
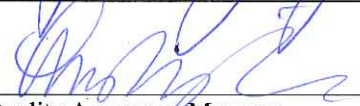
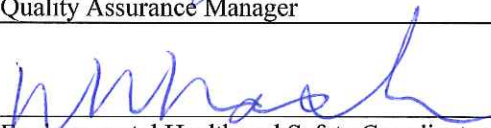



FACILITY SOP ATTACHMENT

SOP NUMBER: IR-WET-SULFIDE_SPEC, Rev 2 (10/31/2012)		CHANGE FORM ID: CF1	
SOP TITLE: SULFIDE, COLORIMETRIC, METHYLENE BLUE (STANDARD METHOD 4500 S2-B, C, D & H)			
REASON FOR ADDITION OR CHANGE (Use additional sheets if necessary): Revise wavelength used. SOP currently references 625nm from the now-discontinued EPA 376.2. SM4500S2- D specifies 664nm. (A sequence of samples was analyzed on 03/11/13 at both wavelengths and found to yield nearly identical results.)			
CHANGE OR ADDITION (Use additional sheets if necessary): The spectrophotometer wavelength setting referenced in SOP sections 2.0, 6.1.1, 10.6.1, 10.7, and 11.3.3 has been changed from 625nm to 664nm.			
Prepared By: D. Dawes			
*APPROVED BY:			
 _____ Technical Review Signature		_____ Date <u>4/11/13</u>	
 _____ Quality Assurance Manager		_____ Date <u>4-1-2013</u>	
 _____ Environmental Health and Safety Coordinator		_____ Date <u>04/01/2013</u>	
 _____ Laboratory Director		_____ Date <u>04/02/2013</u>	


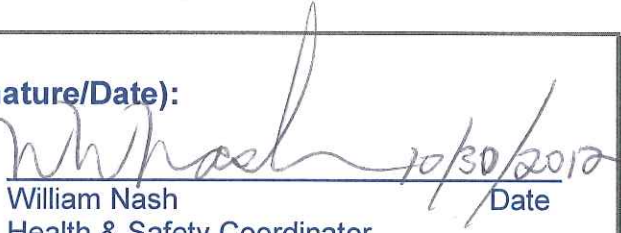


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**Title: SULFIDE, COLORIMETRIC, METHYLENE BLUE
SM 4500- S2 B, C, D & H**

Approvals (Signature/Date):			
 Tung Nguyen Technical Manager	10/30/12 Date	 William Nash Health & Safety Coordinator	10/30/2012 Date
 Maria Friedman Quality Assurance Manager	10-30-2012 Date	 Trevor Brenner Laboratory Director	10/31/12 Date

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1.0 SCOPE AND APPLICATION

1.1 This SOP is applicable to the measurement of total sulfide, dissolved sulfide, and un-ionized hydrogen sulfide in drinking, surface and saline water, domestic and industrial wastes in accordance with Standard Methods 4500-S₂-. The following procedures are included:

- Separation of Soluble and Insoluble Sulfides (4500-S²⁻ B)
- Sample Treatment to Remove Interfering Substances or to Concentrate (4500-S²⁻ C)
- Methylene Blue spectrophotometric determination (4500-S²⁻ D)
- Calculation of Un-ionized Hydrogen Sulfide (4500-S²⁻ H)

1.2 See Attachment 1 for method reporting limits and batch QC acceptance criteria.

1.3 On occasion clients may request modifications to this SOP. These modifications are handled following the procedures outlined in "Validation of Methods" in the Quality Assurance Manual

2.0 SUMMARY OF METHOD

Sulfide samples are received preserved with sodium hydroxide and zinc acetate to form a sulfide precipitate. A representative aliquot of the sample is centrifuged to condense the precipitate; the supernatant is decanted to remove potential interferences, and then brought back up to the original volume. The aliquot is then remixed. Sulfide reacts stoichiometrically with dimethyl-p-phenylenediamine (p-aminodimethyl aniline) in the presence of ferric chloride to produce methylene blue. The methylene blue dye is measured on the spectrophotometer at a wavelength of 625 nm.

3.0 DEFINITIONS

3.1 Definitions

3.1.1 Total sulfide includes dissolved H₂S and HS⁻, as well as acid-soluble metallic sulfides present in suspended matter.

3.1.2 Dissolved sulfides are what remain after suspended solids have been removed by flocculation and settling.

3.1.3 There are no additional specific definitions associated with this test. See the laboratory QA manual and methods SM 4500-S²⁻ B, C, D & H for general definitions.

4.0 INTERFERENCES

4.1 Color and turbidity may interfere with the observations of color.

4.2 Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert sulfide to an un-measurable form.

4.3 Strong reducing agents interfere by preventing the formation of the blue color. These interferences may be at least partially removed by precipitating the sulfide with zinc, removing the supernatant liquid and replacing the liquid with DI water.

5.0 SAFETY

Employees must abide by the policies and procedures in the Corporate Environmental Health and Safety Manual (CW-E-M-001) and this document. This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toe, nonabsorbent shoes are a minimum.

5.1 Specific Safety Concerns or Requirements

Personal Protective Equipment Required: Safety Glasses/Face Shield, Lab coat, Nitrile Gloves

Sodium Sulfide will form Hydrogen Sulfide (HS) gas if combined with water moisture or strong acids. Inhalation of HS gas may be fatal.

