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Standard Operating Procedure for Determination of Silicate in Fresh, Estuarine, and Coastal Waters Using the Molybdosilicate Method

(Reference Method: SM 4500-SiO2 E-2011)

Document #: NASLDoc-025

Revision 2025-1
Replaces revision 2024-1
Effective May 1, 2025

I attest that I have reviewed this standard operating procedure and agree to comply with all procedures outlined within this document.

Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date
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Employee (Print)	Employee (Signature)	Date

Revised by: ____________________ Date: ____________

Reviewed by: ____________________ Date: ____________

Laboratory Supervisor: ____________________ Date: ____________

Changes affecting Revision 2025-1

- Section 6.1: Added Gallery+ Aqua Master
- Section 7.9: Added wash solution
- Table 2: Added iDOC/DOC
- Section 9.3.5: Added ICV is made from an external source of the calibration standards
- Section 10.2: Added Silicate and Silicate H
- Section 11.1: Added Gallery + Daily Operating Procedure

Determination of Silicate in Fresh, Estuarine, and Coastal Waters
Using the Molybdosilicate Method

1. SCOPE and APPLICATION

1.1. The reaction is based on the reduction of silicomolybdate in acidic solution to heteropoly blue by ascorbic acid. Oxalic acid is added to minimize interference from phosphates. The method is used to analyze all ranges of salinity.

1.2. A Method Detection Limit (MDL) of 0.05 mg Si/L was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.

1.3. The Quantitation Limit/Reporting Limit for Si was set at 0.21 mg Si/L.

1.4. The method is suitable for Si concentrations 0.05 to 10.5 mg Si/L.

1.5. This procedure should be used by analysts experienced in the theory and application of aqueous inorganic analysis. Three months experience with an analyst, experienced in the analysis of silicate in aqueous samples, is required.

1.6. This method can be used for all programs that require analysis of dissolved silicate.

1.7. This procedure references SM 4500-SiO_2 E-2011.

2. SUMMARY

2.1. Filtered samples are mixed with oxalic acid, ammonium molybdate, and sulfuric acid. The resulting silicomolybdate is reduced to heteropoly molybdenum blue by the addition of ascorbic acid. The oxalic acid is added to destroy molybdophosphoric acid formed from phosphorus in the sample.

3. DEFINITIONS

3.1. Acceptance Criteria **-** Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2. Accuracy **-** The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3. Aliquot **-** A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4. Analytical Range – the analytical range is 0.21 to 10.5 mg Si/L. The overall analytical range is comprised of two distinct yet overlapping concentration ranges. A separate calibration is performed for each range. These ranges include 0.21 to 2.1 mg Si/L, and 1.05 to 10.5 mg Si/L. Two ranges are utilized so that samples can be analyzed on the most appropriate scale possible.

3.5. Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6. Blank **-** A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7. Calibrate **-** To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8. Calibration **-** The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9. Calibration Blank – A volume of reagent water fortified with the same matrix as the calibration standards, without analyte added.

3.10. Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.11. Calibration Method **-** A defined technical procedure for performing a calibration. (NELAC)

3.12. Calibration Standard **-** A substance or reference material used to calibrate an instrument. (QAMS)

  3.12.1. Initial Calibration Standards (STD) **-** A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

  3.12.2. Initial Calibration Verification (ICV) **-** An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

  3.12.3. Continuing Calibration Verification (CCV) **-** An individual standard which is analyzed after every 18-23 field sample analysis.

3.13. Certified Reference Material (CRM) **-** A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025).

3.14. Corrective Action **-** Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.15. Deficiency - An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.16. Demonstration of Capability -A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.17. Detection Limit **-** The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.18. Duplicate Analyses **-** The analyses or measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage external to the laboratory (EPA-QAD)

3.19. External Standard (ES) - A pure analyte (Sodium silicofluoride (Na_2SiF_6)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.20. 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, preservation and storage, as well as with laboratory procedures.

3.21. Holding Time **-** The maximum time which samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.22. Laboratory Duplicates (LD1 and LD2) - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.23. Laboratory Reagent Blank (LRB) - A matrix blank that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.24. Laboratory Control Sample (LCS) **-** A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standards or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.25. Limit of Detection (LOD) -The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. Also referred to as MDL. (ACS)

3.26. Limit of Quantitation (LOQ) - The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired. Also referred to as Quantitation Limit.

3.27. Linear Dynamic Range (LDR) - The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.28. 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.

