concentration for which the future recovery is predicted to fall between 50-150% recovery with 99% confidence. Single laboratory LCMRLs for perchlorate were 0.022 µg/L for MRM using *m/z* 83 and 0.056 µg/L for SIM using *m/z* 101. These values are also provided in Table 2. The procedure used to determine the LCMRL is described elsewhere.[1]

1.4 Laboratories using this method are not required to determine an LCMRL, but they will need to demonstrate that their laboratory MRL for this method meets the requirements described in Section 9.2.4.

1.5 Detection limit (DL) is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero[2]. The DL is dependent on sample matrix, fortification concentration, and instrument performance. Determining the DL for analytes in this method is optional (Sect. 9.2.5). The DLs for perchlorate were 0.005 µg/L in LC/MRM mode and 0.008 µg/L in the LC/SIM Mode. These values are also provided in Table 2.

1.6 This method is intended for use by analysts skilled in the operation of LC/MS instrumentation and the interpretation of the associated data.

2. SUMMARY OF METHOD

2.1 Water samples are collected in the field using a sterile filtration technique. Prior to analysis, isotopically enriched perchlorate ($Cl^{18}O_4^-$) is added to the sample as an internal standard. The sample is injected without cleanup or concentration onto a chromatographic column (Dionex IonPak® AS-21 or equivalent), which separates perchlorate from other anions and background interferences. Perchlorate is subsequently detected by negative electrospray ionization mass spectrometry. A remote controlled valve is used to divert early eluting cations and anions to waste. Prior to the elution of perchlorate, the valve is switched sending the chromatographic eluent to the mass spectrometer. This diversion helps prevent unnecessary fouling of the electrospray source. Perchlorate is quantified using the internal standard technique.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A sequence of samples, which are analyzed within a 30-hour period and include no more than 20 field samples. Each Analysis Batch must also include all required QC samples, which do not contribute to the maximum field sample total of 20. The required QC samples include:

Laboratory Reagent Blank (LRB)
Continuing Calibration Check Standards (CCCs)
Laboratory Fortified Blank (LFB)
Laboratory Fortified Sample Matrix (LFSM)
Laboratory Fortified Sample Matrix Duplicate or Laboratory Duplicate (LFSMD or LD)

3.2 CALIBRATION STANDARD (CAL) – A solution of the target analyte prepared from the primary dilution standard solution or stock standard solution and the internal standard. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analyte, which is analyzed periodically to verify the accuracy of the existing calibration.

3.4 DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination (Sect. 9.2.5), and accurate quantitation is not expected at this level.[2]

3.5 INTERNAL STANDARD (IS) – A pure compound added to all standard solutions and field samples in a known amount. It is used to measure the relative response of the method analyte. The internal standard must be a compound that is not a sample component.

3.6 LABORATORY DUPLICATES (LDs) – Two sample aliquots (LD_1 and LD_2), taken in the laboratory from a single sample bottle, and analyzed separately with identical procedures. Analyses of LD_1 and LD_2 indicate precision associated specifically with the laboratory procedures by removing variation contributed from sample collection, preservation and storage procedures.

3.7 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which a known quantity of the method analyte is added. The LFB is analyzed exactly like a sample including the preservation procedures in Section 8. Its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of a field sample to which a known quantity of the method analyte is added. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured value in the LFSM corrected for background concentrations.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the field sample used to prepare the LFSM which is fortified and analyzed identically to the LFSM. The LFSMD is used instead of the Laboratory Duplicate to assess method precision and accuracy when the occurrence of the target analyte is infrequent.

3.10 LABORATORY FORTIFIED SYNTHETIC SAMPLE MATRIX (LFSSM) – An aliquot of the Laboratory Synthetic Sample Matrix (Sect. 7.2.4) which is fortified with perchlorate. This QC sample is processed like a field sample (Sect. 8.1) and is used to confirm that the analyst has adequate chromatographic resolution between sulfate and perchlorate.

3.11 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water 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 the apparatus.

3.12 LABORATORY SYNTHETIC SAMPLE MATRIX BLANK (LSSMB) – An aliquot of the Laboratory Synthetic Sample Matrix (Sect. 7.2.4) that is not fortified with perchlorate. This QC sample is processed like a field sample (Sect. 8.1) and is used to determine if the method analyte or other interferences are present in the LSSM solution.

3.13 LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which the future recovery is predicted to fall between 50-150% recovery with 99% confidence.[1]

3.14 MATERIAL SAFETY DATA SHEETS (MSDS) – These sheets contain written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.15 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value for the target analyte in a sample following analysis. This concentration must meet the criteria defined in Section 9.2.4 and must be no lower than the concentration of the lowest calibration standard for the target analyte.

3.16 MULTIPLE REACTION MONITORING (MRM) – A mass spectrometric technique where a parent ion is first isolated then subsequently fragmented into a product ion(s). Quantitation is accomplished by monitoring a specific product ion.

3.17 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing the method analyte prepared in the laboratory from stock standard solutions and diluted as needed to prepare Calibration Standards and other analyte solutions.

3.18 QUALITY CONTROL SAMPLE (QCS) – A solution containing the method analyte at a known concentration which is obtained from a source external to the laboratory and different from the source of calibration standards. The QCS is used to verify the calibration standards/curve integrity.

3.19 REAGENT WATER (RW) – Purified water which does not contain any measurable quantity of the target analyte or interfering compounds at or above 1/3 the MRL for the target analyte.

3.20 SELECTED ION MONITORING (SIM) – A mass spectrometric technique where only select ions are monitored. This technique helps to increase sensitivity.

3.21 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing the method analyte that is prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware thoroughly and rinse with reagent water. As an alternative to glassware, disposable high-density polyethylene equipment may be used.

NOTE: Perchlorate has been detected in some common laboratory detergents. If background contamination is a problem, detergents used during glassware cleaning should be investigated as a potential source of contamination.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware. These interferences may lead to discrete artifacts and /or elevated baselines in the chromatograms. All laboratory reagents and equipment must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for the target analyte) under the conditions of the analysis. This may be accomplished by analyzing LRBs as described in Section 3.11. **Subtracting blank values from sample results is not permitted.**

4.3 Matrix interferences may be caused by contaminants that are present in the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Water samples high in organic carbon or dissolved solids may lead to elevated chromatographic baselines or interfering peaks.

4.3.1 Hydrogen sulfate that is formed from the ^{34}S isotope of sulfur ($H^{34}SO_4^-$, *m/z* 99) is a common interference. It can interfere with the qualitative identification of perchlorate if there is poor chromatographic resolution between sulfate and perchlorate. This interference is particularly troublesome when using the SIM mode of detection, as the *m/z* of perchlorate (which is required for confirmation) is *m/z* 99. When using MRM detection, this ion, at modest concentrations, does not interfere with the identification or quantitation of perchlorate.

4.4 Equipment used for sample collection and storage has the potential to introduce interferences. The potential for interferences from these devices must be investigated during the Initial Demonstration of Capability (Sect. 9.2) by preparing and analyzing a Laboratory Reagent Blank (LRB). This procedure should be repeated each time that a new brand or lot of equipment is used to ensure that background contamination does not interfere with the identification and quantitation of perchlorate.

4.5 The percent of ^{18}O enrichment of the internal standard may vary between manufacturers. Poor isotopic enrichment of the internal standard may lead to sample contamination by $Cl^{16}O_4^-$. Consequently, it must be demonstrated that solutions containing a working level of the internal standard do not contain unlabeled perchlorate at concentrations greater than 1/3 of the MRL. This is initially confirmed during the analysis of the Laboratory Reagent Blank (LRB) during the IDC and is monitored in each Analysis Batch.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of MSDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available. [3-5]

6. EQUIPMENT AND SUPPLIES (References to specific brands or catalog numbers are included as examples only and do not imply endorsement of the product.)