5.2 Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. **NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the MSDS for each of the materials listed in the table.** A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
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Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Sulfuric Acid	Corrosive Oxidizer Dehydrator Poison Carcinogen	1 mg/m ³ - TWA	Inhalation produces damaging effects on the mucous membranes and upper respiratory tract. Symptoms may include irritation of the nose and throat, and labored breathing. Symptoms of redness, pain, and severe burn can occur. Contact can cause blurred vision, redness, pain and severe tissue burns. Can cause blindness.
Iodine solution	Irritant	N/A	May cause irritation to the respiratory tract, skin. May cause nausea, vomiting, diarrhea and cramps.
Sodium Hydroxide	Corrosive	2 mg/m ³ - Ceiling	Severe irritant. Effects from inhalation of dust or mist vary from mild irritation to serious damage of the upper respiratory tract, depending on severity of exposure. Symptoms may include sneezing, sore throat or runny nose. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes, and with greater exposures it can cause burns that may result in permanent impairment of vision, even blindness.
Aluminum Chloride	Irritant Corrosive	2 mg/m ³	Inhalation of dust may irritate the respiratory tract due to corrosive nature. Ingestion may cause irritation in mouth and esophagus and burns to mucous membrane. Skin contact may cause irritation with redness and pain. Eye contact may cause severe pain, blurred vision and tissue damage.
Amine-sulfuric acid.	Corrosive Oxidizer Dehydrator Poison Carcinogen	1 mg/m ³ TWA	Inhalation produces damaging effects on the mucous membranes and upper respiratory tract. Symptoms may include irritation of the nose and throat, and labored breathing. Symptoms of redness, pain, and severe burn can occur. Contact can cause blurred vision, redness, pain and severe tissue burns. Can cause blindness.
Sodium Sulfide	Corrosive	10 ppm- TWA 15 ppm- STEL	Will form Hydrogen Sulfide (HS) gas if combined with water, moisture or strong acids. Inhalation of HS gas may be fatal. HS gas is also flammable and can cause a flash fire. Symptoms include painful conjunctivitis, headache, nausea, dizziness, coughing and, in extreme cases, pulmonary edema and possible death. Irritant. Contact with skin can produce serious caustic burns with painful inflammation and possible destruction of tissue. Inflammation, tearing and pain may be expected. Severe contact can cause destruction of tissue.
1 – Always add acid to water to prevent violent reactions.			
2 – Exposure limit refers to the OSHA regulatory exposure limit.			

6.0 EQUIPMENT AND SUPPLIES

6.1 Instrumentation

6.1.1 Spectrophotometer, 625 nm wavelength

6.2 Supplies

6.2.1 50 mL plastic bottles

6.2.2 Dispensers

- 6.2.3 125 mL plastic containers
- 6.2.4 1 L plastic container
- 6.2.5 250 mL volumetric flasks
- 6.2.6 40 mL sample vials
- 6.2.7 250 mL Erlenmeyer flasks
- 6.2.8 Disposable plastic droppers
- 6.2.9 Plastic test tubes
- 6.2.10 Cuvettes, 1 cm light path.
- 6.2.11 15 mL conical bottom centrifuge tubes, plastic
- 6.2.12 40 mL clear glass VOA vials

7.0 REAGENTS AND STANDARDS

7.1 Reagents

All purchased and prepared reagents must be made from a traceable (NIST) source material, if available, and documentation of this traceability must be maintained by the laboratory

- 7.1.1 Zinc Acetate, $Zn(C_2H_3O_2)_2$, reagent grade
- 7.1.2 Sodium Hydroxide, reagent grade
- 7.1.3 Aluminum Chloride, $AlCl_3$, reagent grade
- 7.1.4 Sulfuric acid, H_2SO_4 , reagent grade
- 7.1.5 Amine-sulfuric acid reagent - purchased
- 7.1.6 Ferric chloride, $FeCl_3$, reagent grade
- 7.1.7 Diammonium hydrogen phosphate solution, $(NH_4)_2HPO_4$, reagent grade
- 7.1.8 Sodium sulfide Na_2S , reagent grade (two sources are required)
- 7.1.9 Laboratory Reagent Grade Water

7.2 Standards

All purchased standards must be accompanied by a Certificate of Analysis (C of A) which is kept available at the laboratory in order to demonstrate traceability of the standard to certified (NIST-traceable, if available) source material. All prepared standards must be made from a traceable (NIST) source material, if available, and documentation of this traceability must be maintained by the laboratory

- 7.2.1 Iodine solution (approx. 0.025N) – purchased and standardized daily prior to use
- 7.2.2 Sodium thiosulfate (0.025N), $Na_2S_2O_3 \cdot 5 H_2O$ – purchased

8.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Sample container, preservation techniques and holding times may vary and are dependent on sample matrix, method of choice, regulatory compliance, and/or specific contract or client requests. Listed below are the holding times and the references that include preservation requirements.