3.29. May **-** Denotes permitted action, but not required action. (NELAC)

3.30. Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Standard Methods).

3.31. Must **-** Denotes a requirement that must be met. (Random House College Dictionary)

3.32. Photometer **-** measures the absorbance of the solution in the cell in a multicell cuvette. Light passes from the lamp through the condensing lenses to the interference filter. The plane surface of the first condensing lens is coated with a material which reflects heat and infrared light. The filters are mounted on a filter wheel. There are 15 positions for filters. Each filter corresponds to a wavelength of interest. The 660 nm filter is specified by the test definition for silicate. After passing through the filter the light is converted into a stream of light pulses by a chopper. Then the light is directed via a quartz fiber through a focusing lens and a slit to the beam divider. The beam divider divides the light into two parts. A specified portion is reflected to the reference detector, which monitors the light level fluctuations. The remaining major portion of the light beam goes through the liquid in the cell to the signal detector, which measures the amount of light absorbed.

3.33. Precision **-** The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.34. Preservation – Refrigeration, freezing and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.35. Quality Control Sample (QCS) -A sample of analyte of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also referred to as CRM.

3.36. Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.37. Sample Segment – Bar-coded metal tray that holds up to fourteen four milliliter auto analyzer vials containing samples or standards. The user identifies each vial in the operating software.

3.38. Sample Segment Holder – An automated temperature-controlled carousel that contains up to six sample segments. This carousel spins in clockwise or counterclockwise manner to move the sample segments into position for analysis. This carousel format allows for continuous processing.

3.39. Sensitivity **-** The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.40. Shall **-** Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.41. Should **-** Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.42. Standard Reference Material (SRM) - Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also referred to as CRM.

3.43. Test Definition – A photometric test consisting of a user defined testing sequence, reagent additions, calibration standards, incubations and absorption results.

3.44. Test Flow – Functions to define the parameter for reagent and sample dispensing, dilution, incubation and measurement

4. INTERFERENCES

4.1. Because both apparatus and reagents may contribute silica, avoid using glassware as much as possible and use reagents low in silica. Phosphate interference can be eliminated by the addition of oxalic acid.

4.2. Suspended matter in the sample will scatter light as it passes through the cuvette to the detector. High blank responses will result. The identified sample will be reanalyzed.

4.3. Blemishes in the cuvette, as result of the manufacturing process, will result in high blank responses. The identified sample will be reanalyzed.

5. SAFETY

5.1. Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes must always be worn. In certain situations, it may also be necessary to use gloves and/or face shield. If solutions or chemicals come in contact with eyes, flush with water continuously for 15 minutes. If solutions or chemicals come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the Chesapeake Biological Laboratory (CBL) Associate Director of Administration and Facilities Maintenance of the incident. Contact the CBL Associate Director of Administration and Facilities Maintenance if additional treatment is required.

5.2. The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials and procedures.

5.3. Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4. The following hazard classifications are listed for the chemicals regularly used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS)

Table 1

<table>
<tr><th>Chemical</th><th>Health Hazard</th><th>Fire Hazard</th><th>Instability Hazard</th><th>Specific Hazard</th><th></th></tr>
<tr><td>Sulfuric acid</td><td>4</td><td>0</td><td>2</td><td>ACID,COR</td><td></td></tr>
<tr><td>Oxalic acid</td><td>3</td><td>0</td><td>0</td><td>ACID,COR</td><td></td></tr>
<tr><td>Ascorbic acid</td><td>1</td><td>0</td><td>0</td><td>ACID</td><td></td></tr>
<tr><td>Ammonium molybdate</td><td>2</td><td>0</td><td>0</td><td>IRRITANT</td><td></td></tr>
<tr><td>Sodium silicofluoride</td><td>2</td><td>0</td><td>0</td><td>IRRITANT</td><td></td></tr>
<tr><td>Bleach</td><td>3</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td colspan="6">On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)</td></tr>
<tr><td colspan="6">HAZARD RATING

Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY</td></tr>
</table>

6. EQUIPMENT AND SUPPLIES

6.1. Aquakem 250 or Gallery+ Aqua Master multi-wavelength automated discrete photometric analyzer. Control software operates on a computer running Microsoft Windows 7 or newer operating system.

