METHOD 327.0 DETERMINATION OF CHLORINE DIOXIDE AND CHLORITE ION IN DRINKING WATER USING LISSAMINE GREEN B AND HORSERADISH PEROXIDASE WITH DETECTION BY VISIBLE SPECTROPHOTOMETRY

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METHOD 327.0
DETERMINATION OF CHLORINE DIOXIDE AND CHLORITE ION IN DRINKING WATER USING LISSAMINE GREEN B AND HORSERADISH PEROXIDASE WITH DETECTION BY VISIBLE SPECTROPHOTOMETRY

1. SCOPE AND APPLICATION

1.1 This is a spectrophotometric method for the analysis of chlorine dioxide and chlorite* in finished drinking waters. It is primarily intended to be used at drinking water utilities in conjunction with daily monitoring requirements.

1.2 The experimentally determined Detection Limits (DLs) for chlorine dioxide and chlorite are provided in Section 17, Table 1. The DL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.(1) Detection Limits are technique and instrument dependent. The DL differs from, and is lower than, the Minimum Reporting Level (MRL) (Sect. 3.10). Method performance was demonstrated over a combined concentration range of 0.2 mg/L to 2.2 mg/L. Precision and accuracy data are presented in Section 17, Tables 1-3.

1.3 Chlorine dioxide is a volatile, reactive gas. Care was taken during method development to minimize steps that could result in the loss of chlorine dioxide during analysis. Analyst technique can affect method precision and accuracy.

2. SUMMARY OF METHOD

2.1 A drinking water sample is collected headspace-free in a 16-mL amber glass vial. A second sample is collected and sparged with an inert gas to remove all traces of chlorine dioxide and then transferred to a second 16-mL amber vial. A third vial is filled with reagent water. A 1.0-mL aliquot of water is removed from each vial, and a 1.0-mL aliquot of a concentrated citric acid buffer containing glycine is added. The samples are capped and gently mixed, and then a second 1.0-mL aliquot is removed and a 1.0-mL aliquot of a Lissamine Green B (LGB)/Horseradish Peroxidase (HRP) reagent is added. The HRP catalyzes the conversion of chlorite to chlorine dioxide.(2,3) Chlorine dioxide rapidly oxidizes the LGB, reducing its absorption in the red region of the visible spectrum in proportion to the chlorine dioxide concentration.(4,5) A visible spectrophotometer is used to measure the absorbance of the reagent water blank and sample absorbance at 633 nm, which is the absorbance maximum for LGB in the citric acid/glycine buffer. The absorbance difference between the reagent water blank and the samples is used to calculate the concentrations of chlorine dioxide, using an external standard calibration

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*Throughout this document chlorite ion (ClO_2^-) is referred to as 'chlorite' for simplicity, even though the use of 'chlorite ion' is more accurate.

curve, determined using chlorite standards. The unsparged sample is used to determine the total chlorite and chlorine dioxide concentration and the sparged sample is used to determine the chlorite concentration. The chlorine dioxide concentration is the difference between these two values.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples that is processed and analyzed together and includes no more than 10 Field Samples. An analysis batch must also include at least one Method Blank and all required QC samples. The Method Blanks and QC samples do not contribute to the maximum Field Sample total of 10.

3.2 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution. 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(s), which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

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 of precision (Sect. 9.2.3), and accurate quantitation is not expected at this level.[1]

3.5 FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection and storage, as well as laboratory procedures.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFSM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFSM corrected for background concentrations.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the field sample used to prepare the LFSMD, fortified, processed and analyzed identically. The LFSMD is used instead of the Field Duplicate to access method precision when the occurrence of target analytes is low.

3.8 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data, including storage, spill, and handling precautions.

3.9 METHOD BLANK (MB) – An aliquot of reagent water that is free from all method analytes and is processed and analyzed with the samples in the analysis batch. The absorbance value of the MB is used to calculate the absorbance change caused by the reaction of chlorine dioxide with LGB.

3.10 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This concentration can be no lower than the concentration of the lowest continuing calibration standard for that analyte and can be used only if acceptable quality control criteria for this standard are met.

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

3.12 REAGENT WATER – A high-purity water, typically either distilled or deionized, that is free from organic and inorganic contaminants and all method analytes.

3.13 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 Free available chlorine reacts with LGB to discolor the solution. Glycine reacts with the free available chlorine to form chloroaminoacetic acid, thereby eliminating the potential for this type of interference.

4.2 Laboratory glassware can potentially exhibit a demand for chlorine dioxide.[5] While this was not observed during method development, it could bias the analytical results and lead to poor accuracy and/or precision. This potential problem can be avoided if sample collection vials are cleaned and reused as described in Section 8.1.2.

4.3 Chlorite concentrations are calculated after the samples are sparged to remove chlorine dioxide. Samples that are not efficiently sparged to remove the chlorine dioxide will yield chlorite values that are higher than the true value.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely identified; each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made

available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[6-8]

5.2 This method does not require the preparation of chlorine dioxide standards, which could have inhalation hazards. It also does not require the preparation of chlorite standards from a recrystallized solid standard, which can have an explosion hazard. Because of the significant potential dangers associated with these materials, they are not recommended for use in conjunction with this method. The chlorite standard cited in Section 7.3.1 does not pose an explosion hazard.

6. EQUIPMENT AND SUPPLIES

6.1 VISIBLE SPECTROPHOTOMETER – A single- or double-beam spectrophotometer that uses a monochromator to make accurate absorbance measurements at discrete wavelengths (Agilent Model 8453 or equivalent) equipped with a standard, rectangular 1-cm path length cell is recommended. The spectrophotometer must have a linear response and acceptable photometric accuracy up to an absorbance value (A) of 1.6.

6.1.1 Nondispersive spectrophotometers (e.g., filter-based, light emitting diode-based, etc.) with alternate cuvette shapes and pathlengths are allowed as long as they meet the method QC requirements. These devices must have filters with transmission maximum near 633 nm and must have a linear response and acceptable photometric accuracy up to an absorbance value of 1.6.

6.1.2 Longer pathlength cells may be used for applications that require lower DLs; however, the concentration of the Buffered LGB Stock Solution (Sect. 7.2.4) must be adjusted accordingly. Shorter pathlengths are not recommended.

6.2 CUVETTES – A matched set of 1-cm glass cuvettes with polytetrafluoroethylene (PTFE) stoppers (Fisher Cat. #: 22200-350 or equivalent). Alternate path lengths and/or cell shapes are allowed as long as the method QC criteria are met.