Matrix	Sample Container	Min. Sample Size	Preservation	Holding Time	Reference

Waters Total sulfide	Polyethylene bottles, or glass	100 mL	4drops of 2N $Zn(C_2H_3O_2)_2$ and NaOH pH ≥ 9 ; Cool $> 0 > 6$ °C	7 Days from collection	40 CFR Part 136.3
Water dissolved sulfide ^{1,2}	Polyethylene bottles or glass	250 mL	NaOH Cool $> 0 > 6$ °C ¹	7 days from flocculation	SM 4500 S ²⁻

NOTE 1: Water samples for dissolved sulfide should be flocculated in the field, decanted into a container preserved with zinc acetate and sodium hydroxide. If field flocculation is not possible, samples must be preserved in sodium hydroxide, flocculated at the laboratory as soon as possible (no more than 48-72 hours), and further preserved with zinc acetate.

NOTE 2: If requested by client, total or dissolved sulfide may be determined on a soil or solid sample after performing a DI water leach at a 1:10 preparation. The holding time will be 7 days from preparation. Note that, as sulfide is not a stable analyte, soil results may be biased low.

9.0 QUALITY CONTROL

9.1 Sample QC

The following quality control samples are prepared with each batch of samples. Each of these QC samples may be re-analyzed once if it doesn't pass, prior to sample analysis, in order to verify the failure wasn't due to a physical or mechanical problem.

9.1.1 Method Blank (MB)

Prepare and analyze a method blank (MB) for each matrix and with every batch of 20 samples, or less. Check that there are no analytes detected at or above the reporting limit in the method blank. If the method blank shows contamination, re-prepare all samples in the batch unless:

- The samples are ND, flag and write an NCM.
- The sample result is $> 10x$ the blank level; flag and write an NCM

9.1.2 Laboratory Control Sample (LCS)

Prepare and analyze a primary source laboratory control sample (LCS) for every batch of 20 samples or less. The LCS recovery must be within laboratory acceptance limits (see attachment 1). If the LCS is outside of these limits, re-prepare the entire batch and/or re-calibrate the system unless the LCS recovery is above the upper limit and samples are ND, in which case flag and write an NCM.

LCS Duplicate (LCD) is performed only when insufficient sample is available for the MS/MSD or when requested by the client/project/contract.

9.1.3 Matrix Spike and Matrix Spike Duplicate

The sample selection for MS/MSD is random, unless specifically requested by a client.

Prepare and analyze a matrix spike (MS) and a matrix spike (MSD) duplicate for each matrix and with every batch of 20 samples, or less. The recovery and relative percent difference must be within laboratory acceptance limits (see attachment 1), unless there is matrix interference. In the case of matrix interference, flag and write an NCM.

9.2 Instrument QC

The following instrument QC samples are run with each analytical sequence. Each of these QC samples may be re-analyzed once if it does not pass, in order to verify the failure wasn't due to a physical or mechanical problem. Re-analysis must be performed before the analysis of any associated batch QC or client samples.

9.2.1 Initial Calibration Verification (ICV)

Immediately after the initial calibration, analyze the secondary source calibration verification. Verify that its response is within $\pm 20\%$ of the true value.

- If not, re-prepare the ICV standard.
- If the ICV is still out of control, re-calibrate the system

9.2.2 Continuing Calibration Verification (CCV)

Analyze a CCV standard after every 10 samples or less and at the end of the sequence. The recovery must be within $\pm 20\%$ of the true value. If the CCV is outside recovery limits, re-analyze once. If it is still out, the instrument must be recalibrated and samples not bracketed by acceptable CCVs must be reanalyzed unless the CCV is out high, in which case any ND samples may be reported with an NCM.

Follow every CCV with a CCB (calibration blank). The CCB must read less than the method reporting limit. If the CCB is above the reporting limit, re-analyze once. If it is still out, the instrument must be recalibrated and samples not bracketed by acceptable CCBs must be reanalyzed unless the CCB is out high, in which case any ND samples may be reported with an NCM.

9.2.3 Calibration Acceptance Summary

Prepare a calibration curve by plotting the absorbance of the standards against the sulfide concentrations. The resulting correlation coefficient (r) must be ≥ 0.995 . If this criterion is not met, re-prepare the calibration standards and repeat the calibration.

Perform the initial calibration daily.

Refer to the "Calibration Curves" SOP for more information on calibrations.

10.0 **PROCEDURE**

10.1 Reagent Preparation

10.1.1 Diammonium hydrogen phosphate solution, $(\text{NH}_4)_2\text{HPO}_4$

Prepare the diammonium hydrogen phosphate solution, $(\text{NH}_4)_2\text{HPO}_4$, as needed. Add 50 grams of $(\text{NH}_4)_2\text{HPO}_4$ to 100 mL of Laboratory Reagent Grade water. Mix well, and store in a labeled glass bottle with dispenser. Shelf life is 6 months, store at room temperature.

10.1.2 Ferric Chloride (FeCl_3)

The ferric chloride solution is normally purchased pre-made. If it is unavailable, prepare the ferric chloride (FeCl_3) solution by adding 100 grams $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to 40 mL of Laboratory Reagent Grade Water. Mix well, and store in a labeled 125 mL poly container. Follow vendor storage and shelf life specifications

10.1.3 Aluminum Chloride (AlCl_3)

Prepare the aluminum chloride (AlCl_3) solution as needed. Add 100 grams of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ to 144 mL of Laboratory Reagent Grade Water. Mix well, and store in a labeled glass bottle with dispenser. Shelf life is 6 months, store at room temperature.

