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Hach Method 8131 – Sulfide Check (Screen)

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1. Scope and Application

1.1. The sulfide test is based on the ability of hydrogen sulfide and acid-soluble metallic sulfides to convert N, N-dimethyl-p-phenylenediamine oxalate directly to methylene blue. The intensity of the methlylene color developed is directly proportional to the amount of sulfide present in the original sample. The absorbance is read at 664nm. Method is modified using Hach reagents.

1.2. Restricted Procedure

1.2.1. This procedure is restricted to use by an analyst experienced in the operation of a HACH DR/4000 Spectrophotometer. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method (See SOP reference 13.4).

2. Definitions

2.1. Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

2.2. Primary Source (PS) – A standard that is used to make up the calibration points of a curve.

2.3. Second Source (SS) – A standard made from a manufacturer other than that of the primary source.

2.4. Initial Calibration Verification (ICV) – An ICV is a second source standard that is used to verify the correctness of the primary source calibration curve. The ICV is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.5. Continuing Calibration Check (CCC) or Continuing Calibration Verification (CCV) – A standard used to verify that the response of the instrument has not changed since initial calibration. The CCC is run at a level equal to that the midpoint on the calibration curve.

2.6. Calibration Blank (CB), Initial Calibration Verification Blank (ICB), Method Blank (MBLK), MDLB or Continuing Calibration Blank (CCB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

3. Interferences

3.1. Color and turbidity may interfere with observations of color or with photometric readings.

3.2. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide to an unmeasurable form.

4. Safety

4.1. Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

5. Apparatus and Equipment

5.1. 250 ml plastic bottles

5.2. HACH DR 4000 Spectrophotometer (SPEC01)

5.3. Volumetric flask: various sizes

5.4. Volumetric pipette: various sizes

5.5. Micro pipettes: various sizes

5.6. 10 mm path length, 80 µl, glass flow cell

5.7. DR/4000 1 –Inch Cell Adapter

5.8. 25 ml sample cell

5.9. Stoppers

6. Reagents

6.1. Reagent Water

6.1.1. Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest (Deionized, HPLC, Milli-Q water, or equivalent. Milli-Q water has a resistivity of 18.2[MΩ.cm] @ 25°C and a TOC of 50 ug/L or less).

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6.3. Hach Sulfide Reagent 1

6.3.1. Hach Item #181632

6.4. Hach Sulfide Reagent 2

6.4.1. Hach Item #181732

6.5. Sodium Sulfide Stock Solution(1000 mg/L S^{-2})(PS) Primary Source

6.5.1. This standard is purchased from a commercially available source.

6.6. Sodium Sulfide Intermediate Solution(1000 ug/L S^{-2})

In a 1L volumetric flask, add 1.0 ml of Sodium Sulfide Stock Solution (1000 mg/L S^{-2}) and dilute to volume with reagent water. Invert to mix. Prepare standard fresh daily.

6.7. 0.25 N NaOH Solution

6.7.1. Dissolve 5g NaOH in reagent water and bring to volume in a 500 ml volumetric flask. Prepare fresh weekly.

6.8. Calibration Standards

6.9. Prepare working standards from Sodium Sulfide as shown in chart. The calibration standards range from 0.00 ug/L – 25 ug/L. After the standards are brought to volume, 1 ml of 0.25 N NaOH (See SOP section 6.6) per 200 ml of standard is added. Prepare standards fresh daily.

Table 6.9.1.- Working Standards

Sodium Sulfide Intermediate Solution (ml)	Final volume in ml	Concentration S^{-2} (ug/L)	0.25 N NaOH Solution (ml)
0.5	250	2.0	1.25
1.0	200	5.0	1.0
2.0	200	10.0	1.0
3.0	200	15.0	1.0
4.0	200	20.0	1.0
5.0	200	25.0	1.0

6.10. CB/ICB/MBLK/CCB

6.11. Prepared by adding 1 ml of 0.25N NaOH per 200 ml of reagent water. Adjust 0.25N NaOH accordingly.

6.12. Prepare fresh daily.

6.13. Volumes and amounts of reagents, chemicals, and standards may be altered as long as final concentrations remain the same. Sample volumes and injection amounts may be altered as long as required detection limits can be met and sample/reagent ratios remain the same.

6.14. ICV Sulfide Stock Solution (SS Second Source)

6.15. ICV Sulfide Stock Solution (1000 mg/L S^{-2})

6.16. The ICV stock standard is typically a standard intended as a "QC Sample" but used as a second source standard instead.