6.1 SAMPLE CONTAINERS – 125-mL sterile high-density polyethylene (HDPE) bottles (I-Chem 125-mL sterile HDPE bottle, Fisher Cat. No. N411-0125 or equivalent).

6.2 SAMPLE FILTERS – Sterile sample filters (Corning 26-mm surfactant free cellulose acetate 0.2-um filter, Fisher Cat. No. 09-754-13 or equivalent). If alternate filters are used they should be certified as having passed a bacterial challenge test.[6] Additionally, if alternate filters or different lots of the recommended filters are used, they must be tested using a LRB

and a LFB fortified at the MRL as outlined in Section 9.2 to insure that they do not introduce interferences or retain perchlorate.

6.3 SYRINGES – Sterile, silicone free disposable syringes (Henke Sass Wolf 20-mL Luer lock, Fisher Cat. No. 14-817-33 or equivalent).

6.4 VOLUMETRIC FLASKS – Class A, suggested sizes include 5, 100, 250, 500 and 1000 mL for preparation of standards and mobile phase.

6.5 GRADUATED CYLINDER – Suggested size 25-mL.

6.6 AUTO PIPETTES – Capable of delivering variable volumes from 25 µL to 2000 µL.

6.7 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.8 NITROGEN – High purity compressed gas used for desolvation in the mass spectrometer. The gas purity and pressure requirements will depend on the instrument manufacturers' specifications.

6.9 ARGON – High purity compressed gas used in the collision cell of the mass spectrometer. The gas purity and pressure requirements will depend on the instrument manufactures'specifications.

6.10 SAMPLE PRETREATMENT CARTRIDGES – Cartridges may be needed to clean up samples that can not be analyzed due to high levels of background interferences. Single-use, disposable cartridges (OnGuard-II Ba^{2+} Dionex, Cat. No. 57093 or equivalent) may be used to remove sulfate from the sample.

6.11 LIQUID CHROMATOGRAPHY ELECTROSPRAY MASS SPECTROMETRY SYSTEM (LC/ESI/MS)

6.11.1 LC COLUMN – LC column, 2.1 x 250 mm (Dionex IonPak®AS-21 or equivalent). Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9.1) may be used.

NOTE: The IonPak®AS-21 columns are shipped with a sodium hydroxide mobile phase. During method development new columns were flushed with 200 mM methylamine mobile phase for approximately 30 minutes before being connected to the mass spectrometer. Additionally, the retention time of perchlorate on a new IonPak® AS-21 would decrease over several days as the column equilibrated. This shift in retention time was inconsequential to the analysis due to the presence of the internal standard.

6.11.2 LC SYSTEM – (Waters 2690 or equivalent) The LC system must be compatible with a basic mobile phase (approximately pH 12). If the LC system contains Vespel® components, such as injection valve rotor seals, these components must be replaced with a material suitable for high use at high pH. Suitable materials for high pH include Tefzel®, PEEK™, Teflon®, UHMWPE and KEL-F®. Consult the instrument

manufacturer if there are questions regarding the use of a high pH mobile phase in the system. Additionally, the system must be capable of consistently injecting up to100-µL volumes and delivering mobile phase at a constant flow rate of 0.350 mL/min.

6.11.3 MASS SPECTROMETER – (Micromass QuattroMicro or equivalent) The MS must be capable of providing electrospray ionization with negative ion detection. Due to the potential for $H^{34}SO_4^-$ interference, MRM detection is recommended.

6.11.4 DATA SYSTEM – An interfaced data system is required to acquire, store, and output MS data. The computer software should have the capability of processing stored LC/MS data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the ion abundance of any specific ion between specified time or scan number limits. The software must be able to construct a linear regression or quadratic calibration curve, and calculate analyte concentrations using the internal standard technique.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used in all analyses. Unless otherwise indicated, it is intended that all reagents will conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available. Other grades may be used, as long as the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 METHYLAMINE – (CASRN 74-89-5) – (40 wt.%) in water (Aldrich Cat. No. 426466 or equivalent).

7.1.2 METHYLAMINE MOBILE PHASE (200 mM) – Add 20 mL of the 40 wt.% methylamine solution in water to a 1L volumetric flask. Dilute to volume with reagent water. During method development, the mobile phase was found to be stable for at least 48 hours. Laboratories are encouraged to institute their own quality control (QC) procedures to determine when solutions need to be replaced.

7.1.3 REAGENT WATER – Purified water that does not contain any measurable quantity of the target analyte or interfering compounds at or above $^1/_3$ the MRL for the target analyte (Sect. 3.19). The reagent water used during method development was generated from tap water using a Millipore ELIX-3 followed by a Millipore Gradient A10 system.

7.1.4 SODIUM CHLORIDE – (NaCl, CASRN 7647-14-5) – Fisher Cat. No. S-271 or equivalent.

7.1.5 SODIUM BICARBONATE – ($NaHCO_3$, CASRN 497-19-8) – Fluka Cat. No. 71627 or equivalent.

7.1.6 SODIUM SULFATE – (Na_2SO_4, CASRN 7757-82-6) – Fluka Cat. No. 71959 or equivalent.

7.2 STANDARD SOLUTIONS – When a compounds' purity is assayed to be 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Stock standard solutions are estimated to be stable for one year and any fortified or dilute solutions made from the stock standards are stable for at least 30 days. **Although estimated stability times for standard solutions are given, laboratories should use standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTION – This method uses the internal standard $NaCl^{18}O_4$.

7.2.1.1 INTERNAL STANDARD STOCK STANDARD (ISSS) (100 µg/mL $Cl^{18}O_4^-$) – Prepare, or purchase commercially, an ISSS at a concentration of 100 µg /mL. To prepare this solution from a solid standard, weigh out 12.1 mg of $NaCl^{18}O_4$ into a 100-mL volumetric flask and dilute to 100 mL with reagent water. The internal standard used during method development was custom synthesized by Isotech (Miamisburg, OH). Subsequently, several manufacturers offer this product as a standard item. Because the degree of ^{18}O enrichment could vary between manufacturers, it must be confirmed during the IDC (Sect. 9.2) and in subsequent LRBs that this standard does not contribute unenriched perchlorate to the sample. Any background contribution from the IS must be < 1/3 of the perchlorate MRL when added at working levels (the concentration of the internal standard used during method development was 1 ng/mL).

7.2.1.2 INTERNAL STANDARD FORTIFICATION SOLUTION (77.0 ng/mL $Cl^{18}O_4$) – Place 77.0 µL of the internal standard ISSS into a 100-mL volumetric flask and dilute to volume with reagent water. The addition of 25 µL to 1.9 mL of sample will produce a final concentration of 1.0 ng/mL. All field samples and CAL standards must have the same amount of internal standard added.

7.2.2 PERCHLORATE STANDARD SOLUTIONS – Obtain the analyte as a solid standard of $NaClO_4$ or as a commercially prepared solution from a reputable standard manufacturer. Prepare the perchlorate stock, primary and secondary standards as described below.

7.2.2.1 PERCHLORATE STOCK STANDARD SOLUTION (PSSS) (100 µg/mL ClO_4^-) – To prepare this solution from a solid $NaClO_4$ standard, weigh out 12.3 mg of $NaClO_4$ into a 100-mL volumetric flask and dilute to volume with reagent water.

7.2.2.2 PERCHLORATE PRIMARY DILUTION SOLUTION (PPDS) (1.0 µg/mL ClO_4^-) – Prepare the perchlorate PDS by adding 1.0 mL of the Stock Standard Solution to a 100-mL volumetric flask and dilute to volume with reagent water. This solution is used to prepare the Perchlorate Secondary Dilution Standard and the Calibration Standards below.

7.2.2.3 PERCHLORATE SECONDARY DILUTION SOLUTION (PSDS) (5.0 ng/mL ClO_4^-) – Prepare the 5 ng/mL Analyte SDS by adding 500 µL of the Analyte PDS to a 100-mL volumetric flask and dilute to volume with reagent water. This solution is used to prepare the Calibration Standards below.