10.1.4 Zinc Acetate Zn(C₂H₃O₂)₂

Prepare the zinc acetate solution, Zn(C₂H₃O₂)₂, as needed. Add 220 grams of Zn(C₂H₃O₂)₂ • 2H₂O to 870 mL of Laboratory Reagent Grade Water. Mix well, and store in a labeled 1L poly container. Shelf life is 6 months, store at room temperature.

10.1.5 Sodium Hydroxide, 6N

Prepare 6N NaOH solution by adding 24.0g NaOH pellets to a 100mL volumetric flask partially filled with reagent grade water. Swirl to mix, allow to cool, and bring to volume. Shelf life is 6 months, store at room temperature.

10.1.6 Sulfuric acid, 1:1

Carefully added concentrated sulfuric acid to an equal volume of reagent grade water. Swirl to mix, allow to cool, and bring to volume. Shelf life is 6 months, store at room temperature. Document the preparation of the reagents properly in the LIMS.

10.2 Standard Preparation**10.2.1 Sodium Sulfide primary stock solution Na₂S (~111 mg/L)**

Add approximately 100 mL of Laboratory Reagent Grade water to a 250 mL volumetric flask. Add 0.1875g of Na₂S • 9 H₂O to the flask and swirl until dissolved. Dilute to 250 mL with Laboratory Reagent Grade water. Prepare daily.

10.2.2 Sodium Sulfide secondary stock solution Na₂S (~111 mg/L)

This source must be from a different vendor. Prepare the secondary source sulfide stock solution by adding approximately 100 mL of Laboratory Reagent Grade water to a 250 mL volumetric flask. Add 0.1875g of Na₂S • 9 H₂O to the flask and swirl until dissolved. Dilute to 250 mL with Laboratory Reagent Grade water. Prepare daily.

Document the preparation of the standards properly in the LIMS.

10.3 Verification of normality of Iodine and Sulfide stocks solutions**10.3.1 Verify the normality of the iodine solution daily.**

Titrate the purchased iodine solution with .025N sodium thiosulfate solution until the amber color fades to yellow.

Add starch indicator solution and continue to titrate drop by drop until the blue color disappears. Calculate the Iodine Concentration using the following equation:

$$\text{Normality I}_2 = \frac{V_T \times N_T}{\text{Sample Vol. (mL)}}$$

Where:

V_T = Volume (mL) of Titrant solution

N_T = Normality of Titrant solution

Record the Normality of the Iodine solution in the sulfide spread sheet.

10.3.2 Verify Sulfide Stocks (primary and secondary) daily.

Aliquot 100 mL of DI water and 10 mL of the sulfide stock into a clean Erlenmeyer flask. Add 10 mL of 0.025N Iodine solution and mix

Titrate with .025N $\text{Na}_2\text{S}_2\text{O}_3$ until amber color turns yellow.

Add approximately 10 drops of starch indicator and continue to titrate until the blue color goes clear.

Calculate the Stock Concentration using the following equation:

$$C_S = \frac{[(V_I \times N_I) - (V_T \times N_T)] \times 16}{\text{Sample Vol (L)}}$$

Where:

C_S = Concentration of Sulfide stock in mg/L

V_I = Volume (mL) of Iodide solution

N_I = Normality of Iodide solution

V_T = Volume (mL) of Titrant solution

N_T = Normality of Titrant solution

Record the Normality of the Sulfide stock solutions in the sulfide spread sheet.

10.4 Sample Preparation

10.4.1 Check and record the pH on Total/Dissolved Sulfides logbook log sheet or preparation bench sheet. The pH of the samples must be at least 9. If the pH of the sample is not at least 9 preserve the sample with 6N NaOH and document this with an NCM.

10.4.2 NOTE: All samples and batch QC are prepared in 40 mL vials. Because these are not volumetric devices, it is imperative that all vials are from the same vendor and that samples and batch QC are prepared by filling the vials completely (no headspace) to ensure uniform volume.

10.4.3 Dissolved Sulfide

NOTE: Preparation for dissolved sulfide is accomplished by flocculation with an aluminum chloride solution. This should be performed in the field. If it is not, the laboratory will flocculate and preserve as soon as possible (no more than 48-72 hours). After flocculation, dissolved sulfide samples are decanted, preserved with zinc acetate and must be analyzed within seven days from date of flocculation.

- Add 2 drops of 6N sodium hydroxide to a 40 mL sample vial
- Fill the vial with the sample, leaving just enough space to add aluminum chloride reagent.
- Prepare a Method Blank and LCS by filling two vials with a similar volume of laboratory reagent grade water. To the LCS vial, add 200 μL sulfide primary stock solution. Spike level is nominally 0.5 mg/L
- Prepare an MS/MSD pair by filling two additional vials with the selected source sample and adding 200 μL sulfide primary stock solution. Spike level is nominally 0.5 mg/L
- Add 2 or more drops of aluminum chloride. (Adjust the amount so the resulting precipitate is not excessively bulky or clumped and settles readily.)
- If necessary, add additional drops of reagent grade water to the sample vial until the liquid forms a convex bulge atop the vial. Cap. There must be no headspace.