6.3 ANALYTICAL BALANCE – Used to accurately weigh analytical standard materials (±0.1 mg sensitivity).

6.4 TOP LOADING BALANCE – Used to accurately weigh reagents (±10 mg sensitivity).

6.5 WEIGHING BOATS – Plastic, disposable - used to weigh reagents.

6.6 WEIGHING FUNNEL – Used to weigh and transfer LGB and HRP (Fisher Cat. #: 19958-3 or equivalent).

6.7 PIPETTES – Use 0.5-mL and 1.0-mL Class A, volumetric pipettes (Fisher Cat. #: 13-651-11 or equivalent) and/or variable volume single channel pipettes with disposable plastic tips.

6.7.1 Use a variable volume, single channel pipette (Brinkman Eppendorf Research Pro Pipette, Fisher Cat. #:21-378-84 or equivalent) with a volume of 500 to 5000 L to deliver volumes of 1.0 mL or larger.

6.7.2 Use a variable volume, single channel pipette (Brinkman Eppendorf Research Pro Pipette, Fisher Cat. #:21-378-83 or equivalent) with a volume of 50 to 1000 L to deliver volumes less than 1.0 mL.

6.8 VIALS – 16-mL amber glass vials with PTFE-lined screw caps (Fisher Cat. #: 03-391-8E or equivalent) used to collect samples.

6.9 ERLENMEYER FLASKS – 500 mL and 250 mL glass, used to prepare reagents.

6.10 BOTTLES – Amber glass bottles with PTFE-lined screw caps or opaque, high-density polyethylene (HDPE) bottles of various sizes to store reagents and standards.

6.11 BEAKERS – 100 mL (Fisher Cat.#: 02-540H or equivalent), used to collect and sparge samples for chlorite analyses.

6.12 VOLUMETRIC FLASKS – Class A, of varying sizes.

6.13 SPARGING APPARATUS – A cylinder of nitrogen or helium equipped with a regulator that is attached to an appropriate length of Tygon tubing and a disposable, Pasteur pipette. Glass gas-dispersion tubes with fritted cylinders or disks may also be used as long as they are rinsed between uses.

7. REAGENTS AND STANDARDS

7.1 INTENDED USE OF REAGENTS AND STANDARD SOLUTIONS – Sections 7.2 and 7.3 describe reagents, solvents and standards that are compatible with a 1-cm rectangular cuvette. In this configuration, and with these reagents, this method has a linear range of 0.2 - 2.2 mg/L total chlorite and chlorine dioxide. If longer pathlength cells are used to obtain concentration data at lower levels, the Buffered LGB Stock Solution (Sect. 7.2.4) must be adjusted accordingly.

7.2 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society[9], where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.2.1 REAGENT WATER – Purified water (typically either deionized or distilled) which does not contain any chlorine dioxide or chlorite or compounds that absorb light at 633 nm. The absorbance of the reagent water at 633 nm for a

1-cm pathlength (against a cuvette containing air) should be 5 milli-absorbance units (mA) or less.

7.2.2 CONCENTRATED CITRIC ACID/GLYCINE BUFFER – Add 9.0 g of trisodium citrate dihydrate ($C_6O_7H_5Na_3 \cdot 2H_2O$) (Sigma Cat. #: C3434 or equivalent), 5.0 g disodium hydrogencitrate sesquihydrate ($C_6O_7H_6Na_2 \cdot 1.5H_2O$) (Sigma Cat. #: 35908-4 or equivalent) and 1.0 g glycine (Sigma Cat. #: G7162 or equivalent) to a 250-mL Erlenmeyer flask. Add 125 mL of reagent water and mix thoroughly. Add one drop of chloroform (or about 50 µL) (Aldrich HPLC Grade, Cat. #: 36,692-7 or equivalent) and store this solution at ≤ 6 °C.

7.2.2.1 Care must be taken to ensure that all crystalline material is completely dissolved either by using a stir bar or by swirling the contents of the flask manually for several minutes.

7.2.2.2 The pH of the concentrated buffer stock solution should be confirmed with a pH electrode to be in the pH range of 6.0 - 6.2 upon dilution by adding a 1.0-mL aliquot of the buffer to 15 mL of reagent water. The pH of the concentrated stock solution may be adjusted, if necessary, by adding small amounts of the $C_6O_7H_6Na_2 \cdot 1.5H_2O$ to lower the pH value or $C_6O_7H_5Na_3 \cdot 2H_2O$ to increase the pH value.

7.2.2.3 Chloroform acts as an antimicrobial agent. If the buffer solution turns cloudy, which is indicative of microbial growth, it must be discarded.

7.2.3 LISSAMINE GREEN B (LGB) CONCENTRATED STOCK SOLUTION IN REAGENT WATER – Accurately weigh 240 mg of LGB (Aldrich Cat. #: 19958-3 or equivalent) in a glass weighing funnel (Sect. 6.6). Transfer the LGB to a 250-mL wide neck volumetric flask with reagent water and dilute to the mark using reagent water. Add a small PTFE stirbar and allow the solution to stir overnight. Transfer the solution to a 500-mL amber glass bottle which has a PTFE-lined cap and store this solution at ≤ 6 °C. If stored properly, this solution should be stable for 2 months.

7.2.3.1 The minimum acceptable purity for the LGB solid is 70%. Higher purity is recommended. Although Aldrich specifies that their LGB has a minimum purity of 60%, the lot of LGB used in this work was 73% pure according to the manufacturer. This information can be obtained from Aldrich by contacting a technical service representative.

7.2.3.2 Confirm that the LGB solid is at least 70% pure as follows. Add 6 mL of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2) to a 100-mL volumetric flask. Fill the flask about half full with reagent water and then add a 1.0-mL aliquot of the LGB Concentrated Stock

Solution. Fill the flask to the mark with reagent water and mix thoroughly. Allow the flask to equilibrate at room temperature for 15 - 20 minutes, and then mix again. Transfer an aliquot of this solution to a cuvette with a 1-cm pathlength. The absorbance of this solution must be ≥ 0.99.

7.2.4 BUFFERED LGB STOCK SOLUTION – Prepare a dilution of the LGB Concentrated Stock Solution in the Concentrated Citric Acid/Glycine Buffer. **The concentration of this solution should be confirmed prior to mixing it with the Horseradish Peroxidase (HRP) Stock Solution to prepare the Combined LGB/HRP Reagent (Sect. 7.2.6). This step will eliminate waste of the HRP enzyme, which is the most expensive reagent in the method.** The procedure below is used to determine that the Buffered LGB Stock Solution has been prepared at the appropriate concentration so that when it is used in the procedure it will yield a Method Blank absorbance in the required absorbance range of 1.5 ± 0.1.