7.2.2.4 PERCHLORATE FORTIFICATION SOLUTIONS (PFS) (100, 200 and 1000 µg/mL) – The Analyte Fortification Solutions are prepared by dilution of the Perchlorate Secondary Dilution Solution and are used to fortify the LFB, LFSSM, the LFSMs and the LFSMDs with method analytes. It is recommended that multiple concentrations be prepared so that the fortification levels can be rotated or adjusted to the concentration of the target analyte in the native samples.

7.2.3 CALIBRATION (CAL) STANDARDS – Prepare a calibration curve from dilutions of the perchlorate PDS and the perchlorate SDS using a minimum of five Calibration Standards that span the concentration range of interest. The lowest concentration calibration standard must be at or below the MRL. An example of the dilutions used to prepare the Calibration Standards used to collect the data in Section 17 are shown in the table below.

PREPARATION OF CALIBRATION CURVE STANDARDS				
CAL Level	**Vol. of Analyte PDS (µL)**	**Vol. of Analyte SDS (µL)**	**Final Vol. of CAL Std. (mL)**	**Final Conc. of CAL Std. (ng/mL)**
1		400	100	0.020
2		1000	100	0.050
3		2000	100	0.10
4	50		100	0.50
5	100		100	1.0
6	500		100	5.0

A constant amount of the internal standard is added to each Calibration Standard (1.0 ng/mL in the final volume). This is accomplished for each standard by taking 1.9 mL of the calibration standard and placing it in a 2.0-mL autosampler vial and adding 25 µL of the 77 ng/mL Internal Standard Fortification Solution.

NOTE: CAL standards are not processed with the sample collection equipment. This step must be omitted for the CALs in order to identify any potential losses associated with the sample filtration or collection protocols.

7.2.4 LABORATORY SYNTHETIC SAMPLE MATRIX (LSSM) – Prepare a LSSM that contains the common anions chloride, sulfate and bicarbonate at 1000 mg/L follows.

7.2.4.1 Weigh out 1.40 g of $NaHCO_3$, 1.48 g of Na_2SO_4, and 1.54 g of NaCl (Fluka 71627, Fluka 71959, Fisher S-271, respectively or equivalent). Add these to a 1-L volumetric flask using a powder funnel and dilute to volume using reagent water.

7.2.5 LABORATORY FORTIFIED SYNTHETIC SAMPLE MATRIX (LFSSM) – An aliquot of the Laboratory Synthetic Sample Matrix fortified using the Perchlorate Fortification Solutions prepared above (Sect. 7.2.2.4).

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices.[7]

8.1.2 When sampling from a cold water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually approximately 3 to 5 minutes). Collect a representative sample from the flowing system using a beaker of appropriate size. Use this bulk sample to generate individual samples as needed. A volume of at least 20-mL is required for each individual sample.

8.1.3 When sampling from an open body of water, fill a beaker with water sampled from a representative area. Use this bulk sample to generate individual samples as needed. A volume of at least 20-mL is required for each individual field sample.

8.1.4 Once representative samples are obtained, they must be filtered to remove any native microorganisms. Perchlorate is known to be susceptible to microbiological degradation by anaerobic bacteria[8]. Samples are filtered to remove microbes and stored with headspace to minimize the possibility that anaerobic conditions develop during storage. At a minimum, leave the top one third of the bottle empty to reduce the potential for degradation by any remaining anaerobic organisms.

8.1.4.1 Remove a sterile syringe (Sect. 6.3) from its package and draw up 20 mL of the bulk sample. Remove a sterile syringe filter (Sect 6.2) from its package without touching the exit Luer connection. Connect the filter to the syringe making sure that no water from the syringe drops on the exterior of the filter. Open a sterile sample container (Sect. 6.1) without touching the interior. Using gentle pressure, pass the sample through the filter into the sample container. During this process do not let the syringe or filter make contact with the sample container. Following filtration, seal the sample container tightly, label and prepare the container for shipment. Syringes and filters are single use items and must be discarded after each sample.

8.2 SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection. Samples should be confirmed to be at or below 10 °C when they are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until analysis. Samples should not be frozen.

8.3 SAMPLE HOLDING TIMES – Samples should be analyzed as soon as possible. Samples that are collected and stored as described in Sections 8.1 and 8.2 may be held for a maximum of 28 days.

9. QUALITY CONTROL

9.1 Quality Control (QC) requirements include the Initial Demonstration of Capability and ongoing QC requirements that must be met when preparing and analyzing field samples. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Section 17, Tables 5 and 6. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 METHOD MODIFICATIONS – The analyst is permitted to modify the separation technique, LC columns, mobile phase composition, LC conditions and MS conditions. However, each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.2 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 meet the calibration requirements outlined in Section 10. Requirements for the initial demonstration of laboratory capability are described in the following sections and are summarized in Table 5.

9.2.1 DEMONSTRATION OF LOW SYSTEM BACKGROUND – Analyze a LRB and a LSSMB after processing both through all sample collection steps outlined in Section 8.1. Confirm that the blanks are reasonably free of contamination and that the criteria in Section 9.3.1 are met. The LRB and LSSMB must be filtered using the same sample collection devices that are used for field samples.

9.2.1.1 Concentration dependent carry-over is manifest by signals in subsequent samples that increase proportionally to the concentration of the previously injected standard. Analysis of reagent water blank (non-filtered) must be performed after the highest CAL standard during the IDC to determine if carry-over is present. The results for this sample must meet the criteria outlined in section 9.3.1. If the reagent water blank fails to meet the criteria there is likely system carry-over. The source can often be traced to injection valve problems or an inadequate autosampler rinse protocol. System carry-over should be eliminated by determining the source of the problem and taking corrective action.

9.2.2 DEMONSTRATION OF PRECISION – Prepare and analyze 7 replicate LFBs and LFSSMs. These samples should be fortified near the midrange of the initial calibration curve. All samples must be processed using the sample collection devices described in Section 8.1. The percent relative standard deviation (%RSD) of the concentrations of the replicate analyses must be ≤ 20%.

$$\%\ \text{RSD} = \frac{\text{Standard Deviation of Measured Concentrations}}{\text{Average Concentration}} \times 100$$

9.2.3 DEMONSTRATION OF ACCURACY – Using the same sets of replicate data generated for section 9.2.2, calculate the average percent recovery. The average percent recovery of the replicate analyses must be within 80-120% of the true value.

$$\%\ \text{Recovery} = \frac{\text{Average Measured Concentration}}{\text{Fortified Concentration}} \times 100$$

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The lowest calibration standard used to establish the initial calibration (as well as the low-level Continuing Calibration Check) must be at or below the concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm or validate the MRL following the procedure outlined below.

9.2.4.1 Fortify and analyze seven replicate Laboratory Fortified Blanks at the target MRL concentration. All samples must be processed using the sample collection devices described in Section 8.1. Calculate the mean (Mean) and standard deviation (S) for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963S$$

where S is the standard deviation, and 3.963 is a constant value for seven replicates.[1]

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

The Upper PIR Limit must be ≤150 percent recovery.

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

The Lower PIR Limit must be ≥ 50 percent recovery.

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

9.2.4.3 The 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.

9.2.5 DETECTION LIMIT DETERMINATION *(optional) – While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

Analyses for this procedure should be done over at least 3 days. Prepare at least 7 replicate LFBs processing the reagent water through all sample collection steps outlined in Section 8.1. Use the solutions described in Section 7.2.2.4 to fortify at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. Analyze the seven replicates through all steps of Section 11.

NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the DL using the following equation:

$$DL = St_{(n-1,1-\alpha = 0.99)}$$

where:

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

n = number of replicates

S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing DL calculations.

9.3 ONGOING QC REQUIREMENTS – This section describes the ongoing QC criteria that must be followed when processing and analyzing field samples. Table 6 summarizes these requirements.