- Shake the sample vigorously for 1 minute. Be sure the sample is inverted several times during the shaking process. If desired they may be placed in the TCLP extractor and tumbled for 3 minutes.
- Let the sample settle for 5-15 minutes or until the liquid is reasonably clear. If it takes longer than 15 minutes then add an additional 2 drops of each reagent and re-shake.
- Draw off or decant the supernatant liquid and place into a second 40mL vial containing 3 drops of zinc acetate.
- Fill the vial with reagent grade water until the liquid forms a convex bulge atop the vial. Cap and mix well
- Analyze the sample for dissolved sulfide by following the Total Sulfide analysis procedure.

10.4.4 Total Sulfide

- Add 3 drops of zinc acetate solution and 1 drop of 6N sodium hydroxide to a 40 mL VOA vial.
- Fill with sample to the very top and the liquid forms a convex bulge atop the vial. Cap. There must be no headspace present after the addition of the sample.
- Prepare a Method Blank and LCS by filling two vials with a similar volume of laboratory reagent grade water. To the LCS vial, add 200 μ L sulfide primary stock solution. Spike level is nominally 0.5 mg/L
- Prepare an MS/MSD pair by filling two additional vials with the selected source sample and adding 200 μ L sulfide primary stock solution. Spike level is nominally 0.5 mg/L
- Mix the sample by rotating back and forth vigorously about a transverse axis until any visible precipitate is uniformly distributed.
- Centrifuge all samples and batch QC for 10 minutes at 2900 RPM.
- After centrifugation, carefully, pour off the clear top layer and discard it. Be sure not to pour off any of the precipitate that might be stirred up during the decantation. It is more important to retain all the precipitate rather than to discard all the supernatant.
- Refill the VOA vial with Laboratory Reagent Grade water until the liquid forms a convex bulge atop the vial. Cap and mix well.
- The sample is now ready for analysis of total sulfides.

10.4.5 Un-ionized Sulfide

- Determine dissolved sulfide as described above.
- Determine sample conductivity following IR-WET-COND using an unpreserved aliquot.
- Use the Ionization Constant table (attachment 2) to determine the pK' (at 20°C) based on sample conductivity (use the conductivity value closed to that measured for the sample).
- Calculate un-ionized sulfide as H₂S using the formula listed in Section 11.

10.5 Calibration

Prepare a series of standards by pipeting suitable volumes of standardized sulfide solution into 7.5 mL of Laboratory Reagent Grade water in plastic test tubes. The standard is processed in the same way as the samples using the same reagents. Prepare the calibration standard at the following concentrations (**these concentrations are approximate depending on the actual concentration of the standardized sulfide solution**): 0, 0.05, 0.10, 0.30, 0.50, 0.80 and 1.0 mg/L.

Nominal Std Conc (mg/L)	Vol Na ₂ S stock (μL)*	Amine reagent	FeCl ₃	(NH ₄) ₂ HPO ₄	H ₂ SO ₄
Blank	NA	NA	3 drops	1.6 mL	0.5 mL
0.05	3.75	0.5 mL	3 drops	1.6 mL	0.0
0.10	7.50	0.5 mL	3 drops	1.6 mL	0.0
0.30	22.5	0.5 mL	3 drops	1.6 mL	0.0
0.50	37.5	0.5 mL	3 drops	1.6 mL	0.0
0.80	60.0	0.5 mL	3 drops	1.6 mL	0.0
1.0	75.0	0.5 mL	3 drops	1.6 mL	0.0

* Based on a sulfide stock concentration of 100 mg/L. Although the low (reporting limit) standard must be prepared at the exact concentration listed, all other calibration points can be prepared using easier-to-pipette volumes such as 20, 40, 60, 75 μL and using the resultant concentration in the calibration curve.

Calculate the actual concentration using the following equation:

$$C_{\text{std}} = \frac{C_s \times V_{\text{std}}}{V_w}$$

Where:

C_{std} = Actual concentration of the calibration standard (mg/L)

C_s = Concentration of stock sulfide solution

V_w = Volume of reagent water into which the standards are made (7.5 mL)

Record the actual calibration standard concentrations in the sulfide spreadsheet.

10.6 Sample Analysis

Set up test tube rack with enough test tubes for the samples, calibration curve and QA/QC samples.

10.6.1 Color development

- Into each test tube add 7.5 mL of Laboratory Reagent Grade Water for the calibration standards, For all others, add 7.5 mL of prepared sample or batch QC (method blank, LCS, MS and MSD) to each test tube
- Add 0.5 mL of the amine-sulfuric acid reagent to each sample and QA/QC samples including the method blank.
- Add 3 drops of FeCl₃ to all of the samples and QA/QC, including the method blank. Cover with parafilm and invert tube once to mix.
- Let the samples sit for three minutes.
- Add 1.6 mL of the (NH₄)₂HPO₄ solution to all of the samples and QA/QC samples. Cover with parafilm and invert once to mix.
- If precipitation occurs in a sample, add a few drops of amine-sulfuric acid reagent to re-dissolve the sulfide.
- Read the absorbance of all of the samples and QA/QC samples on the spectrophotometer at a wavelength of 625nm and calculate the final concentration from the calibration curve.

- All samples greater than the highest calibration standard must be diluted. Samples greater than 20 mg/L must be diluted a minimum of 20-fold.