7.2.4.1 For LGB with a purity of 73%, transfer 40 mL of the LGB Concentrated Stock Solution (Sect. 7.2.3) to a wide neck 100-mL volumetric flask. Add 6 mL of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2), dilute to the mark with reagent water, and mix thoroughly. For LGB that has lower purity, the volume of the Concentrated Stock Solution can be estimated using the equation

$$V_{LGB} = 40\left(\frac{73}{P_{LGB}}\right)$$

where V_{LGB} is the volume of the LGB Concentrated Stock Solution required, and P_{LGB} is the purity of the LGB (obtained from the manufacturer) expressed as a whole number.

7.2.4.2 Confirm that the Buffered LGB Stock Solution concentration is in the correct range as follows. Fill a 16-mL vial with reagent water, remove 1.0 mL of the water and add 1.0 mL of the of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2). Cap the vial and shake. Remove 0.5 mL of the buffered reagent water and add 0.5 mL of the Buffered LGB Stock Solution. Recap the vial and mix the contents thoroughly by shaking. Transfer an aliquot of this solution to a 1-cm cuvette and determine the absorbance at 633 nm. The absorbance of this solution at 633 nm must be in the range of 1.5 ± 0.1. If not, the Buffered LGB Stock Solution should be reprepared from the LGB Concentrated Stock Solution. The appropriate volume of the LGB Concentrated Stock Solution can be calculated using the equation

$$V_{req} = V_{added}\left(\frac{1.5}{A_{meas}}\right)$$

where V_{req} is the volume of the LGB Concentrated Stock Solution that is required to prepare the new solution, V_{added} is the volume of the LGB Concentrated Stock Solution originally added (40 mL in Sect. 7.2.4.1), and A_{meas} is the measured absorbance.

7.2.4.3 If the analyst is using the method to determine concentrations < 0.2 mg/L, a longer pathlength cuvette is required. The Buffered LGB Stock Solution concentration must be adjusted accordingly so that the absorbance in the longer pathlength cuvette, when checked as described in Section 7.2.4.2, is in the absorbance range of 1.5 ± 0.1. To calculate the amount of LGB Concentrated Stock Solution required for the solution, simply divide the recommended volume in Section 7.2.4.1 by the pathlength.

7.2.4.4 This stock solution is used to prepare the Combined LGB/HRP Reagent and is not preserved with chloroform. This solution should not be kept or stored for more than one day.

7.2.5 BUFFERED HORSERADISH PEROXIDASE (HRP) STOCK SOLUTION – Weigh out 60 - 70 mg of Type II HRP (Sigma Cat. #: P8250 or equivalent) using a glass weighing funnel (Sect. 6.6) and an analytical balance. Transfer the HRP to a wide-neck, 50-mL volumetric flask by washing it from the funnel with reagent water. The HRP is a lyophalized powder that can be lost during transfer if care is not taken to minimize air currents. Carefully rinse the walls of the funnel with reagent water to ensure complete transfer. Add 6 mL of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2) and then dilute the flask contents to the mark with reagent water and mix thoroughly.

7.2.5.1 The HRP must have a minimum activity of 150 units per mg of solid using the purpurogallin method.[10] The Type II HRP sold by Sigma meets this criterion. According to Sigma, their HRP is stable for up to 1 year if stored as a solid at ≤ -20 °C.[11] Other sources of HRP may also be suitable, but they have not been evaluated.

7.2.5.2 This stock solution is used to prepare the Combined LGB/HRP Reagent and is not preserved with chloroform, and therefore should not be kept or stored for more than one day.

7.2.6 COMBINED LGB/HRP REAGENT – Confirm the concentration of the Buffered LGB Stock Solution according to Section 7.2.4.2 prior to performing this step. This will prevent wasting the Buffered HRP Stock Solution, which is

the most expensive reagent in this method. Combine 50 mL of the Buffered LGB Stock Solution (Sect. 7.2.4) with the entire volume of Buffered HRP Stock Solution (Sect. 7.2.5) in an amber glass bottle with a PTFE-lined cap. Do not rinse the 50-mL volumetric flask containing the Buffered HRP Stock Solution to transfer the remaining droplets as quantitative transfer of this reagent is not necessary. The volume of the combined reagent is sufficient for approximately 100 tests (e.g., blanks, samples, calibrations, etc.). Add one drop of chloroform (or about 50 µL). This solution may be used for 14 days if stored at ≤ 6 °C.

7.2.7 HELIUM OR NITROGEN (≥99.995%) – UHP Grade or purer, used to sparge samples to remove chlorine dioxide.

7.3 STANDARD SOLUTIONS – Because chlorite is converted to chlorine dioxide using the HRP reagent, this method uses chlorite standards to calibrate for both chlorine dioxide and chlorite (Sect. 2.1). Chlorite standard solutions may be prepared from certified, commercially available solutions or from solid compounds. Preparation of chlorite standards from a recrystallized solid of high purity (95%+) is not recommended as this form of chlorite can pose an explosion hazard. Solution concentrations listed in this section were used to develop this method and are included as an example. **Even though stability times for standard solutions are suggested in the following sections, laboratories should use standard QC practices to determine when Standard Solutions described in this section should be replaced.**

7.3.1 CHLORITE STOCK STANDARD SOLUTION (1000 mg/L) – Certified chlorite standard solutions are commercially available (Absolute Standards, Cat. # 54109 or equivalent). Commercial standards should be stored at ≤ 6 °C in an amber glass bottle or high density polyethylene (HDPE) container to prolong standard life. If stored properly, this solution should be stable for 6 months.

7.3.2 CHLORITE PRIMARY DILUTION STANDARD (PDS) (10 mg/L) – This solution is used to prepare Calibration Standards and Continuing Calibration Check Standards. Partially fill a 500-mL volumetric flask with reagent water. Add 5.0 mL of the Chlorite Stock Standard Solution (Sect. 7.3.1) using a pipette and fill the flask to the mark using reagent water. Invert and shake the flask several times to ensure that the solution is completely mixed and then transfer the flask contents to an amber bottle with a PTFE-lined cap or an HDPE container. This solution should be stored at ≤ 6 °C. If stored properly, this solution should be stable for 3 months.