9.3.1 LABORATORY REAGENT BLANK (LRB) – A LRB is analyzed during the IDC and is required with each Analysis Batch (Sect. 3.1) to confirm that background contaminants are not interfering with the identification or quantitation of perchlorate. If the LRB produces a peak within the perchlorate retention time window, identify the source of contamination and eliminate the interference before processing samples. The LRB must contain the IS at the same concentration used to fortify all field samples and CAL standards. LRBs must be processed in the same manner as field samples including exposure to all sample collection devices (i.e., sterile filtration into sample containers). If samples are collected using devices that have not been previously evaluated by the laboratory, duplicates of the sample collection devices must be sent with the samples so a LRB (and a LFB) may be processed in the laboratory. If perchlorate or other interferences are detected in the LRB at concentrations ≥ 1/3 of the MRL, then all data for the analyte must be considered invalid for all samples in the Analysis Batch.

NOTE: Although quantitative data below the MRL may not be accurate enough for data reporting, such data are useful in determining the magnitude of a background interference. Therefore, blank contamination levels may be estimated by extrapolation, when the concentration is below the lowest calibration standard.

9.3.2 CONTINUING CALIBRATION CHECK (CCC) – CCC standards are analyzed at the beginning of each Analysis Batch, after every ten field samples and at the end of the Analysis Batch. See Section 10.4 for concentration requirements and acceptance criteria.

9.3.3 LABORATORY FORTIFIED BLANK (LFB) – A LFB is required with each Analysis Batch. The LFB fortification level must be rotated between low, medium, and high from batch to batch. The low concentration LFB must be as near as practical to the MRL. Results of LFBs fortified at ≤ MRL must be within 50-150% of the true value. Results of LFB analysis from all other concentrations must be 80-120% of the true value. If the LFB results do not meet these criteria, then all data for perchlorate must be considered invalid for all samples in the Analysis Batch.

NOTE: LFBs must be processed in the same manner as field samples including exposure to all sample collection devices (i.e., sterile filtration into sample containers). If samples are collected using devices that have not been previously evaluated by the laboratory, duplicates of the sample collection devices must be sent with the samples so a LFB (and a LRB) may be processed in the laboratory.

9.3.4 INTERNAL STANDARD (IS) – The analyst must monitor the peak area of the internal standard in all injections of the Analysis Batch. The IS response (as indicated by peak area) for any chromatographic run must not deviate by more than ± 30% of the IS area measured during the first CCC of the Analysis Batch. If the IS area counts for a sample do not meet this criterion, inject a second aliquot of the suspect sample as part of the same or a new Analysis Batch.

9.3.4.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.3.4.2 If the IS area counts of the reinjected aliquot still do not meet the IS criterion, check the IS area of the most recent CCC. If the IS criterion is met in the CCC but not the sample, report the sample results as suspect/matrix.

9.3.4.3 If the IS area criterion is not met in both the sample and the CCC, instrument maintenance such as sample cone cleaning may be necessary. Perform the appropriate instrument maintenance and then reinject the sample in a subsequent analytical batch.

9.3.5 ISOTOPE AREA COUNT RATIO ACCEPTANCE CRITERIA – All CAL standards, QC samples and field samples must meet the area count ratio requirement for confirmation of perchlorate. Perchlorate has a strong M+2 ion due to the presence of the ^{37}Cl isotope. The analyst must confirm that the ratio of the molecular ion to its M+2 ion

(99/101) for the SIM technique, or the ratio of their product ions (83/85) for the MRM technique, are within ± 25% of the theoretical value of 3.08 (2.31 to 3.85)[9]. If a CAL standard or QC sample fails this requirement, remedial action is required. Necessary actions may include mass calibration, column replacement/regeneration or instrument cleaning. If the area count ratio for a field sample falls outside this range, the cause is frequently interference from sulfate in the sample matrix. In this case the sample may require dilution or pretreatment with a barium cartridge to remove the sulfate (see Sect 11.3.4.2 regarding required remedial action).

9.3.6 RELATIVE RETENTION TIME ACCEPTANCE CRITERIA – All CAL standards, QC samples and field samples must meet the Relative Retention Time (RRT) requirement for confirmation of perchlorate. The RRT of perchlorate can be calculated using the equation

$$\frac{\text{Retention Time of Perchlorate Ion}}{\text{Retention Time of Internal Standard Ion}} = \text{RRT}$$

The RRT must be 1.0 ± 2% (0.98-1.02) for a peak to be identified as perchlorate.

9.3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of a LFSM (Sect. 3.8) is required in each Analysis Batch. The LFSM is used to assess whether the sample matrix affects method accuracy. If a variety of different sample matrices are analyzed regularly, for example drinking water from groundwater and surface water sources, performance data should be collected for each source.

9.3.7.1 Within each Analysis Batch, a minimum of one field sample is fortified as an LFSM for every 20 samples analyzed. The LFSM is prepared by fortifying a sample with an appropriate amount of the Perchlorate Fortification Solution (Sect. 7.2.2.4). The fortification should be delivered in the smallest volume possible to minimize dilution of the sample. Select a spiking concentration that is greater than or equal to the native background concentration, if known. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data and rotate through low, medium and high calibration concentrations when selecting a fortifying concentration.

9.3.7.2 Calculate the perchlorate recovery (%REC) using the equation

$$\%\text{REC} = \frac{(\text{A} - \text{B})}{\text{C}} \times 100$$

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.3.7.3 Recoveries for samples fortified at concentrations ≤ MRL should be 50-150%. Recoveries for samples fortified at all other concentrations should be 80-120%. If

the accuracy for a LFSM falls outside the designated range, and the laboratory performance for perchlorate is shown to be in control in the CCCs, the recovery is judged to be matrix biased. The result for perchlorate in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7.3.1 Field Samples that have native perchlorate concentrations below the MRL and are fortified at concentrations at or near the MRL should be corrected for the native levels in order to obtain meaningful %REC values. **This is the only case where background subtraction of results below the MRL is permitted.**

9.3.8 LABORATORY DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LD or LFSMD) – Within each Analysis Batch, a minimum of one Laboratory Duplicate (LD) or Laboratory Fortified Sample Matrix Duplicate (LFSMD) must be analyzed. Laboratory Duplicates check the precision associated with laboratory procedures. If perchlorate is not routinely observed in field samples, a LFSMD should be analyzed rather than a LD.

9.3.8.1 Calculate the relative percent difference (RPD) for duplicate measurements (LD_1 and LD_2) using the equation

$$\text{RPD} = \frac{\left|\text{LD}_1 - \text{LD}_2\right|}{\left(\text{LD}_1 + \text{LD}_2\right)/2} \times 100$$

9.3.8.2 RPDs for Laboratory Duplicates should be ≤ 20%. Greater variability may be observed when Laboratory Duplicates have perchlorate concentrations that are within a factor of ≤ 2 x MRL. At these concentrations, Laboratory Duplicates should have RPDs that are ≤ 50%. If the RPD of the analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC, the precision is judged to be matrix influenced. The result from the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.8.3 If a LFSMD is analyzed instead of a Laboratory Duplicate, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

$$RPD = \frac{\left|LFSM - LFSMD\right|}{\left(LFSM + LFSMD\right)/2} \times 100$$

9.3.8.4 RPDs for duplicate LFSMs should be ≤ 20%. Greater variability may be observed when fortified LFSMs have analyte concentrations that are within ≤ 2 x MRL. LFSMs at these concentrations should have RPDs that are ≤ 50%. If the RPD of the analyte falls outside the designated range, and the laboratory performance for the

analyte is shown to be in control in the CCC, the precision is judged to be matrix influenced. The result from the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.4 QUARTERLY INSTRUMENT PERFORMANCE CHECK USING THE LSSMB AND LFSSM – Analysis of a LFSSM (Sect. 3.10) must be performed at least quarterly to assess instrumental performance with respect to samples high in common anions. An aliquot of LFSSM (fortified at the mid-range of the calibration curve) must be processed and analyzed as a sample along with a LSSMB. Both solutions must be from the same stock of LSSM. Results for the LSSMB and LFSSM should meet the criteria set forth in Sections 9.3.1 and 9.3.3, respectively. If the LSSMB contains perchlorate at a concentration ≥ 1/3 the MRL, then the source of the contamination should be identified and eliminated. If the LFSSM does not meet the QC acceptance criteria for LFB recovery, instrument maintenance or column replacement may be required.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable MS mass calibration and initial analyte calibration are required before any samples are analyzed. If the initial calibration is successful, continuing calibration checks are required at the beginning and end of each period in which analyses are performed, and after every tenth sample. Verification of mass spectrometer mass calibration should be repeated each time a major instrument modification is made or maintenance is performed.