6.2. Freezer, capable of maintaining -20 ± 5° C.

6.3. Refrigerator, capable of maintaining 4 +/- 2ºC.

6.4. Lab ware – All reusable lab ware (glass, Teflon, plastic, etc.) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% HCl (v/v) acid rinse, followed by 4-6 reagent water rinses. This laboratory cleans all lab ware that has held solutions containing ammonium molybdate with 10% NaOH (w/v) rinse.

7. REAGENTS AND STANDARDS

7.1. Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2. Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.

7.3. Oxalic Acid Solution -

Oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)	100g
Reagent water	up to 1000mL

In a 1000mL plastic volumetric flask, dissolve 100g of oxalic acid in ~400mL reagent water and dilute to 1000mL with reagent water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot number and balance ID number in the Analytical Reagent log book. Store the flask at room temperature in the dark and make every 12 months.

7.4. Ascorbic Acid Solution -

Oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)	1.25g
Ascorbic acid ($C_6H_8O_6$), U.S.P. quality	25g
Reagent water	up to 250mL

In a 250mL plastic volumetric flask, dissolve 1.25g of oxalic acid in ~100mL of reagent water. Add 25g of ascorbic acid and mix until dissolved. Dilute to 250mL with reagent water. Write name of preparer, preparation date, reagent manufacturers, manufacturers' lot numbers and balance ID number in the Analytical Reagent log book. Store at 4ºC and make every 4 months.

7.5. Ammonium Molybdate Solution -

Ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$]	3.0g
Reagent water	up to 100mL

In a 100mL plastic volumetric flask, dissolve 3.0g ammonium molybdate in ~80mL of reagent water. Dilute to 100mL with reagent water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot number and balance ID number in the Analytical Reagent log book. Store in the dark at room temperature. Reagent is stable for 48 hours.

7.6. Sulfuric Acid Solution, 0.7 N –

Sulfuric acid (H_2SO_4), concentrated (sp. Gr. 1.84)	4.0mL
Reagent water	up to 1000mL

In a 1000mL plastic volumetric flask, add 4.0 mL of concentrated sulfuric acid to ~300mL of reagent water. Dilute to 1000mL with reagent water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot number in the Analytical Reagent log book. Store at 4°C and make every 6 months.

7.7. Stock Silicate Standard, 10,000uM

Sodium silicofluoride (Na_2SiF_6), dried at 45°C	1.88g
Reagent water	up to 1000mL

In a 1000mL plastic volumetric flask, dissolve 1.88g of sodium silicofluoride in ~400mL of reagent water. Dilute to 1000mL with reagent water (1ml contains 10umoles Si). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number and balance ID number in the Analytical Standard log book. Store in a plastic container at room temperature. Make fresh every 6 months.

7.9 Cleaning/Wash Solution –

Bleach	55.0 mL

In a 100 mL volumetric flask, dilute 55.0 mL of bleach to volume with 45 mL reagent water to yield a concentration of 55% bleach. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Reagent is stable for 6 months.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Water collected for Si should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2. Water collected for Si should be refrigerated at 4° C. The sample container should be clean and sample rinsed.

8.3. Refrigerated Si samples may be stored longer than 28 days. It has been shown that refrigerated QCS samples up to a year old still fall well within the control limits.

9. QUALITY CONTROL

9.1. The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks field duplicates, and calibration standards analyzed as samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2. Initial Demonstration of Capability

9.2.1. The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2. Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDLs.

9.2.3 Method Detection Limits (MDLs) – Initial MDLs should be established for Silicate using a spiked water sample, typically two to 10 times the estimated MDL and no more than ten times higher than the estimated MDL. Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDLs must include data/calculations from all instruments.

9.2.3.1 Make all computations as specified in section 12 of the analytical method and express the final results in the method-specified reporting units.

9.2.3.2 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

9.2.3.3 Compute the MDLs (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\propto=0.99)} S_S$$

Where:

MDLs = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student’s t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.

S_S = sample standard deviation of the replicate spiked sample analyses.

9.2.3.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDLb does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of “ND” (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDLb equal to the highest method blank result. If more than 100 method blanks are available, set

MDLb to the level that is no less than the 99^{th} percentile of the method blank results. For “n” method blanks where $n \geq 100$, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result.

Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

If all of the method blanks for an individual analyte give numerical results, then
calculate the MDLb as:

$$MDL_b = \overline{X} + t(n-1, 1-\propto=0.99)S_b$$

Where:
MDL_b = the MDL based on method blanks
$\overline{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)
$t_{(n-1,\ 1-\alpha = 0.99)}$ = the Student’s t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_b = sample standard deviation of the replicate method blank sample analyses.