7.3.3 CALIBRATION STANDARDS – At least three Calibration Standards are required to establish an initial calibration curve. Five Calibration Standards are recommended. The concentration of the Calibration Standards must span the intended reporting range. An example of the dilutions that were used to prepare the standards used in the development of this method is included in the table below. All Calibration Standards are to be prepared from the Chlorite Primary Dilution Standard (Sect. 7.3.2) using Class A volumetric or variable-volume

pipettes and volumetric flasks. Transfer an appropriate aliquot of the Chlorite PDS (10 mg/L) (Sect. 7.3.2) to the appropriate flask and dilute to the mark with reagent water. Mix the contents of the flask thoroughly and transfer to an amber glass bottle or opaque plastic container. These solutions should be stored at ≤ 6 °C. If stored properly, these solutions should be stable for 3 months.

Chlorite PDS Volume (mL)	Final Volume (mL)	Calibration Std. Conc. (mg/L)
10	500	0.20
25	500	0.50
50	500	1.00
75	500	1.50
100	500	2.00

7.3.4 CONTINUING CALIBRATION CHECK (CCC) STANDARDS – Calibration Standards prepared above may also be used as CCC Standards.

7.3.5 LFSM FORTIFICATION SOLUTION (100 mg/L) – This solution is used to fortify Field Samples for the Laboratory Fortified Sample Matrix requirement (Sect. 9.5). Partially fill a 100-mL volumetric flask with reagent water. Add 10.0 mL of the Chlorite Stock Standard Solution (Sect. 7.3.1) using a pipette, and then fill the flask to the mark using reagent water. Invert and shake the flask several times to ensure the solution is completely mixed and then transfer the contents of the flask to an amber bottle with a PTFE-lined cap or an HDPE container. This solution should be stored at ≤6 °C. If stored properly, this solution should be stable for 3 months.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE VIAL PREPARATION

8.1.1 VIAL SELECTION – The procedure and reagent concentrations were developed using the vials listed in Section 6.8. The manufacturer lists the volume of these vials to be 16 mL. The procedure used to select appropriate vials is described below. If alternate vials are used that have an average volume that differs from 16 mL by more than 10%, reagent concentrations and volumes must be adjusted accordingly.

8.1.1.1 Select an appropriate number of vials based on anticipated sample load requirements. Vials that do not have appropriate volumes will be rejected during this process, so begin the selection process with 10-15% more vials than needed. Vials may be reused after cleaning. Method development was conducted with approximately 30 vials.

8.1.1.2 Label and cap each 16-mL vial and determine the tare weight with the PTFE-lined cap. Fill the vial with water and recap. Dry the external walls of the vial and confirm that there is no headspace by inverting the vial and looking for an air bubble. Weigh the full vial and record the water weight. Convert the water weight to volume of the vial by assuming 1.0 g of water is equal to 1.0 mL.

8.1.1.3 Determine the average volume for the vials tested, and select the vials that are closest in volume. Discard any vial that has a volume that is greater than 1% above or below the average value. During development of this method, the average volume was 16.4 mL and vials were accepted that were in the volume range of 16.4 ± 0.1 (or within 0.63%). Approximately 10% of the vials were rejected.

8.1.2 VIAL CLEANING – Vials may be reused. Prior to reuse they should be thoroughly cleaned with soap and water and rinsed several times with reagent water. Vials (without caps) can be dried in an oven at 140 °C. **Reusing vials may help avoid any potential for bias associated with chlorine dioxide demand of the glassware (Sect. 4.2).**

8.2 SAMPLE COLLECTION

8.2.1 Prior to sample collection, remove the aerator (if present) to minimize loss of chlorine dioxide during sample collection. Open the tap and allow the system to flush until the water temperature has stabilized (usually 3-5 minutes). Reduce the tap flow just before collecting the samples.

8.2.2 Two aliquots must be collected at each sampling point if both chlorine dioxide and chlorite are to be determined. Both aliquots require sample processing to halt the continued formation and/or reproportionation of the method analytes. Two pipette tips (or pipettes) will be required for this procedure. Tips can be used repeatedly to perform the same functions with each Field Sample. This is not expected to cause significant contamination due to carryover. However, care should be taken to ensure that fluid is not retained in (or on) the pipette tip prior to performing the next transfer. These pipette tips (or pipettes) are given numbers for clarity. The pipette tips are summarized in the table below with their function. These tips may be saved for use in Section 11.

Pipette Tip #	Used to
1	Remove sample and remove buffered sample
2	Transfer citric acid/glycine buffer

8.2.3 FIELD SAMPLE COLLECTION TO DETERMINE CHLORINE DIOXIDE AND CHLORITE – Fill the 16-mL vial completely so that no headspace remains after capping the vial. This is most easily done by slightly overfilling the vial. Invert the capped vial to ensure that the vial does not contain an air bubble (or headspace). Recollect the sample if a bubble is noted. Remove a 1.0-mL aliquot of the Field Sample from the vial using pipette tip 1 and add a 1.0-mL aliquot of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2) using pipette tip 2. Free available chlorine is removed in this step. Gently mix the vial by inverting it three times. Clearly label the sample. This aliquot will be used to determine the total chlorine dioxide and chlorite concentration.

Note: When removing the 1.0-mL aliquot from the vial, carefully place the pipette tip into the vial in such a way that the sample does not overflow out of the vial. As sample is withdrawn into the pipette, the tip should be gently lowered so that it is always below the surface of the sample.

8.2.4 FIELD SAMPLE COLLECTION TO DETERMINE CHLORITE – Collect a second aliquot (approximately 40 mL) in an open container with a large surface area such as a 100-mL beaker. Containers like bottles are not efficiently sparged even over long times and should not be used. This aliquot will be used to determine the chlorite concentration and must be sparged immediately after collection. **Field Samples that are not completely sparged will yield chlorite concentrations that are high.**

8.2.4.1 Sparge the sample using a constant flow of nitrogen or helium gas until the chlorine dioxide is removed. This can be accomplished using a lecture bottle or gas cylinder with a regulator equipped with a suitable length of Tygon tubing attached to a Pasteur pipette or a gas-dispersion tube with a fritted cylinder or disk (Sect. 6.13). A new pipette should be used for each sample. Glass gas-dispersion tubes should be rinsed thoroughly between samples.

8.2.4.1.1 Sparging a sample contained in a beaker with nitrogen at a flow rate of 250 mL/minute for 15 minutes using a Pasteur pipette removed over 97% of the chlorine dioxide.