6.15.1 This stock standard must be from a different source than the stock standard used to make the calibration standards.

6.16.2. This standard is purchased from a commercially available source. The purchased standard is stable until opened. New stock is purchased for each calibration.

6.17. ICV Intermediate Sulfide Solution (1000 ug/L S^{-2})

6.17.1. A 1 ml aliquot of the ICV Sulfide Stock Solution (see SOP section 6.13.) is pipetted into a 1L volumetric flask and diluted to volume with reagent water. After the ICV is brought to volume, add 5 ml of 0.25N NaOH (See SOP section 6.6).

6.17.2. The ICV solution must be prepared fresh daily.

6.18. ICV 15.0 ug/L Solution

6.18.1. A 3 ml aliquot of the ICV Intermediate Sulfide Solution (see SOP section 6.16.) is pipetted into a 200 ml volumetric flask and diluted to volume with reagent water. After the ICV is brought to volume, add 1 ml of 0.25N NaOH (see SOP section 6.6).

6.18.2. This solution must be prepared fresh daily.

7. Sample Collection

7.1. Samples are collected in a 250 ml plastic bottle.

7.2. Sample bottle should be completely full with no head space.

7.3. Chemical preservation is not required.

7.4. Analyze sample as soon as possible.

7.5. Sample temperature should be maintained at 0- 6°C (not frozen) immediately after collection and until analysis.

8. Calibration

8.1. Calibration Standards

8.1.1. A commercially prepared stock standard is purchased. Once opened it must be re-standardized by the iodometric method. This stock is then used to make six

calibration standards. The standards are used to make a calibration curve by correlating absorbance of each standard vs. concentration. The correlation coefficient must be at least 0.995 using a linear regression. An alternate source standard, where available, should be used to verify initial calibration of the measurement system.

8.2. Calibration Curve

8.2.1. The HACH DR 4000 Sulfide curve is calibrated every six months or when the second source calibration verification fails. Minimum acceptable correlation is 0.995 using a linear regression.

8.3. Calibration Verification

8.3.1. An Initial Calibration Verification standard (ICV) and an Initial Calibration Blank (ICB) must be analyzed immediately after the calibration standards. The ICV standard must be prepared with a stock from a different source than the standards used in the calibration of the instrument. The ICV value must be within 10% of its true value and the ICB value must be less than the method RL or the run will have to be repeated.

8.3.1.1. The ICV is only analyzed after the initial calibration. A daily ICV is not required for the sulfide check due to the short holding time of the standards.

8.3.2. A Continuing Calibration Check (CCC) and a Continuing Calibration Blank (CCB) must be analyzed after the initial calibration of the instrument. The CCC must be within 10% of its expected value and the CCB must be less than the RL. The CCC may come from the same source as the calibration standards. If the CCC does not meet acceptance criteria, then all samples affected by the out of control CCC are to be rerun.

8.3.2.1. Since the method is only used as a check, a CCC is only analyzed after the initial calibration. It is not analyzed daily due to the short holding time of the standards.

9. Quality Control

9.1. Refer to Table 14.1 for Reporting Limits (RLs), Table 14.2 for Quality Control Acceptance Criteria, and Table 14.3 for Quality Control Procedures associated with this method.

10. Procedure

10.1. HACH Calibration Procedure

10.1.1. Turn on instrument and allow the system to go through diagnostic check. Let the instrument warm up for 30 minutes before performing analysis.

10.1.2. Select “User methods” type in 1 and select the edit tab. Scroll through method and locate the calibration table line. Select the edit tab. The previous calibration standards and absorbance will be displayed.

10.1.3. Delete all standards and corresponding absorbance values.

10.1.4. Type in each standard and hit the enter button after each entry.

10.1.5. Select the entry-done tab.

10.1.6. Measure 25 ml of each standard into each sample cell.

10.1.7. Using the dispenser provided, add 1ml of Reagent 1 to each of the sample cells. Swirl to mix.

10.1.8. Using the dispenser provided, add 1ml of Reagent 2 to each of the sample cells. Swirl to mix.

10.1.9. Press the "start timer" tab on instrument.

10.1.10. Once the 5 minutes has elapsed, place the 0 µg/L standard into the sample cell labeled "A", then place in the instrument compartment. Close lid and press the zero.