9.2.3.5 The verified MDL is the greater of the MDLs or MDLb. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

9.2.3.6 MDLs should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

9.3. Assessing Laboratory Performance

9.3.1. Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of reagent water treated the same as the samples. Analyte found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment.

9.3.2. Quality Control Sample (QCS)/ Standard Reference Material (SRM)- When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3s of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3. The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4. Control Charts – The Accuracy Control Chart for QCS/SRM samples and reagent blanks is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). These values are derived from stated values of the QCS/SRM. The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter QCS/SRM results on the chart each time the sample is analyzed.

9.3.5. Calibration Verification, Initial and Continuing (ICV/CCV)– Immediately following calibration (ICV) and following every 10 samples (CCV), one calibration verification of 50 µM Si/L (1.4 mg Si/L) for SILCBL/ Silicate, 250 µM Si/L (7.0 mg Si/L) for SILCBLHI/Silicate H is analyzed to assess instrument performance. The ICV is made from an external source of the calibration standards. The CCVs are made from the same material as calibration standards (Na_2SiF_6), and are to be within ± 3*s*. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported.

9.4. Assessing Analyte Recovery

9.4.1. Analyte recovery is assessed through percent recoveries of laboratory spikes.

9.4.2 Percent Recovery = ((Actual value-Original value)/(Spike Concentration added)) X 100. Percent Recovery for each spiked sample should fall within 90-110%.

9.5. Assessing Analyte Precision – Relative Percent Difference

9.5.1. Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference.

9.5.2. RPD = (Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 100

9.6. Corrective Actions for Out-Of-Control Data

9.6.1. Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

9.6.2. Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

9.6.3. Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.

9.6.4. When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.6.5. When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7. General Operation **-** To assure optimal operation and analytical results, the Reagent Blank and CCV are tracked daily in the raw data file, copied to Reagent Blank and CCV Control Charts.

Table 2:

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Coefficient of determination	≥ 0.995	If <0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	± 10%	If QCS value is outside ± 10% of the target value reject the run, correct the problem and rerun samples.	Beginning of run and every 20 samples.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Continuing Calibration Verification (CCV)	± 10%	If outside ±10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 10 samples.
Method Blank/Laboratory Reagent Blank (LRB)	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV and after every 10 samples following the CCV.

Laboratory Fortified Sample Matrix Spike	± 10%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a “matrix induced bias” qualifier.	1/20
Laboratory Duplicate	± 10%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	1/20
iDOC/DOC Mean Percent Recovery	± 10%	If outside of range, correct the problem and repeat. Cannot analyze samples until passing.	Annual
iDOC/DOC Standard Deviation	≥2	If outside of range, correct the problem and repeat. Cannot analyze samples until passing.	Annual

10. CALIBRATION AND STANDARDIZATION

10.1. Calibration - Daily calibration must be performed before sample analysis may begin. Five-point calibrations are used with each of the two sub- calibrations that cover the analytical range. Two working silicate standards are used to produce the calibrators for each set of two calibration curves. The instrument performs serial dilutions of working standards to produce the five calibrators defined for each curve. The following outlines the preparation of the working standards and the following table describes the subsequent serial dilutions the instrument performs to make each standard for each of the two calibration curves.

10.2. **Silicate Working Standards-**

Si (SILCBL/Silicate)

Working Standard	2.1 mg Si/L	(0.75 mL stock standard to 100 mL)
Working CCV	1.4 mg Si/L	(0.5 mL stock standard to 100 mL)

High Si (SILCBLHI/Silicate H)

Working Standard	10.5 mg Si/L	(3.75 mL stock standard to 100 mL)
Working CCV	7.0 mg Si/L	(2.5 mL stock standard to 100 mL)

Write name of preparer, preparation date, Stock Standard preparation date in the Analytical Standard log book. Make fresh every month.