10.6.2 Background Correction

- If there is any visible color to the samples prior to the addition of the reagents, analyze a "color blank" and subtract the color blank absorbance from the sample absorbance.
- A color blank is prepared by taking a second 7.5 mL aliquot of sample and performing the color development steps with the exception of using 1:1 sulfuric acid instead of the amine-sulfuric acid reagent.

10.7 Instrument Conditions

- UV/Vis spectrophotometer, λ - 625 nm
- Cuvettes 1 cm light path

11.0 CALCULATIONS / DATA REDUCTION

11.1 Accuracy

$$\frac{\text{ICV / CCV, LCS \% Recovery}}{\text{known concentration}} = \frac{\text{observed concentration}}{\text{known concentration}} \times 100$$

$$\text{MS \% Recovery} = \frac{(\text{spiked sample}) - (\text{unspiked sample})}{\text{spiked concentration}} \times 100$$

11.2 Precision (RPD)

$$\text{Matrix Duplicate (MD)} = \frac{|\text{orig. sample value} - \text{dup. sample value}|}{[(\text{orig. sample value} + \text{dup. sample value})/2]} \times 100$$

11.3 Concentration

11.3.1 Total or Dissolved Sulfide (mg/L) = C x D

Where:

C = instrument reading (ppm)

D = Dilution Factor

11.3.2 Calculate Un-ionized H₂S as follows:

$$C_{\text{H}_2\text{S}} = S_d \times \left(\frac{1}{10^{\text{pH}-\text{pK}'} + 1} \right)$$

Where:

C = Concentration of H₂S as S²⁻ in mg/L

S_d = Concentration of dissolve sulfide (mg/L)

pH = pH of sample

pK' = Logarithm of practical ionization constant for H₂S (Attachment 2)

11.3.3 Calibration Curve.

Sample sulfide concentrations are calculated from their absorbances using the equation for a line:

$$y = mx + b$$

Where:

y = the concentration of the sample

m = the slope of the line

b = the intercept

x = the absorbance of the sample at 625 nm

The values of m and b are obtained and calculated automatically by spectrophotometer using linear regression from the calibration standards. The value of the absorbance of the sample is then substituted into the linear regression equation and the concentration of the sample is calculated. The concentration of the sample is given in mg/L.

12.0 METHOD PERFORMANCE

12.1 Method Detection Limit Study (MDL)

The method detection limit (MDL) is the lowest concentration that can be detected for a given analytical method and sample matrix with 99% confidence that the analyte is present. The MDL is determined according to the laboratory's MDL procedure as described in laboratory's SOP, IR-QA-MDL. MDLs reflect a calculated (statistical) value determined under ideal laboratory conditions in a clean matrix, and may not be achievable in all environmental matrices. The MDL must be performed annually.

12.2 Demonstration of Capabilities

Every analyst must perform an Initial Demonstration of Capability (IDOC) before performing analyses on any client samples. An IDOC consists of 4 consecutive LCS samples spiked at 1 to 4 times the RL with an average recovery and RSD within laboratory acceptance limits. An on-going DOC (ODOC) must be performed annually. An ODOC can be 4 consecutive LCSs at mid-level or a passing PT.

12.3 Training Requirements

The analyst must have documented training, including reading of the SOP and source methods, conducted by the department manager, senior chemist, or other analyst with training documentation and a passing DOC.

13.0 POLLUTION CONTROL

It is TestAmerica's policy to evaluate each method and look for opportunities to minimize waste generated (i.e., examine recycling options, ordering chemicals based on quantity needed, preparation of reagents based on anticipated usage and reagent stability). Employees must abide by the policies in the "Waste Management and Pollution Prevention" section of the Corporate Environmental Health and Safety Manual (CW-E-M-001).

14.0 WASTE MANAGEMENT

Waste management practices are conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes are disposed of in an accepted manner. Waste description rules and land disposal restrictions are followed. Waste disposal procedures are incorporated by reference to the laboratory's Waste Disposal SOP (IR-EHS-WASTE). The following waste streams are produced when this method is carried out:

Sulfuric Acid waste - Sulfuric acid waste is generated in the cyanide, fluoride, phenols, phosphorous, thiosulfate, volatiles organic acids, residual chlorine and sulfide tests. The waste is collected in 2.5 gal carboy satellite containers. Waste bulked as H₂SO₄/w RCRA metals.

Base waste - This waste is generated from sample preparation for the sulfide method. The waste is collected in a 2.5 gal carboy satellite container. Waste bulked as NaOH/pyridine.

Unused standards All these departments generate unused and expired standards. If the standard is hazardous and can not be collected with one of the waste streams generated in the method, then analyst and technicians take this standard and place it on the shelves labeled "hazardous waste" in the main waste storage area. The standard will be lab packed (example: mercury standard).

If the standard can be collected in the satellite waste container for one of the waste streams of the method, then pour the standard in the right satellite container, rinse the original container, and collect the rinsate in the satellite container. The original container can be placed in the regular trash. (Example, buffer solutions pH 4)

15.0 REFERENCES / CROSS-REFERENCES

15.1 Method 4500-S²⁻ B, C, D, Standard Methods for the Examination of Water and Waste Water, 20th Edition.

Part B – Separation of Soluble and Insoluble Sulfides (flocculation)

Part C – Sample Pretreatment to remove interferences or to concentrate

Part D – Methylene Blue Method

15.2 Method 4500-S²⁻ H, "Calculation of Un-ionized Hydrogen Sulfide," Standard Methods for the Examination of Water and Waste Water, 21st Edition.