8.2.4.1.2 Laboratories should confirm that their procedure used to sparge chlorine dioxide from Field Samples is sufficient by analyzing a sample known to contain high levels of

chlorine dioxide (> 2 mg/L) and evaluating its removal at various sparge times.

8.2.4.2 Transfer an aliquot of the sparged Field Sample to a 16-mL vial. Invert the capped vial to ensure that it is completely filled. If an air bubble is noted, uncap the vial and add additional sparged sample as necessary. Remove a 1.0-mL aliquot of the sparged Field Sample from the vial using pipette tip 1 and add a 1.0-mL aliquot of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2) using pipette tip 2. This step removes any free available chlorine from the sample. Gently mix the vial by inverting it three times.

8.2.5 QC SAMPLES – Field Duplicates require collection of an extra 16-mL vial as per Section 8.2.3 and an additional sparged sample as per Section 8.2.4. A portion of the Field Sample sparged in Section 8.2.4 should be used for the Laboratory Fortified Sample Matrix.

8.3 SAMPLE STORAGE – Field Samples and Field Duplicates should be analyzed as soon as possible. Samples may be held for up to 4 hours if collected properly and stored in a cooler at ≤10 °C.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a Quality Control (QC) program. The requirements of this program consist of an Initial Demonstration of Capability (IDC), and subsequent analysis in each analysis batch of a Continuing Calibration Check Standard (CCC). A Laboratory Fortified Sample Matrix (LFSM) and a Field Duplicate Sample are recommended weekly. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 5 and 6. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections. The full IDC must be conducted prior to the analysis of any Field Samples each time a new analyst uses the method. Sections 9.2.1 and 9.2.2 must be repeated each time a new LGB Concentrated Stock Solution (Sect. 7.2.3) is prepared. An acceptable Initial Calibration (Sect. 10.2) must be conducted prior to performing the IDC.

9.2.1 INITIAL DEMONSTRATION OF ACCURACY – Process and analyze five highest-level Calibration Standards and five reagent water Method Blanks through the procedure described in Section 11.2. Calculate the chlorite concentration for each Calibration Standard as described in Section 12. The

average concentration for the five replicates must be ± 30% of the fortified value.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Using the same set of replicate data generated for Section 9.2.1, calculate the standard deviation and relative standard deviation (*RSD*) of the replicate values. The *RSD* is calculated using the equation

$$RSD = \frac{S}{\overline{X}} \times 100\%$$

where

S is the standard deviation for the replicate values,
and $\overline{X}$ is the average value for the replicates.

The RSD of the results of the replicate analyses must be less than 20%.

9.2.3 DETECTION LIMIT (DL) DETERMINATION – Prepare and analyze at least seven replicate CCCs at a chlorite concentration estimated to be near the DL using the procedure described in Section 11.2 for total chlorite and chlorine dioxide. For a 1-cm pathlength, the CCCs must be fortified with chlorite in the concentration range of 0.20 to 0.35 mg/L or at a concentration no more than 3- to 5-times the calculated DL. Calculate the DL using the equation

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

where

$t_{(n - 1, 1 - \alpha = 0.99)}$ is the Student's t value for the 99% confidence level with n-1 degrees of freedom,
n is the number of replicates,
and *S* is the standard deviation of replicate analyses.

Selected t values can be found in the table below.

Number of Replicates	7	8	9	10	11
Student's t value	3.143	2.998	2.896	2.821	2.764

9.2.3.1 The DL for this method is a function of pathlength and analyte concentration. Longer pathlengths will result in improved sensitivity and should decrease the DLs for chlorine dioxide and chlorite. DLs for a 1-cm pathlength calculated by fortifying reagent water with chlorite in the concentration range of 0.20 to 0.35 mg/L should be ≤ 0.25 mg/L.

9.2.3.2 Detection Limit is a statistical determination of precision only.[1] If the DL replicates are fortified at a low enough concentration, they may result in a calculated DL that is higher than the fortified concentration. Therefore no precision and accuracy are specified for the DL.

9.3 CONTINUING CALIBRATION CHECK (CCC) STANDARDS – A mid-level CCC is prepared as described in Section 7.3.4 and analyzed with each analysis batch or each day samples are analyzed. For a 1-cm pathlength, this CCC should have a chlorite concentration near 1.0 mg/L. The calculated chlorite concentration for the CCC (Sect.12.5) must be ± 30% of the expected value. If this criterion is not met, the Field Sample(s) should be re-collected and reanalyzed after establishing acceptable CCC recovery (Sect. 10.3.2).

9.4 FIELD DUPLICATE (FD) – A Field Duplicate is recommended weekly for both chlorine dioxide and chlorite. Calculate the relative percent difference (RPD) between the Field Sample (FD1) and the Field Duplicate (FD2) as shown below. The RPD for samples with concentrations greater than the lowest calibration standard should not exceed 30%. The RPD at concentrations at or near the lowest calibration standard (at or below 0.30 mg/L for a 1-cm pathlength) should not exceed 50%.

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \times 100\%$$

If the *RPD* for the Field Sample and the Field Duplicate falls outside the designated range, and the laboratory performance for that analyte meets the QC performance criterion in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the Field Sample is labeled "suspect/matrix" to inform the data user that the results are suspect due to matrix effects.

9.5 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A Laboratory Fortified Sample Matrix is recommended weekly.

9.5.1 Collect a Field Sample for the LFSM as described in Section 8.2.4 and process it as described in Section 11.4. Fortify the Field Sample using the LFSM Fortification Solution (Sect. 7.3.5) with a chlorite concentration that is greater than the expected concentration based on historical levels, being careful not to exceed the upper limit of the initial calibration curve. This should be done in a manner that does not increase the sample volume by more than 1%.

9.5.2 Calculate the recovery (*R*) for each analyte using the equation

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

where *A* is the measured concentration in the fortified sample, *B* is the measured concentration in the unfortified sample, and *C* is the fortification concentration.

9.5.3 Recoveries from samples fortified at or above their native concentration should range between 70 and 130% unless the samples were fortified at low levels (e.g., total concentrations at or below 0.30 mg/L) where 50 to 150% recoveries are acceptable. If the accuracy of the LFSM falls outside the designated range, and the laboratory performance for chlorite is shown to be in control (e.g., the QC criterion for the CCC was met), the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled "suspect/matrix" to inform the data user that the results are suspect due to matrix effects.