Note: CAL solutions and CCC standards are not processed with the sample collection devices. This step must be omitted for the CALs and CCCs in order to identify any potential losses associated with the sample filtration or collection devices.

10.2 MASS SPECTROMETER CALIBRATION AND OPTIMIZATION

10.2.1 MASS CALIBRATION – Calibrate the mass spectrometer according to the manufacturer's recommendations. The user should be aware that many current LC/MS instruments are designed to analyze macromolecules having large *m/z* ratios. Subsequently, many of the LC/MS calibration procedures are designed to cover the full scanning range of the instrument. Since this method uses the lower portion of the mass range, it may be necessary to use alternate calibration compounds that provide ions of a lower *m/z* ratio to properly define the mass calibration over the range needed for the analysis of perchlorate. For our studies, a sodium iodide/rubidium iodide mixture was used as a calibration compound. It is recommended that the analyst contact the instrument manufacturer regarding appropriate mass calibration standards. Mass calibration must be verified prior to the analysis of field samples by injecting a high-level calibration standard and acquiring a full scan continuum mass spectrum that covers the *m/z* range of the target analyte. The perchlorate ion ($^{35}ClO_4^-$) should have a *m/z* of 99 ± 0.3, the ($^{37}ClO_4^-$) isotope should have a *m/z* of 101 ± 0.3 and the internal standard ($^{35}Cl\ ^{18}O_4^-$) should have a *m/z* of 107 ± 0.3.

10.2.2 OPTIMIZING MS PARAMETERS – LC/MS instruments have a large number of

instrumental parameters that must be optimized. Each LC/MS system will have different optimal conditions which are influenced by the source geometry and system design. Due to the differences in design, the recommendations of the instrument manufacturer should be followed when tuning the instrument. MS conditions should be established by infusing a solution of the target compound into the mobile phase while the analyst tunes (or varies) the MS parameters using the same mobile phase flow rate and composition as in the final analysis. The response for the parent ions must be optimized for MS analysis and three MS/MS transitions must be optimized for the MRM analysis of perchlorate. These transitions are listed in the following table.

Precursor Ion (*m/z*)	Fragment Lost (*m/z*)	Product Ion (*m/z*)
$^{35}ClO_4$ (99)	^{16}O (16)	$^{35}ClO_3$ (83)
$^{37}ClO_4$ (101)	^{16}O (16)	$^{37}ClO_3$ (85)
$^{35}Cl^{18}O_4$ (107)	^{18}O (18)	$^{35}Cl^{18}O_3$ (89)

10.2.3 INSTRUMENT CONDITIONS – Operating conditions are described in Section 17 Table 1. Conditions different from those described may be used if the QC criteria in Section 9.2 are met. Different conditions include alternate LC columns, mobile phases and MS conditions.

10.3 INITIAL CALIBRATION – During method development daily calibrations were performed, however, it is permissible to verify the calibration with daily CCCs. Calibration must be performed using peak areas and the internal standard technique. Calibration using peak heights is not permitted.

10.3.1 CALIBRATION STANDARDS – Prepare a set of at least five CAL standards as described in Section 7.2.3. The lowest concentration of the calibration standards must be at or below the MRL. The MRL must be confirmed using the procedure outlined in Section 9.2.4 after establishing the initial calibration. Additionally, field samples must be quantified using a calibration curve that spans the same concentration range used to collect the IDC data (Sect. 9.2).

10.3.2 CALIBRATION – The LC/MS system is calibrated using peak areas and the internal standard technique. Concentrations may be calculated through the use of a linear or quadratic calibration curve. Quantitation ions are listed in Table 1 for both the MRM and SIM detection techniques.

10.3.3 CALIBRATION ACCEPTANCE CRITERIA – The validation of the calibration is determined by calculating the concentration of the analyte from each of the analyses used to generate the calibration curve. Calibration points that are ≤ MRL should calculate to be 50-150% of their true value. All other calibration points should calculate to be 80 to 120% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. In this case, corrective action should be taken to reanalyze the calibration standards, restrict the range of calibration, or select an alternate method of calibration.

10.3.4 INITIAL CALIBRATION VERIFICATION - Analyze a QCS sample fortified near the midpoint of the calibration range. The QCS sample should be from a source different than the source of the calibration standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared and analyzed just like a CCC. The acceptance criterion for the QCS is that the calculated amount of perchlorate must be 80-120% of the true value. If the measured analyte concentration does not meet this criterion, check the entire analytical procedure to locate and correct the problem before analyzing any field samples.

10.4 CONTINUING CALIBRATION CHECKS (CCCs) – The CCC verifies the calibration at the beginning, after every tenth field sample and at the end of each Analysis Batch. CCCs are not counted as samples**. The beginning CCCs for each Analysis Batch must include a CCC at or below the MRL and a CCC fortified at a level near the midpoint of the curve**. These CCCs verify instrument sensitivity and the accuracy of the curve prior to the analyses of samples. Subsequent CCCs should alternate between a low, medium and high concentration.

10.4.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.4.2 Determine that the absolute area of the quantitation ion of the internal standard has not changed by more than ± 30% from the first CCC of the Analysis Batch.

NOTE: The IS area counts of the first CCC of the Analysis Batch should be $\geq$ 50% of the average of the IS area counts of the CAL standards from the initial calibration. If the IS response drifts below 50% of the average determined during the initial calibration, instrument maintenance or ESI/MS detector inlet cleaning is generally required.

10.4.3 Calculate the concentration of the analyte in the CCC. CCCs fortified at ≤ MRL must calculate to be 50-150% of the true value. CCCs fortified at all other levels must calculate to be 80-120%. If these conditions do not exist, then all data for the analyte must be considered invalid, and remedial action (Sect. 10.4.4) should be taken. The remedial action may require recalibration. Any field samples that have been analyzed since the last acceptable calibration verification and are still within holding time should be reanalyzed after adequate calibration has been restored.

10.4.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria requires remedial action. Maintenance such as cleaning an ion source probe, sample cone, ion lenses and/or regenerating or replacing LC columns may be required. Following major maintenance, the analyst must return to the initial calibration step (Sect. 10.3).

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper sample collection and storage (Section 8), ensuring that the instrument is properly calibrated (Section 10) and that all required QC are met (Section 9). This section describes the procedures for sample preparation and analysis.

11.2 SAMPLE PREPARATION

11.2.1 Collect and store field samples as described in Section 8.1. Allow field samples to reach room temperature prior to analysis.

11.2.2 Process all LRBs, LFBs, LFSSMs and LFSSMBs using the sample collection devices described in Section 8.1.

11.2.3 Transfer a 1.9-mL aliqout of each Field or QC Sample to an autosampler vial. Add 25 µL of the IS Fortification Solution (Sect. 7.2.1.2), cap and mix well. Larger amounts of sample may be used, but the amount of internal standard must be increased to give a final concentration that is equal to the CAL IS concentration.

11.3 SAMPLE ANALYSIS

11.3.1 Establish operating conditions as described in Table 1 of Section 17.

11.3.2 Establish a valid initial calibration following the procedures outlined in Section 10.3 or confirm that the calibration is still valid by running a CCC as described in Section 10.4. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2.

11.3.3 Analyze aliquots of Field and QC Samples at appropriate frequencies (Section 9) with the LC/MS conditions used to acquire the initial calibration. At the conclusion of data acquisition, use the same software settings that were used in the calibration procedure to identify peaks in the predetermined retention time windows.