Silicate Calibrators:

Test Name	Working Standard	Dilution Factor	Concentration mg Si/L
SILCBL/Silicate	2.1 mg Si/L	1+9	0.21
	2.1 mg Si/L	1+4	0.42
	2.1 mg Si/L	1+2	0.70
	2.1 mg Si/L	1+1	1.05
	2.1 mg Si/L	1+0	2.10
SILCBLHI/SilicateH	10.5 mg Si/L	1+9	1.05
	10.5 mg Si/L	1+4	2.10
	10.5 mg Si/L	1+2	3.50
	10.5 mg Si/L	1+1	5.25
	10.5 mg Si/L	1+0	10.5

The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met the entire curve can be reanalyzed or individual standards can be reanalyzed. One standard value (original or reanalyzed) for each and every standard is incorporated in the curve. The coefficient of determination (Pearson's r value) for the calibration curve as well as the calculated concentration of each calibrator is reviewed. The calculated value of each calibrator must be within ten percent of the expected value. The coefficient of determination (Pearson's r value) for the calibration curve must be greater than or equal to 0.995.

11. PROCEDURE –

11.1. GALLERY PLUS DAILY OPERATIONS AND QUALITY CONTROL

11.1.1. Turn on the computer. Enter Windows user name and password. Open Gallery Discrete Analyzer software and enter software username and password. Once software is running, turn on instrument and allow connection between instrument and computer to complete.

11.1.2. Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh reagent water. Connect the water line to the reservoir and slide the reservoir into the right slot at the bottom of the instrument.

11.1.3. Remove from refrigerator samples and SRM that will be analyzed that day. Begin daily bench sheet documentation.

11.1.4. Place cuvette waste container into the center slot at the bottom of the instrument. Connect the waste line and insert the waste container into the left slot at the bottom of the instrument.

11.1.5. In the instrument software select F5 tab, 2 Actions, and Extra Wash. – complete at least five extra wash cycles.

11.1.6. Perform Start Up operations by selecting Start Up at the bottom of the F1 Home Page.

11.1.7. Gather working standards and reagents from their appropriate storage area during startup. Assess standards and reagents. Remake anything that has exceeded the time over which it is considered stable.

11.1.8. Once startup is complete, check the instrument water blanks by selecting F5 tab, 6 Water Blank Results. If any of the instrument blanks are outside their predefined and software-controlled limits, the user will be notified on the Home page. User takes corrective action to return instrument functions to controlled limits.

11.1.9. Load reagents, select F3 tab, 2 Reagents, select an open box, insert new vial, select the appropriate reagent from list. Load reagents into reagent carousel inside the refrigerated reagent compartment located under the right panel.

11.1.10. Load working standards and quality control standards into a sample segment. Identify the standards in their positions by selecting F2 tab, 3 racks, select rack, Add sample, select calibrator and controls, select the corresponding calibrator and control ID. Insert the rack into the sample carousel located under the left side panel.

11.1.11. Select the methods to be calibrated by selecting F4 tab, 1 Cal/QC selection, select calibration tab. Select Silicate and Silicate H as the two methods to be calibrated, confirm that the appropriate calibration standards, check standards, and QC standards are loaded for each calibration. After selecting each method select Calibrate at the bottom of the page. The two methods will now show as pending. Return to the main page.

11.1.12. Begin calibration from the F1 home page, select Start, Begin Calibration. See test flow below for stepwise instrument functions for the analysis of standards and samples.

Test Flow – Method of Analysis, Stepwise

- 100 µL SAMPLE to cuvette
- End point absorbance measurement at 660 nm for sample blank determination
- 31 µL sulfuric acid solution (H2S Si) to cuvette with mixing
- 39 µL ammonium molybdate solution (MOL Si) reagent to cuvette with mixing
- Incubation, 30 seconds
- 62 µL oxalic acid solution (OXA Si) to cuvette with mixing
- Incubation, 30 seconds
- 16 µL ascorbic acid solution (ASC Si) to cuvette with mixing
- Incubation, 600 seconds
- End point absorbance measurement, 660 nm

- Software processes absorbance value and uses calibration curve to calculate analyte concentration (mg/L of Si)
- User is notified if any measured values used to calculate final concentration are outside preset limits. If so, user has options to accept result, rerun the sample or rerun the sample diluted to a user or software specified factor.

11.1.13. Organize samples and reagent blanks while instrument performs calibrations.

11.1.14. As calibration curves are produced by the instrument, review them for acceptability. The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met, either the entire curve shall be reanalyzed or individual standards shall be reanalyzed, depending on the violation. One standard value (original or reanalyzed) for each and every calibrator is incorporated in the curve. Select F4 tab, 3 Calibration Results, Accept, Rerun, or Reject as needed.

11.1.15. Once calibration curves are accepted, samples are loaded into the sample segments and segments are loaded into the instrument for analysis. Load samples select F2 tab, 3 Racks, select segment, Add sample, Fill Rack, select sample, enter sample ID.