10.1.11. Press the "Read" tab when the absorbance is stable, this will lock in the 0 µg/L standard. Remove standard, pour back into original sample cell, rinse cell labeled "A" with reagent and continue to next step.

10.1.12. Pour the 2 µg/L standard into the sample cell labeled "A", then place in the instrument compartment. Then close lid. Press the "Read" tab when the absorbance is stable. This will lock in the 2 µg/L standard.

10.1.13. Remove standard. Pour back into original sample cell, rinse cell labeled "A" with reagent water and continue to next step.

10.1.14. For the remaining standards repeat step 10.1.12.

10.1.15. Once the last standard is read, a calibration curve will be displayed. Verify that the r (correlation coefficient) value is ≥ 0.995 ($r^2 \geq 0.990$).

10.1.16. Press the "Entry done" tab, this will return the user to the start screen.

10.1.17. Press the "exit" button and save the calibration. The calibration will be good for six months or until the QC fails to meet requirements.

10.2. Sample Analysis

10.2.1. Samples and standards should be warmed to room temperature.

10.2.2. Close the lid. Turn on instrument and allow the instrument to go through system diagnostic check. The instrument should be on for more than thirty minutes before analyzing samples.

10.2.3. Select user method one and hit enter. Then select Option, then MORE and change units to ug/L.

10.2.4. Pour 25 ml of reagent water into sample cell (this will be used to zero the instrument), and 25 ml of each sample into other sample cells. Add 0.125 ml of 0.25N NaOH solution to each sample cell and swirl to mix.

10.2.5. Using the dispenser provided, add 1ml of Reagent 1 to each of the sample cells. Swirl to mix.

10.2.6. Using the dispenser provided, add 1 ml of Reagent 2 to each of the sample cells. Swirl to mix.

10.2.7. Press the "start timer" tab.

10.2.8. Once 5 minutes has elapsed, pour the blank (reagent water) into the sample cell labeled "A", then place in the instrument compartment, close lid and press the "zero" tab. Remove blank, pour back into original sample cell, rinse cell labeled "A" with reagent water and proceed to next step.

10.2.9. Pour first sample into the sample cell labeled "A", then place in the instrument compartment and close the lid. When the concentration value stabilizes, press the "read" tab. The value will be printed out. Repeat process for the remaining samples and QC.

11. Calculations

11.1 The weighted external standard calibration is calculated by the instrument software and is documented in the instrument software.

11.2. Percent Drift, %Drift

$$\%\text{Drift} = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.2.1. Where:
Concentration $_{\text{Calculated}}$ = Concentration calculated from result
Concentration $_{\text{Expected}}$ = Theoretical concentration of the standard

11.3. Calculation of Dilution Factors

$$C \times D = F$$

11.3.1 Where:
C = concentration from instrument in ug/L
D = dilution factor, if any
F = final concentration in ug/L

12. Waste Management

12.1. See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, reference 13.4.

13. References

13.1. Plan & Fire Safety Plan, Rev. 2, March 2, 2009 or later. HACH Method 8131, based on the USEPA Methylene Blue Method.

13.2. EPD Laboratory Quality Assurance Plan, online revision.

13.3. GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.

13.4. GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015, online revision.

13.5. GA EPD Laboratory Safety Plan – EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

14. Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Table 14.1 RLs for Method HACH 8131

Parameter/Method	Analyte	Matrix (aqueous)	
		RL	Unit
HACH 8131	Sulfide Check	2	µg/L

Table 14.2. Default QC Limits for HACH Method 8131

QC Type	Analyte	Accuracy (%R)		Precision (%RPD
		LCL	UCL	
LCS/LCSD	Sulfide Check	NA		NA
MS/MSD	Sulfide Check	NA		NA

Note: This method is only used as a check for the presence of Sulfide for Buford Hatchery. No LCS/LCSD or MS/MSD are prepared/analyzed.

Table 14.3 Summary of Calibration and QC Procedures for Method HACH 8131

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
HACH 8131	Sulfide Check	Six point initial calibration	Initial calibration prior to sample analysis	Correlation coefficient ≥ 0.995 linear regression	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per calibration if available	Sulfide Check value must be within 10% of expected value	Correct problem then repeat initial calibration	

Table 14.3 Summary of Calibration and QC Procedures for Method HACH 8131

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
HACH 8131	Sulfide Check	Method Blank (MBLK)	One per batch	Value must be less than 2 µg/L	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to re-analyze, flag with a "B"

Updates to Previous Version:
Updated for online revision.