15.3 CA-Q-S-005, Calibration Curves (General)

15.4 IR-QA-MDL, Determination of Method Detection Limits

16.0 METHOD MODIFICATIONS

Item	Method	Modification
1	4500-S ²⁻ C	All samples are pre-treated (by precipitating the sulfide present with zinc acetate, decanting the supernatant, and replacing it with an equal volume of DI water) rather than only samples that contain strong reducing agents (sulfite, thiosulfate, iodide, etc.)
2	4500-S ²⁻ H	The calculation for unionized hydrogen sulfide is performed automatically in LIMS and is based only on freshwater samples (Ionic strength ≤ 0.1M)

17.0 ATTACHMENTS

17.1 **Attachment 1:** Analysis Information

17.2 **Attachment 2:** Logarithm of Practical Ionization Constant for H₂S

17.3 **Attachment 3:** Data Review Checklist

17.4 **Attachment 4:** Total/Dissolved Sulfides logbook (flocculation and pH)

17.5 **Attachment 5:** Standardization and Calibration Standard Spreadsheet

18.0 REVISION HISTORY

18.1 **Revision 0, dated 15 February 2008**

- Integration for TestAmerica and STL operations
- Changed un-ionized sulfide procedure to use formula instead of chart
- This revision supersedes 4500_S.SOP, revision 2 (01/16/03)

18.2 **Revision 1, dated 12 March 2010**

- Renamed IR-WET-SULFIDE_SPEC; supersedes IR-WET-376, revision 0, 02/15/08
- Removed reference to EPA 376.2 per the 04/11/07 EPA Method Update Rule (MUR)
- Addition of Safety sections 5.1, 5.2, and table in 5.2.
- Addition of Pollution Control wording.
- Addition of Waste Management wording
- Additional information on the calculation of the sulfide concentration in the samples.
- Revision of Calibration Standards
- Revision of Demonstration of Capabilities
- Addition of Data Review Checklist
- Revised by RW and LH

18.3 **Revision 2, dated 31 October 2012**

- This revision supersedes IR-WET-SULFIDE_SPEC, revision 1 (03/12/10) and IR-WET-SULFIDE_SPEC-CF1 (03/18/11)
- Added Total/Dissolved Sulfides logbook (flocculation and pH).
- Added Standardization and Calibration Standard Spreadsheet
- Specified the volumetric limitations of 40 mL VOA vials
- Clarified that dissolved sulfide, once prepared, is analyzed in the same manner as total sulfide
- Added the preparation of batch QA to both total sulfide and dissolved sulfide procedures
- Replaced unionized sulfide calculation from 4500 S²⁻ F to 4500 S²⁻ H
- Added reference to parts B, C, D and H in Scope and Application section
- Revised Data Review Checklist
- Revised by RW, DD and LH

Attachment 1
Analysis Information

TestAmerica Irvine							08/28/2012	
Analytical Method Information								
Analyte	MDL	Reporting Limit	Surrogate %R	Duplicate RPD	Matrix Spike %R	Matrix Spike RPD	Blank Spike / LCS %R RPD	
Sulfide, in Water (SM 4500-S² D)								
Preservation: 4 C, NaOH + Zn Acetate								
Container: 500 mL Poly								
Amount Required: 100 ml								
Hold Time: 7 days								
Sulfide	0.020	0.10 mg/l			70 - 130	30	80 - 120	20
Sulfide, in Soil (SM 4500-S² D Mod.)								
Preservation: 4 C, Cool								
Container: 4 oz Jar								
Amount Required: 50 grams								
Hold Time: 7 days from prep								
Sulfide	0.20	1.0 mg/kg			70 - 130	30	80 - 120	20

TestAmerica Irvine							08/28/2012	
Analytical Method Information								
Analyte	MDL	Reporting Limit	Surrogate %R	Duplicate RPD	Matrix Spike %R	Matrix Spike RPD	Blank Spike / LCS %R RPD	
Hydrogen Sulfide (un-ionized) in Water (SM 4500-S² H)								
Preservation: None								
Container: NA								
Amount Required: NA								
Hold Time: 7 days								
Sulfide as H ₂ S	0.024	0.10 mg/l			70 - 130	30	80 - 120	20
Hydrogen Sulfide (un-ionized) in Soil (SM 4500-S² H)								
Preservation: None								
Container: NA								
Amount Required: NA								
Hold Time: 7 days								
Sulfide as H ₂ S		1.0 mg/kg			70 - 130	30	80 - 120	20

TestAmerica Irvine							08/28/2012	
Analytical Method Information								
Analyte	MDL	Reporting Limit	Surrogate %R	Duplicate RPD	Matrix Spike %R	Matrix Spike RPD	Blank Spike / LCS %R RPD	
Sulfide, Dissolved in Water (SM 4500-S² D)								
Preservation: 4 C, NaOH								
Container: 500 mL Poly								
Amount Required: 100 ml								
Hold Time: 7 days from floc								
Dissolved Sulfide	0.020	0.10 mg/l			70 - 130	30	80 - 120	20