11.1.16. Analysis can be requested two ways. Add analysis requests to racks, select F2 tab, 3 Racks, Request to rack, select test from Bi-Z list, select Save. OR. Add analysis request to individual samples, select F2 tab, 1 samples, select sample from list, select test from Bi-Z list, select Save.

11.1.17. Quality Control samples are pre-programmed in the method to be analyzed, following Table 2. The first samples analyzed should be ICV (initial calibration verification) samples. There should be one sample for each calibration curve, of a concentration close to the middle of each range. The following are ICV samples for each curve: CRM 1.0 mg Si/L for Silicate, and 7.0 mg Si/L for Silicate H. CCV (Continuing Calibration Verification) samples (one for each of the two calibration ranges) follow every 10 samples. Standard Reference Material (SRM) samples as well as Laboratory Reagent Blanks (LRB) are analyzed after every 10 samples, following CCV. Throughout the analytical batch, samples are chosen as laboratory duplicates and laboratory spikes to assess analyte precision and analyte recovery, respectively. The total number of duplicates and spikes performed will be equal to or greater than five percent of the total number of samples in the analytical batch.

11.1.18. As sample analysis is complete, results must be reviewed and accepted manually. If results fall outside acceptance limits, the sample should be reanalyzed. If sample result exceeds the highest standard of the highest calibration range, the samples can be automatically diluted by the instrument and reanalyzed. If the result is such that it will fall within a higher calibration range, it should be reanalyzed in the higher range. If the result is such that it will fall within a lower calibration range, it should be reanalyzed within the lower range. Result can be accessed by selecting F2 tab, 4 Results, Accept, Reject, and Rerun as needed.

11.1.19. Upon completion of all analysis, results should be saved to a daily report file. Select F2 tab, 5 Reports, All results to file. The file is then named by the run date. The daily report file for analytical batch of January 2, 2017 would be named 010217. The file is converted to Microsoft Excel for data work up and copied to a removable flash drive. Remaining samples are discarded.

11.1.20. All reagents are removed from the reagent chamber and returned to their appropriate storage areas. Reagents that have exceeded their stability period are discarded.

11.1.21. Initiate the shutdown procedure. Insert wash solution, select F2 tab, 3 racks, select rack, Add sample, select controls, select WashSol. Clear Daily Files, select F5 tab, 2 Actions, select Clear Daily Files. Initiate Standby from the home tab F1 select standby at the bottom of the screen. Once Standby is complete, remove the Wash Solution from the instrument. Exit the software, select F5 tab, 2 Actions, Exit, select Yes. Turn off instrument and computer.

11.1.22. The waste is flushed down the drain with copious amounts of tap water. The waste cuvette box is moved to the fume hood and covered. The instrument is wiped clean of drips or splashes.

11.2. AQUAKEM 250 DAILY OPERATIONS AND QUALITY CONTROL

11.2.1. Turn on the computer. Computer will automatically initiate Konelab software. Once software is running, turn on instrument and allow connection between instrument and computer to complete.

11.2.2. Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh reagent water.

11.2.3. Remove from refrigerator samples and SRM that will be analyzed that day. Begin daily bench sheet documentation.

11.2.4. Place cuvette waste box into cuvette waste sliding drawer.

11.2.5. Once water reservoir is full, use instrument software to click More, Instrument Actions, and Perform Water Wash. – complete at least five water wash cycles

11.2.6. After performing water washes, clean the dispensing needle by performing test washes. Click More, Instrument Actions, More, Adjustment Program. Once in the Adjustment Program click, 4-Dispensing Unit, 1-Dispenser, 8-Test Wash. Perform 8 to 10 Test Washing. When complete, press “Q” to quit until you are able to bring up the Main Page.

11.2.7. Perform Start Up operations by clicking Start Up at the bottom of the Main Page.

11.2.8. Gather working standards and reagents from their appropriate storage area during startup. Assess standards and reagents. Remake anything that has exceeded the time over which it is considered stable.

11.2.9. Once startup is complete, check the instrument water blanks by clicking More, Instrument Actions, More, Check Water Blank. If any of the instrument blanks are outside their predefined and software-controlled limits, the user will be notified on the main menu page. User takes corrective action to return instrument functions to controlled limits.

11.2.10. Load reagents into reagent carousel and place into refrigerated reagent compartment. Reagent position can be found by clicking reagents at the top of the main page.