11.3.4 COMPOUND IDENTIFICATION – Establish an appropriate retention time window for the target analyte and internal standard so they may be identified in QC and field sample chromatograms. Because the retention time will vary with the ionic strength of the sample the absolute retention time windows may need to be wider than usual.

11.3.4.1 High ionic strength samples will cause retention times to decrease and introduce band broadening. This is unavoidable due to the high elutropic strength of the matrix in these samples. Because of these effects, the relative retention time of perchlorate is used as one component for qualitative identification (Section 9.3.6). The relative retention time must be 1.0 ± 2% (0.98-1.02) for a peak to be identified as perchlorate.

11.3.4.2 Perchlorate has a strong M+2 ion due to the presence of ^{37}Cl. The analyst must confirm that the ratio of the molecular ion to its M+2 ion (99/101) for the SIM technique or the ratio of their daughter ions (83/85) for the MRM technique are within ± 25% of the theoretical value of 3.08 (2.31 to 3.85). If this ion ratio requirement is not met in any QC sample, then all samples in the Analysis Batch

are considered invalid and must be reanalyzed after reestablishing acceptable instrument performance.

11.3.4.2.1 If the isotope area ratio criteria are not met when using MRM detection the analyst should report the associated values as "suspect due to lack of confirmation."

11.3.4.2.2 When using SIM detection, remedial action is required if the following conditions exist. If a peak is detected at *m/z* 101, its concentration is ≥ MRL, the relative retention time criterion is met and the isotope area ratio requirement is not met. In this case, sample pretreatment must be performed. The most frequent cause of ratio failure is poor chromatographic resolution between sulfate and perchlorate. If the ratio failure is due to a high sulfate level in the sample, dilution or sulfate removal using a commercial barium pretreatment cartridge (Sect. 6.10) may be successful. Consult the manufacturer's instructions for preparation of the barium pretreatment cartridge prior to use with samples. Generally, the procedure requires rinsing the cartridge with a minimum volume of reagent water. It has been found that rinsing with approximately twice the recommended volume of water yields better results. Add IS to the sample prior to sample pretreatment using the cartridges. Additionally, chromatographic conditions may be changed to eliminate the coelution, however the IDC must be repeated using the new conditions.

NOTE: If cartridges are used, a reagent water blank with IS should be processed to insure that the cartridges don't contribute interferences or retain perchlorate.

11.3.4.2.3 Following pretreatment, reanalyze the sample as part of the same or a subsequent Analysis Batch. If the ratio criteria of the reinjected sample pass, report the results of the reinjected sample. If the ratio criteria are still not met the analyst should report the associated values as "suspect due to lack of confirmation."

11.3.5 EXCEEDING CALIBRATION RANGE –The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, the sample may be diluted with reagent water, with the appropriate amount of internal standard added to match the original level, and the diluted sample reinjected. Incorporate the dilution factor into final concentration calculations. The dilution will also affect analyte MRLs.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify the analyte in the Field and QC Samples as described in Section 11.3.4.

12.1.1 Sulfate has an ion at *m/z* 99 ($H^{34}SO_4$) that can interfere with confirmation if the chromatography is not optimized or if the sulfate concentration in the native samples is

very high. The SIM version of this method is significantly more susceptible to interferences from hydrogen sulfate than the MRM version of this method. The M + 2 ion (*m/z* 101) **must** be used for quantitation of perchlorate when using SIM.

12.2 Calculate analyte concentrations using the multipoint calibration established in Section 10.3. Report only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (Sect. 11.3.5).

12.3 Calculations must use all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

12.4 Prior to reporting data, the laboratory is responsible for assuring that QC requirements have been met or that any appropriate qualifier is documented.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND DETECTION LIMITS – Tables for these data are presented in Section 17. Instrumental conditions and monitored ions are presented in Table 1. The LCMRL for perchlorate is presented in Table 2 and was calculated using a procedure described elsewhere[1]. Single laboratory precision and accuracy data are presented in Tables 3 and 4. Table 5 summarizes the requirements for the initial demonstration of capability (IDC) and Table 6 summarizes the requirements for the required ongoing quality control. Figure 1 shows a representative chromatogram from a MRM analysis. Figure 2 shows a representative chromatogram from a SIM analysis and Figure 3 shows a total ion chromatogram (*m/z* 99, 101 and 107) of a LSSM that contains the common anions chloride, sulfate and bicarbonate at 1000 mg/L.

13.2 SECOND LABORATORY DEMONSTRATION -- The performance of this method was demonstrated by a second laboratory, with results similar to those reported in Section 17. The authors wish to acknowledge the work of the U.S. EPA National Risk Management Research Laboratory, Ada, OK and the U.S. EPA New England Laboratory, North Chelmsford, MA for their participation in the second laboratory demonstration.

14. POLLUTION PREVENTION

14.1 For information about pollution prevention that may be applicable to laboratory operations, consult “Less is Better: Laboratory Chemical Management for Waste Reduction” available from the American Chemical Society’s Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036, or on-line at http://www.ups.edu/community/storeroom/Chemical_Wastes/wastearticles.htm

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, see the publications of the American Chemical Society's Laboratory Environment, Health & Safety Task Force on the Internet at http://membership.acs.org/c/ccs/labehs/publications.htm. Or see "Laboratory Waste Minimization and Pollution Prevention," Copyright © 1996 Battelle Seattle Research Center, which can be found at http://www.p2pays.org/ref/01/text/00779/index2.htm.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INSTRUMENTAL CONDITIONS, RETENTION TIMES, QUANTITATION IONS

Micromass QuattroMicro Mass Spectrometer Settings:

Parameter	LC/MRM	LC/SIM
Ion Source	Electrospray	Electrospray
Polarity	Negative Ion	Negative Ion
Capillary Voltage	0.58 kV	0.58 kV
Cone Voltage	40 V	40 V
Extractor	3 V	3 V
RF Lens	0.3 V	0.3 V
Source Temperature	120 °C	120 °C
Desolvation Temperature	320 °C	320 °C
Cone Gas Flow	60 L/hr	60 L/hr
Desolvation Gas Flow	N_2 800 L/hr	N_2 800 L/hr
LM 1 Resolution	15	15
HM 1 Resolution	15	15
Ion Energy	0.6 V	0.6 V
Entrance	1	50
Collision	24	2
Exit	1	50
LM 2 Resolution	14	15
HM 2 Resolution	14	15
Ion Energy	1 V	1 V
Multiplier	650 V	650 V
Gas Cell Pressure	Ar 3.54e-3 mbar	Off
Function dwell time	300 ms	300 ms
Data Smoothing (mean)	Window/Smoothes, 3/2	Window/Smoothes, 3/2

Chromatographic conditions: LC; Waters 2690, column; Dionex IonPak® AS-21 2.1 x 150 mm 7 μm particle diameter, injection volume; 100 μL, mobile phase; 200 mM methylamine isocratic at 350 μL /min.

Monitored Ions and Retention Times

Analyte	*Precursor Ion (*m/z*)	†Product Ion (*m/z*)	RT (min)
$^{35}ClO_3$	99	83	8.68
$^{37}ClO_3$	101	85	8.68
$^{35}Cl^{18}O_3$ (IS)	107	89	8.65

*In SIM analysis only the precursor ions are monitored and *m/z* 101 **must** be used for quantitation.
†In MRM analysis either perchlorate product ion may be used for quantitation.