10. CALIBRATION

10.1 An acceptable initial calibration must be established before Field or QC Samples are analyzed. After initial calibration is successful, a Continuing Calibration Check (CCC) is required during each analysis batch or each day samples are analyzed.

10.2 INITIAL CALIBRATION – An initial calibration curve must be generated prior to conducting the IDC and prior to analyzing any Field Samples. An initial calibration is also required after preparing new Combined LGB/HRP Reagent as described in Section 7.2.6 (at least every 2 weeks). An initial calibration curve is established by plotting the absorbance differences (ΔA) between the Method Blank and a number of Calibration Standards versus chlorite concentrations in the Calibration Standards. Studies conducted during method development indicated that calibration curves generated for chlorite and chlorine dioxide using the Combined LGB/HRP Reagent (Sect. 7.2.6) were similar enough to allow the calibration for both analytes using chlorite. This procedure is described below.

10.2.1 Prepare at least three Chlorite Calibration Standards that span the intended reporting range as described in Section 7.3.3. Five Calibration Standards are recommended.

10.2.1.1 The high-level calibration standard should not exceed a concentration of 2.2 mg/L. At higher concentrations, the response becomes nonlinear.

10.2.2 Fill a 16-mL sample vial with each Calibration Standard and process each vial along with at least one Method Blank (MB) according to the procedure outlined in Section 11.2. Two MBs are recommended.

10.2.3 Calculate ΔA at 633 nm between the MB and each Calibration Standard. If two MBs are used, use the average MB absorbance value for this calculation.

10.2.4 Plot ΔA for each calibration standard as a function of chlorite concentration (or chlorine dioxide concentration, since chlorite is converted to chlorine dioxide prior to reacting with LGB). This is a procedural calibration technique, where the calibration standards are processed identically to the Field and QC Samples. This eliminates the need to adjust the Calibration Standard concentrations to account for dilutions made during the procedure.

10.2.5 Fit the data using a linear regression and determine the slope and y intercept using the equation

$$\Delta A = \text{Slope}[ClO_2^-] + \text{Int.}$$

where ΔA is the absorbance difference between the blank and the sample, $[ClO_2^-]$ is the chlorite concentration in mg/L, and Slope and Int. are the slope and y intercept for the linear fit, respectively.

10.2.6 The r^2 value for the linear fit should be 0.98 or better. Use the equation for the line to calculate chlorine dioxide and/or chlorite concentration.

10.2.7 Check the Initial Calibration by calculating the concentrations of the Calibration Standards that were used to create the calibration curve. Use the linear regression fit formula for the calculation. Each calculated concentration, except the calculated concentration for the lowest Calibration Standard, should calculate to be 70-130% of its expected value. The lowest Calibration Standard should calculate to be 50-150% of its expected value. Laboratories that have difficulty achieving these criteria will have trouble meeting the QC requirements summarized in Section 9.

10.3 CONTINUING CALIBRATION CHECK (CCC) – A Continuing Calibration Check is required with each Analysis Batch or each day samples are analyzed. This QC sample is processed with the Method Blank(s) and Field Sample(s) in the Analysis Batch to ensure that the data collected in the Analysis Batch are valid.

10.3.1 Process a CCC in an identical manner to the Field Samples as described in Section 11.2. The CCC concentration should be near the mid-point of the initial calibration range. Calibration Standards used to prepare the initial calibration curve may be used for this purpose. For a 1-cm pathlength, the CCC should have a chlorite concentration at or near 1.0 mg/L.

10.3.2 Calculate the CCC concentration using the initial calibration curve generated above (Sect. 10.2) as described in Section 12. The CCC response must be in the range of ± 30% of the expected value. If this criterion is not met, then all data for the Field Samples in the Analysis Batch are invalid. Remedial action should be taken which could require establishing a new initial calibration and may require the preparation of new reagents (the HRP and LGB solutions have the

shortest shelf lives). Field Samples should be re-collected and reanalyzed after re-establishing acceptable QC performance.

11. PROCEDURE

11.1 As noted in Section 2.1, this procedure involves a two-step process for the determination of chlorine dioxide and chlorite in drinking waters. The first step, described in Section 11.2, uses the Combined LGB/HRP Reagent to convert chlorite to chlorine dioxide to measure the total concentration of both analytes. This procedure is used to process Field Samples, Field Duplicates, and CCCs. In the second step, described in Section 11.3, samples are sparged to remove the chlorine dioxide, and are then analyzed to determine the chlorite concentration. Chlorite is not removed during sparging. This procedure is used to process Field Samples and Field Duplicates. (The chlorine dioxide concentration is calculated by subtracting the chlorite concentration determined in the second step from the total concentration determined in the first step.) Section 11.4 describes the processing of LFSMs, which require a slight modification to the procedure described in Section 11.3.

11.1.1 This is a headspace-free procedure. Care taken to minimize headspace will help the analyst to achieve the QC performance criteria summarized in Section 9.

11.2 TOTAL CHLORINE DIOXIDE AND CHLORITE PROCEDURE USED TO PROCESS FIELD SAMPLES, FIELD DUPLICATES AND CCCs

11.2.1 The unsparged samples collected in the 16-mL vials are prepared and analyzed using the procedure described in this section. If samples have been stored (Sect. 8.3), they should be allowed to equilibrate to room temperature.

11.2.2 Fill a 16-mL vial with reagent water and label it as the Method Blank. One MB is required in each Analysis Batch ($\leq$ 10 Field Samples). Two MBs are recommended.

11.2.3 Fill a 16-mL vial with a CCC solution prepared according to the procedure described in Section 7.3.4.

11.2.4 This procedure requires three separate pipette tips (or pipettes). Each tip will be used repeatedly to perform the same function with each Field and QC sample. This is not expected to cause significant contamination due to carryover. However, care should be taken to ensure that fluid is not retained in (or on) the pipette tip prior to performing the next transfer. These pipette tips (or pipettes) are given numbers for clarity. The pipette tips are summarized in the table below with their function.

Pipette Tip #	Used to
1	Remove sample and remove buffered sample
2	Transfer citric acid/glycine buffer
3	Transfer HRP/LGB mixed reagent

11.2.5 Remove a 1.0-mL aliquot from the CCC and MBs using pipette tip 1. Add a 1.0-mL aliquot of the Concentrated Citric Acid/Glycine Buffer (Sect. 7.2.2) to the CCC and MBs using pipette tip 2.