Attachment 2
Dissociation Constant for H₂S in Fresh Water

TABLE 4500-S²⁻-II. CONDITIONAL FIRST DISSOCIATION CONSTANT OF HYDROGEN SULFIDE, FRESH WATER*

Temperature °C	pK'_{FW} at Given Ionic Strength						
	0.00 mol/L	0.005 mol/L	0.01 mol/L	0.02 mol/L	0.03 mol/L	0.05 mol/L	0.10 mol/L
0	7.36	7.33	7.32	7.30	7.29	7.27	7.24
5	7.28	7.25	7.23	7.22	7.21	7.19	7.16
10	7.20	7.16	7.15	7.13	7.12	7.10	7.07
15	7.12	7.09	7.08	7.06	7.05	7.03	7.00
20	7.05	7.02	7.00	6.99	6.97	6.96	6.92
25	6.98	6.95	6.94	6.92	6.91	6.89	6.86
30	6.92	6.89	6.87	6.86	6.84	6.83	6.79

* Values calculated according to Miller¹.

Attachment 3
Data Review Checklist

SULFIDE DATA REVIEW CHECKLIST
SM 4500-S²

Method: _____ Analysis Date: _____ 2nd Level Reviewer: _____

Prep Batch: _____ Analyst Initials: _____ Review Date: _____

Item to Review	Analyst	2 nd Level	Notes/Specific Criteria
Standard preparation:			
Verification of normality of Iodine solution			
Verification of primary Sulfide stock			
Verification of secondary Sulfide stock			
Sample preservation:			
Total sulfide samples preserved with NaOH and Zinc Acetate			
Dissolved sulfide samples preserved with NaOH			
Sample pH recorded on Total/Dissolved Sulfides logbook			
Dissolved sulfide samples flocculation			
Calibration:			
Instrument set at correct wavelength (625nm)			
Correct number of calibration points used (minimum 5 points) + Blank			
Calibration curve meets acceptance criteria, $r \geq 0.995$			
Second source verification (ICV) analyzed and passes (80-120%)			
No reported results above the high calibration standard			
CCVs every 10 samples/end of run and within limits (80-120%)			
CCBs after every CCV and read less than RL			
Sample Preparation Batch:			
All samples prepared and analyzed within hold time of 7 days			
Method Blank reads < RL			
LCS recovery within limits 80-120%			
MS/MSD within limits 70-130%, RPD 30%			
Batch contains no greater than 20 samples			
Data Documentation:			
Copy of prepared logbook			
All standards used are uniquely identified and are not expired			
All errors are crossed out with a single line, initialed and dated			
All flags correctly applied and NCMs written, as required			
The analyst's signature/initials are on the print-out			
Unused data is clearly identified			
All dilution factors applied correctly and entered into LIMS			
Unusual analytical conditions documented in the logbook			
All initial and final results from print-out match LIMS entries			

Attachment 4
Total / Dissolved Sulfides Logbook

TOTAL / DISSOLVED SULFIDES

EPA 9030 & SM4500-S2-B,C,D

Analyst: _____
Date: _____

Dissolved Sulfides					Total Sulfides		
Batch#:					Batch#:		
Bottle	Sample	pH	Flocculation		Bottle	Sample	pH
			Date	Time			

Attachment 5
Standardization and Standard Prep Spreadsheet

TOTAL / DISSOLVED SULFIDES
 EPA 9030/9034 & SM 4500-S²-F

Analyst / Date: _____
 Reviewed by / Date: _____

Stock Standard IDs:

	Iodine	Na ₂ S ₂ O ₃	Amine Sulfuric Acid	Ammonium Phosphate	Zinc Acetate	NaOH	Fe3Cl	Na ₂ S (1 ^o source)	Na ₂ S (2 ^o source)
Concentration:	0.025N	0.025N				6N		N/A	N/A
Standard ID:	IT10368	IU01276	IT06880	IT10370	IT08402	IT09074	IT08284	7110688	8020517

Titration of Iodine solution:

Na ₂ S ₂ O ₃		Iodine	
Vol (ml)	Conc (N)	Vol (ml)	Conc (N)
	0.025		

Titration of Intermediate Sulfide solutions:

Na ₂ S solutions		Iodine		Na ₂ S ₂ O ₃		Na ₂ S	
Vol (ml)	Conc (N)	Vol (ml)	Conc (N)	Vol (ml)	Conc (N)	Vol (ml)	Conc (mg/L)
Cal / LCS /CCV		10	0.0250		0.025		
ICV (2 ^o source)		10	0.0250		0.025		

Concentration of Working Sulfide solution:

Standard Name	Nominal Conc (mg/L)	Stock Source	Vol Req'd for Exact Conc (uL)	Actual Vol of Stock (uL)	Actual Conc (mg/L)
Std 1	0.05	Primary			
Std 2	0.1	Primary			
Std 3	0.3	Primary			
Std 4	0.5	Primary			
Std 5	0.8	Primary			
Std 6	1	Primary			
ICV	0.3	Secondary			
LCS*	0.5	Primary			
MS/MSD*	0.5	Primary			

*40 mL volume