TABLE 2. LOWEST CONCENTRATION MRL AND DLs FOR PERCHLORATE

Analysis Method	Analyte	LCMRL (µg/L)	*DL (µg/L)
LC/MRM	ClO_4^-	0.022	[a]0.005
LC/SIM	ClO_4^-	0.056	[b]0.008

*The DL was calculated from data acquired on a single day using *m/z* 83 (MRM) or *m/z* 101 (SIM)
[a]Fortification 0.010 µg/L
[b]Fortification 0.075 µg/L

TABLE 3. LC/MRM PRECISION AND RECOVERY DATA FOR PERCHLORATE IN VARIOUS MATRICES (n=7 REPLICATES)

Matrix	Unfortified Concentration (µg/L)	Fortified Concentration (µg/L)	Average 83/85 Ratio	Mean % Recovery	% RSD
Reagent Water	<MRL	0.020	2.73	97.7	13.6
	<MRL	0.50	2.97	96.3	0.89
	<MRL	5.0	3.04	101	0.83
Surface Water A	0.51	1.0	3.01	95.1	1.5
	0.51	5.0	3.02	99.4	1.2
Surface Water B	0.45	1.0	3.00	101	1.2
	0.45	5.0	3.02	105	0.70
Ground Water	0.18	0.50	2.96	100	3.6
	0.18	5.0	3.03	103	1.3
*Synthetic High Ionic Strength	<MRL	0.50	2.94	102	1.5
	<MRL	5.0	3.01	102	0.9

* Described in Section 7.2.4

TABLE 4. LC/SIM PRECISION AND RECOVERY DATA FOR PERCHLORATE IN VARIOUS MATRICES (n=7 REPLICATES)

Matrix	Unfortified Concentration (µg/L)	Fortified Concentration (µg/L)	Average 99/101 Ratio	[†]Mean % Recovery	% RSD
Reagent Water	<MRL	0.50	3.07	96.1	1.0
	<MRL	5.0	3.06	102	1.4
Surface Water B	0.46	1.0	3.03	101	1.7
	0.46	5.0	3.04	103	1.1
Ground Water	0.19	0.50	3.05	99	2.6
	0.19	5.0	3.05	100	1.5
*Synthetic High Ionic Strength	<MRL	0.50	2.87	102	2.1
	<MRL	5.0	3.04	103	0.49

*Described in Section 7.2.4
[†]Quantified using *m/z* 101

TABLE 5. INITIAL DEMONSTRATION OF CAPABILITY QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.1	Demonstration of Low System Background	Analyze a LRB and LSSMB prior to any other IDC steps.	Demonstrate that the target analyte is < 1/3 of the MRL and that possible interferences from sampling devices do not prevent the identification and quantitation of perchlorate.
Section 9.2.1.1	Test For System Carryover	Analyze a reagent water blank after the high CAL standard during the IDC calibration.	Demonstrate that the target analyte is < 1/3 of the MRL and that carry-over from previous samples does not prevent the identification and quantitation of perchlorate.
Section 9.2.2	Demonstration of Precision	Analyze 7 replicate LFBs and 7 replicate LFSSMs fortified near the midrange concentration.	%RSD must be ≤ 20%
Section 9.2.3	Demonstration of Accuracy	Calculate average recovery for replicates used in Section 9.2.2.	Mean recovery 80-120% of the true value
Section 9.2.4	Minimum Reporting Limit (MRL) Confirmation	Fortify and analyze 7 replicate LFBs at the proposed MRL concentration. Calculate the mean and the Half Range (HR). Confirm that the Upper PIR and Lower PIR (Sect. 9.2.4.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%

TABLE 6. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.3	Sample Holding Time	28 days when processed and stored according to Sections 8.1 and 8.2.	Sample results are valid only if samples are analyzed within the sample holding time.
Section 10.3	Initial Calibration	Use the internal standard calibration technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Check the calibration curve as described in Section 10.3.3.	When each calibration standard is calculated as an unknown using the calibration curve, the lowest level standard should be within 50-150% of the true value. All other points should be within 80-120% of the true value.
Section 9.3.1	Laboratory Reagent Blank (LRB)	Daily, or with each Analysis Batch of up to 20 field samples, whichever is more frequent.	Results must be < 1/3 the MRL. If the background exceeds 1/3 the MRL, the results for perchlorate in the Analysis Batch are invalid.
Section 10.4	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a Low CCC and a Mid CCC at the beginning of each Analysis Batch. Subsequent CCCs are required after every 10 field samples, and after the last field sample in a batch.	The lowest level CCC must be within 50-150% of the true value. All other points must be within 80-120% of the true value. Results for field samples that are not bracketed by acceptable CCCs are invalid.

TABLE 6. (CONTINUED)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.3.3	Laboratory Fortified Blank (LFB)	Daily, or with each Analysis Batch of up to 20 field samples, whichever is more frequent.	For LFBs fortified at concentrations ≤ MRL, the result must be within 50-150% of the true value. All other LFBs must be within 80-120% of the true value.
Section 9.3.4	Internal Standard (IS)	Compare IS area to the IS area of the first CCC in the Analysis Batch for all QC and field samples.	Peak area counts for the IS must be within ± 30% of the IS area of the first CCC of the Analysis Batch. If the IS area does not meet this criterion, the corresponding perchlorate results are invalid.
Section 9.3.5	Isotope Area Count Ratio	Monitor the isotope ratio for all QC and field samples in the Analysis Batch.	The calculated area count ratio (*m/z* 99/101 for SIM, or *m/z* 85/83 MRM) must be within ± 25% (2.31- 3.85) of the theoretical value of 3.086.
Section 9.3.6	Relative Retention Time	Monitor the relative retention time for all QC and field samples in the Analysis Batch.	The relative retention time for perchlorate vs. the IS must be between 0.98-1.02.
Section 9.3.7	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per Analysis Batch. Fortify the LFSM with perchlorate at a concentration close to but greater than the native concentration (if known). Calculate LFSM recoveries.	For LFSMs fortified at concentrations ≤ MRL, the result must be within 50-150% of the true value. All other LFSMs must be within 80-120% of the true value.

TABLE 6. (CONTINUED)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.3.8	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Laboratory Duplicate(LD)	Analyze at least one LFSMD or LD daily, or with each Analysis Batch of up to 20 field samples, whichever is more frequent.	For LFSMDs or LDs having perchlorate concentrations ≤ 2 x MRL, the RPD must be within 50-150%. All other LFSMDs and LDs must have RPDs within 80-120%.
Section 10.3.4	Quality Control Sample (QCS)	Analyzed with every new calibration curve.	Results must be 80-120% of the true value.

TABLE 7. EXAMPLE SEQUENCE FOR AN ANALYTICAL BATCH

Sample	Sample Description	*Acceptance Criteria
1	CCC ≤ MRL	50-150% of true value
2	Mid Level CCC	80-120% true value
3	LRB	< 1/3 MRL
4	LFB	≤ MRL 50-150% of true value > MRL 80-120% of true value
5	Field Sample-1	
6	Field Sample-2	
7	Field Sample-3	
8	Field Sample-4	
9	Field Sample-5	
10	Field Sample-6	
11	Field Sample-7	
12	Field Sample-8	
13	Field Sample-9	
14	Field Sample-10	
15	CCC (rotate level)	≤ MRL 50-150% of true value > MRL 80-120% of true value
16	LFSM	≤ MRL 50-150% of true value > MRL 80-120% of true value
17	LD or LFSMD	≤ 2 x MRL 50-150% RPD > 2 x MRL 80-120% RPD
18	Field Sample-11	
19	Field Sample-12	
20	Field Sample-13	
21	Field Sample-14	
22	Field Sample-15	
23	Field Sample-16	
24	Field Sample-17	
25	Field Sample-18	
26	Field Sample-19	
27	Field Sample-20	
28	CCC (rotate level)	≤ MRL 50-150% of true value > MRL 80-120% of true value

* Additionally, all samples must meet the isotope area count ratio criteria (Section 9.3.5) and the relative retention time criteria (Section 9.3.6).