Note: Field Samples and Field Duplicates are buffered with the Concentrated Citric Acid/Glycine Buffer at the time of collection to remove the free available chlorine.

11.2.6 Cap the CCC and MB vials and gently mix their contents by inverting them three times. This step buffers the samples to a uniform pH.

11.2.7 Process all Field Samples, Field Duplicates, CCCs and MBs at the same time through the remainder of the procedure described in this section. Be careful not to agitate the samples any more than necessary. Uncap the vial and remove an additional 1.0-mL aliquot using pipette tip 1. Immediately add 1.0-mL of the Combined LGB/HRP Reagent (Sect. 7.2.6) using pipette tip 3. Cap and mix the contents of the vial thoroughly.

11.2.8 Check the vial for headspace by inverting it and looking for an air bubble. If the vial contains air bubbles, the sample must be re-collected (or reprepared in the case of a CCC or MB).

11.2.9 Allow the samples to react at room temperature for at least 20 minutes, but preferably not longer than 40 minutes. Loss of color during this period is normal and is a result of the reaction between LGB and chlorine dioxide.

11.2.10 Thoroughly mix the contents of each vial and then transfer an aliquot to a 1-cm cuvette. The cuvette should first be rinsed with reagent water or an aliquot of the sample. Wipe the outside of the cuvette to remove water droplets and/or finger prints. Use the same cuvette, to measure the visible absorbance at 633 nm for the MB(s), Field Sample(s), Field Duplicate (if processed), and CCC minimizing the time between measurements.

11.3 CHLORITE PROCEDURE USED TO PROCESS FIELD SAMPLES AND FIELD DUPLICATES

11.3.1 This procedure may be used as a stand-alone procedure for the determination of chlorite or in conjunction with the procedure in Section 11.2 for the

determination of chlorine dioxide as well. The sparged samples are analyzed using the procedure described in this section.

11.3.2 Collect, sparge, and preserve each FS or FD to remove chlorine dioxide and free available chlorine as described in Section 8.2.4.

11.3.3 If samples have been stored (Sect. 8.3), they should be allowed to equilibrate to room temperature.

11.3.4 Process the Field Samples and Field Duplicates through all steps described in Sections 11.2.7 through 11.2.10.

11.4 PROCESSING LABORATORY FORTIFIED SAMPLE MATRICES (LFSMs)

11.4.1 This procedure is used for the processing of Laboratory Fortified Sample Matrices.

11.4.2 Collect, sparge, and preserve an FS as described in Section 8.2.4. The sparged sample should be poured into two 16-mL vials. One vial is analyzed for chlorite using the procedure described in Section 11.3 and the other vial is fortified and analyzed according to the procedure described below.

11.4.3 Fortify the Field Sample with the LFSM Fortification Solution (Sect. 7.3.5) that is greater than the expected chlorite concentration based on historical information. **This will require a fourth pipette tip that must not be used for other purposes. Small amounts of the LFSM Fortification Solution can contaminate other Field or QC samples from carryover because this solution is highly concentrated.**

11.4.3.1 Be careful not to exceed the upper concentration of the initial calibration curve when calculating the fortification level. Fortification levels should be in the range of 1 - 2 times the native level so that the chlorite concentration will be within the linear range of the initial calibration curve.

11.4.3.2 As an example, to fortify a Field Sample in a 16.4-mL vial with 1.0 mg/L of chlorite, one would need to add 0.0164 mg (1.0 mg/L x 0.0164 L). This would require a 164- L aliquot of the LFSM Fortification Solution (0.0164 mg/0.100 mg/mL = 0.164 mL or 164 L).

11.4.3.3 If small volumes of the LFSM Fortification Solution are used (1% of the vial volume or less), this aliquot can be delivered without first removing a same-size aliquot to make room for reagents. If this technique is used the analyst must be careful to insert the pipette tip beneath the surface of the vial during delivery of the aliquot and to

check the pipette tip after delivery of the aliquot to ensure that the entire volume has been delivered. Cap the vial once the fortification is complete and mix thoroughly.

11.4.4 Process the LFSM according to the steps described in Sections 11.2.7 through 11.2.10.

12. DATA ANALYSIS AND CALCULATION

12.1 Chlorine dioxide and chlorite concentrations are calculated using an initial calibration curve that is generated using chlorite standards. Slopes for both analytes are similar and, therefore, no conversion factor is required in order to calculate the chlorine dioxide concentrations.

12.2 Generate an initial calibration curve according to Section 10.2 by processing at least three, but preferably five, initial calibration standards through the entire procedure outlined in Section 10.2.

12.3 Establish an Initial Calibration curve as described in Section 10.2. Plot ΔA against the undiluted concentration of the calibration standards.

12.3.1 Samples processed according to the method generated initial calibration curves with slopes in the range of 0.33 - 0.38. Different vial volumes and/or reagent volumes will cause the slopes to differ from this value.

12.4 ClO_2 AND ClO_2^- CALCULATION: Determine the absorbance difference for each Field and QC Sample processed according to Section 11.2, and calculate the total chlorine dioxide and chlorite concentration using the slope and intercept determined in Section 10.2. If more than one MB is prepared and analyzed in the Analysis Batch, use the average MB absorbance when determining the absorbance difference for each sample.

12.5 ClO_2^- CALCULATION: Determine the absorbance difference for each Field and QC Sample processed according to Sections 11.3 and 11.4, and calculate the chlorite concentration using the slope and intercept determined in Section 10.2. If more than one MB is prepared and analyzed in the Analysis Batch, use the average MB absorbance when determining the absorbance difference for each sample.

12.6 ClO_2 CALCULATION: Calculate the chlorine dioxide concentration for each Field and QC sample by subtracting the chlorite concentration (Sect. 12.5) from the total chlorite plus chlorine dioxide concentration (Sect. 12.4).

12.7 Quantitate only those values for which the combined chlorine dioxide and chlorite concentration falls within the concentration range defined by the lowest and highest initial calibration standards.

12.7.1 Pathlength and reagent concentrations were carefully optimized during method development. Analyte concentrations above 2.2 mg/L are in a concentration range that is not linear if the reagent concentrations specified in this method for a 1-cm pathlength are used. For this reason, analysts must not extrapolate beyond the calibration range nor should the initial calibration range be extended beyond this concentration.

12.7.2 Field Samples that exceed this concentration (2.2 mg/L) should be re-collected and carefully diluted by a factor of 2 being careful to minimize unnecessary sample agitation that will result in the loss of chlorine dioxide.

12.7.3 Analytical results obtained from dilutions should be considered estimated values.

12.8 Analyte concentrations are reported in mg/L usually to two significant figures.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND DETECTION LIMITS – Detection Limits for chlorite and chlorine dioxide are presented in Section 17, Table 1 together with method precision and accuracy in reagent water. Single laboratory precision and accuracy are presented in Table 2 for a chlorinated surface water and in Table 3 for a chlorinated ground water.

13.2 SAMPLE STORAGE STABILITY STUDIES – A storage stability study was conducted by fortifying 0.8 mg/L of chlorine dioxide and 1.0 mg/L of chlorite into a chlorinated drinking water sample and analyzing at 0, 2, and 4 hours after storage at 10 °C. The data in Table 4 illustrate that chlorite concentrations are stable over a 4-hour time period when glycine is present to eliminate the free available chlorine residual.

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 NW, Washington, D.C., 20036.

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. 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, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.1.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1: PRECISION AND ACCURACY AND DETECTION LIMITS FOR CHLORINE DIOXIDE AND CHLORITE IN REAGENT WATER

Fortification Concentration		Chlorite			Chlorine Dioxide		
ClO_2^- (mg/L)	ClO_2 (mg/L)	Recovery (%)	RSD (%)	DL[a] (mg/L)	Recovery (%)	RSD (%)	DL[a] (mg/L)
0.25	not fortified	112	12	0.11	not fortified	not fortified	not fortified
0.25	0.95	118	8.5	0.078	116	3.6	not calculated
not fortified	0.26	not fortified	not fortified	not calculated	102	5.1	0.042
1.0	0.26	103	2.9	not calculated	124	16	0.16
1.0	0.94	98.5	3.2	not calculated	111	4.8	not calculated

[a]Based on seven replicates analyzed over one day. Detection Limits were calculated as follows:

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

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, and
S = standard deviation of replicate analyses.

TABLE 2: PRECISION AND ACCURACY FOR CHLORITE AND CHLORINE DIOXIDE IN A CHLORINATED SURFACE WATER[a]

Fortification Level		Chlorite		Chlorine Dioxide	
ClO_2^- (mg/L)	ClO_2 (mg/L)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1.0	not fortified	107	4.2	not fortified	not fortified
not fortified	0.82	not fortified	not fortified	100	1.9
1.0	0.82	109	3.7	91.3	8.9
2.0	not fortified	105	1.4	not fortified	not fortified
not fortified	1.94	not fortified	not fortified	96.9	1.0

[a]The drinking water used in these studies had a free available chlorine concentration of 0.9 mg/L.

TABLE 3: PRECISION AND ACCURACY FOR CHLORITE AND CHLORINE DIOXIDE IN A CHLORINATED GROUND WATER[a]

Fortification Level		Chlorite		Chlorine Dioxide	
ClO_2^- (mg/L)	ClO_2 (mg/L)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1.0	not fortified	110	4.4	not fortified	not fortified
not fortified	0.90	not fortified	not fortified	92.0	3.3
1.0	0.90	107	1.6	93.8	3.0
2.0	not fortified	100	2.7	not fortified	not fortified
not fortified	2.0	not fortified	not fortified	110	1.7

[a]The drinking water used in these studies had a free available chlorine concentration of 0.8 mg/L and a hardness of 325 mg/L ($CaCO_3$).

TABLE 4: STORAGE STABILITY FOR CHLORITE AND CHLORINE DIOXIDE IN A CHLORINATED SURFACE WATER SAMPLE[a]

Holding Time (Hours)	Chlorite Fortified at 1.0 mg/L		Chlorine Dioxide and Chlorite Fortified at a Total Concentration of 1.8 mg/L[b]	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
0	83.3	2.5	97.3	1.4
2	100	6.6	98.1	1.4
4	99.3	2.4	98.1	2.8

[a] The drinking water used in these studies had a free available chlorine concentration of 0.9 mg/L.
[b] Chlorine dioxide and chlorite were fortified into the chlorinated drinking water sample using concentrated standards just prior to preserving the samples so that the concentrations in the surface water were 0.80 and 1.0 mg/L, respectively.

TABLE 5: INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.1	Initial Demonstration of Accuracy	Prior to the analysis of any Field Samples by a new analyst and each time a new LGB Concentrated Stock Solution is prepared, process five highest-level calibration standards. Calculate the average concentration for five standards.	Mean concentration must be within ± 30% of the fortified value.
Section 9.2.2	Initial Demonstration of Precision	Using the same data from Section 9.2.1, calculate the RSD.	RSD must be < 20%.
Section 9.2.3	Detection Limit Determination	Prior to the analysis of any Field Samples by a new analyst, prepare and analyze a minimum of seven replicate CCCs fortified at a chlorite concentration of 0.20 - 0.35 mg/L if using the 1-cm pathlength procedure. Calculate the DL using the equation in Section 9.2.3.	DL should be ≤ 0.25 mg/L when using the 1-cm pathlength procedure.

TABLE 6: QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.3	Sample Storage	Analyze samples as soon as possible. Samples may be stored at ≤ 10 °C for up to 4 hours	Sample results are valid only if samples are analyzed within holding time.
Section 10.2	Initial Calibration	Calibrate with at least three calibration standards prior to the IDC and each time a new Combined LGB/HRP Reagent is prepared. Five calibration standards are recommended. Highest level for the 1-cm pathlength procedure must not exceed 2.2 mg/L.	When each calibration standard is calculated as an unknown using the calibration curve, the result should be 70-130% of the expected value for all except the lowest standard, which should be 50-150% of the expected value.
Sections 9.3 and 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a mid-level CCC each day samples are analyzed. The CCC should be at or near 1.0 mg/L for the 1-cm pathlength procedure.	The calculated chlorite concentration must be ± 30% of the expected value.
Section 9.4	Field Duplicate (FD)	An FD is recommended weekly. Calculate RPD according to Section 9.4.	RPD should not exceed 30% for concentrations above the low-level calibration standard. RPD should not exceed 50% for concentrations at or near the low-level calibration standard (at or below 0.3 mg/L for the 1-cm pathlength procedure).
Section 9.5	Laboratory Fortified Sample Matrix (LFSM)	An LFSM is recommended weekly. Calculate Recovery according to Section 9.5.	Recovery should be 70 - 130% except for samples fortified at low levels (total concentrations at or below 0.3 mg/L for the 1-cm pathlength procedure), where the Recovery should be 50 - 150%.