FIGURE 1
LC/MRM CHROMATOGRAM OF A 0.5 µg/L PERCHLORATE STANDARD

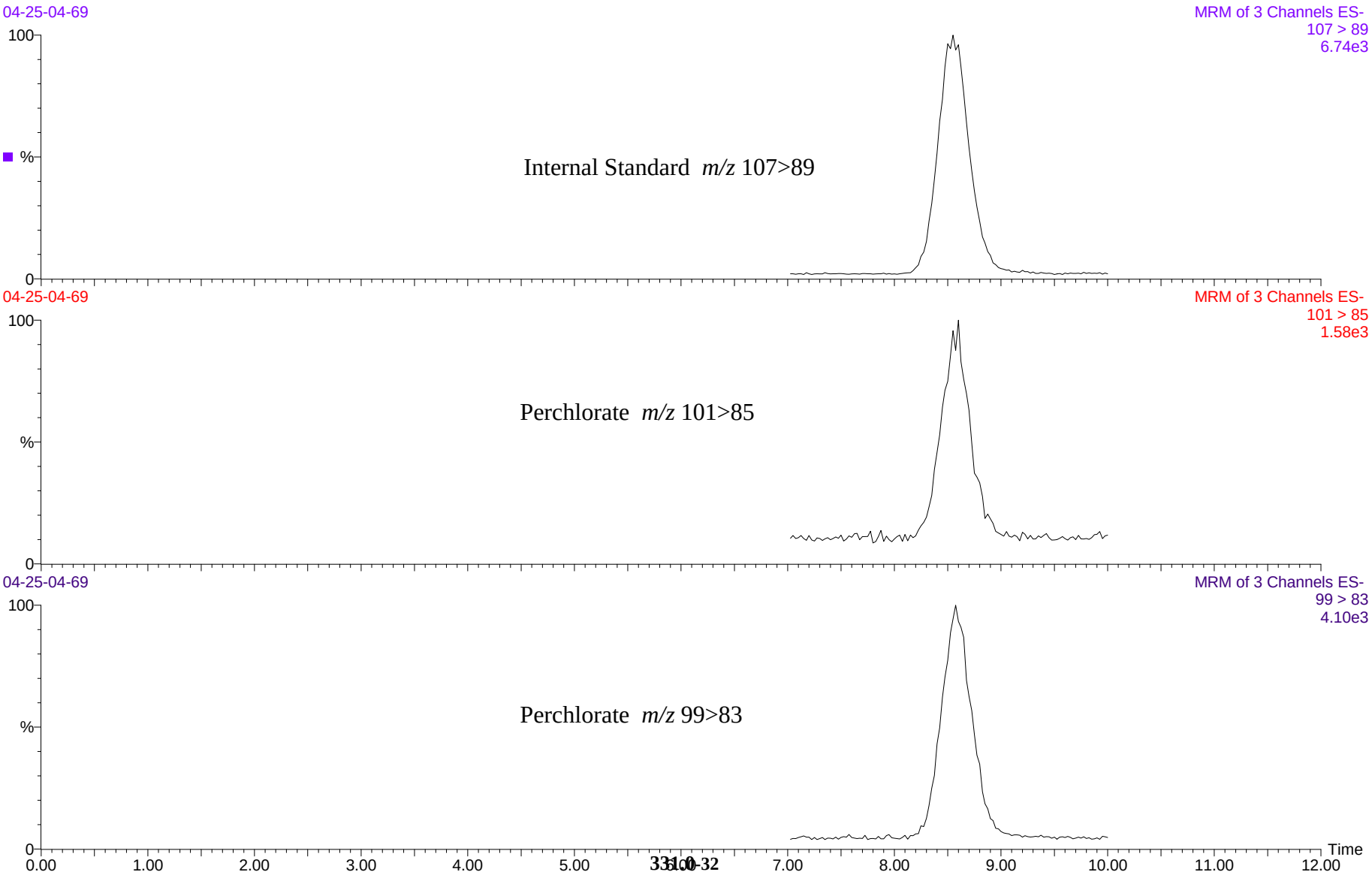

FIGURE 2
LC/SIM CHROMATOGRAM OF A 0.5 µg/L PERCHLORATE STANDARD

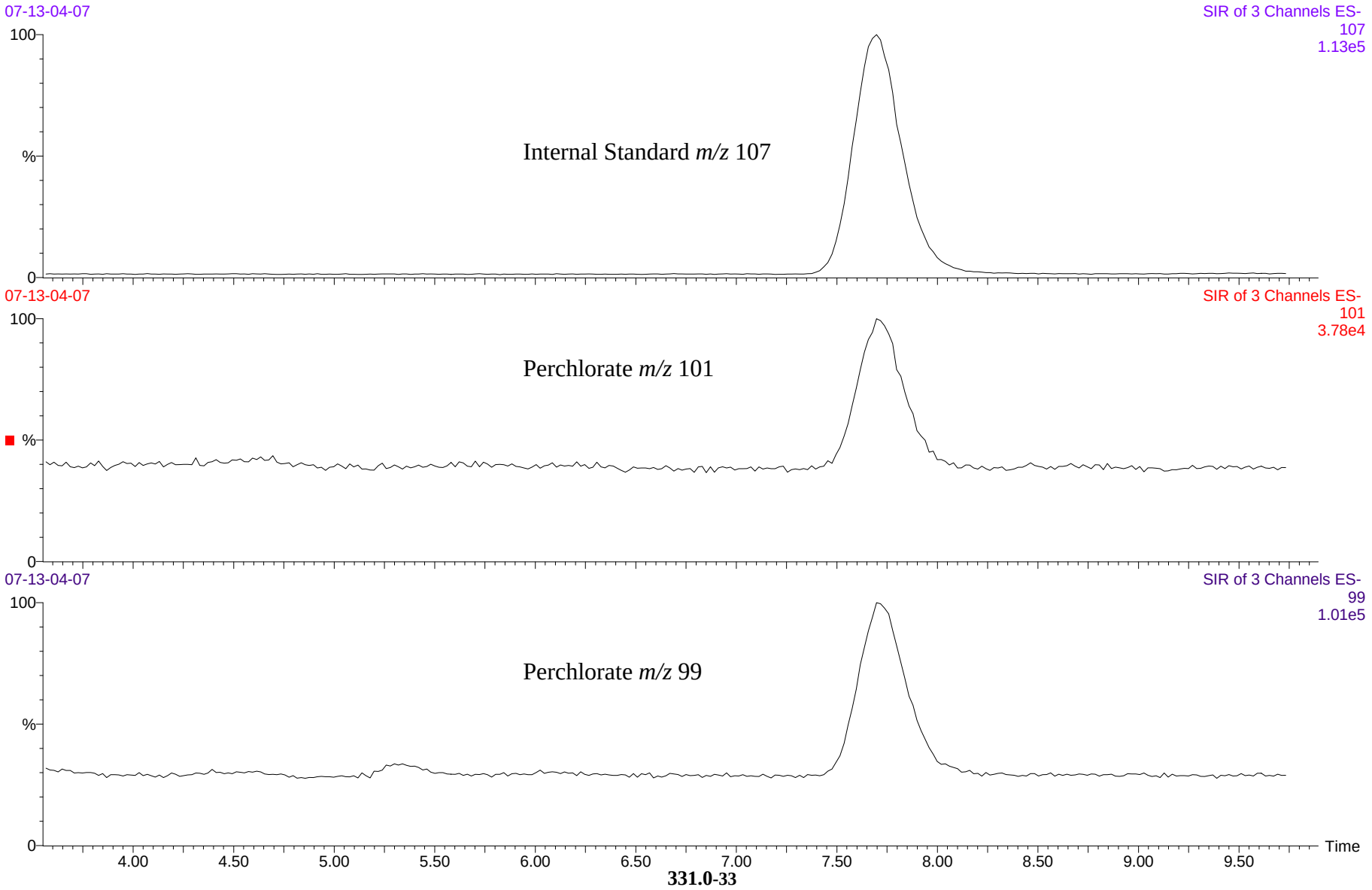

FIGURE 3
LC/SIM TIC CHROMATOGRAM (*m/z* 99, 101,107) OF A LFSSM (1000 mg/L)
FORTIFIED WITH 0.5 µg/L PERCHLORATE