11.2.11. Load working standards into a sample segment, identify the standards in their positions from the drop-down menus at the individual segment positions, and load into instrument. (Click Samples from the top of the Main Page, then click desired segment number).

11.2.12. Select the methods to be calibrated by clicking Calibr. /QC Selection on the bottom of the main page. Click SILCBL and SILCBLHI as the two methods to be calibrated, and then click Calibrate at the bottom of the page. The two methods will now show as pending. Return to the main page.

11.2.13. Begin calibration by clicking Page Up on the keyboard. This may also be a green button on the keyboard – See test flow below for stepwise instrument functions for the analysis of standards and samples.

Test Flow – Method of Analysis, Stepwise

- 100 µL SAMPLE to cuvette
- End point absorbance measurement at 660 nm for sample blank determination
- 31 µL sulfuric acid solution (H2S SILCBL) to cuvette with mixing
- 39 µL ammonium molybdate solution (MOL SILCBL) reagent to cuvette with mixing
- Incubation, 30 seconds
- 62 µL oxalic acid solution (OXA SILCBL) to cuvette with mixing
- Incubation, 30 seconds
- 16 µL ascorbic acid solution (ASC SILCBL) to cuvette with mixing
- Incubation, 600 seconds
- End point absorbance measurement, 660 nm
- Software processes absorbance value and uses calibration curve to calculate analyte concentration (mg/L of Si)
- User is notified if any measured values used to calculate final concentration are outside preset limits. If so, user has options to accept result, rerun the sample or rerun the sample diluted to a user or software specified factor.

11.2.14. Organize samples, reagent blanks, check standards and all quality control samples while instrument performs calibrations.

11.2.15. As calibration curves are produced by the instrument, review them for acceptability. The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met, either the entire curve shall be reanalyzed or individual standards shall be reanalyzed, depending on the violation. One standard value (original or reanalyzed) for each and every calibrator is incorporated in the curve.

11.2.16. Once calibration curves are accepted, samples are loaded into the sample segments and loaded into the instrument for analysis. After the reagent blanks the first samples analyzed should be ICV (initial calibration verification) samples.

There should be one sample for each calibration curve, of a concentration close to the middle of each range. The following are recommended ICV samples for each curve: 1.4 mg Si/L for SILCBL, and 7.0 mg Si/L for SILCBLHI.

11.2.17. Samples are loaded into the segments and analyzed. CCV (Continuing Calibration Verification) samples (one for each of the two calibration ranges) follow every 10 samples. Standard Reference Material (SRM) samples as well as Laboratory Reagent Blanks (LRB) are analyzed after every 10 samples, following CCV. Throughout the analytical batch, samples are chosen as laboratory duplicates and laboratory spikes to assess analyte precision and analyte recovery, respectively. The total number of duplicates and spikes performed will be equal to or greater than ten percent of the total number of samples in the analytical batch.

11.2.18. As sample analysis is complete, results must be reviewed and accepted manually. If results fall outside acceptance limits, the sample should be reanalyzed. If sample result exceeds the highest standard of the highest calibration range, the samples can be automatically diluted by the instrument and reanalyzed. If the result is such that it will fall within a higher calibration range, it should be reanalyzed in the higher range. If the result is such that it will fall within a lower calibration range, it should be reanalyzed within the lower range.

11.2.19. Upon completion of all analysis, results should be saved to a daily report file. Click Report on the bottom of the main page, More, Results To File, and select one row per result. The file is then named by the run date. The daily report file for analytical batch of January 2, 2017 would be named 010217. The file is converted to Microsoft Excel for data work up and copied to a removable flash drive. Remaining samples are discarded.

11.2.20. All reagents are removed from the reagent chamber and returned to their appropriate storage areas. Reagents that have exceeded their stability period are discarded.

11.2.21. Initiate the shutdown procedure, click on Stand By at the bottom of the main page and insert AquaKem Cleaning Solution into the instrument. Daily files are cleared from the instrument software by clicking More, Management, Clear Daily Files. Once prompted remove the AquaKem Cleaning Solution from the instrument. The software is exited and the instrument is turned off. The computer is turned off.

11.2.22. The waste is flushed down the drain with copious amounts of tap water. The waste cuvette box is moved to the fume hood and covered. The instrument is wiped clean of drips or splashes.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Upon completion of all analysis, results are saved to a daily report file. The file is named by the run date. The daily report file for analytical batch of January 1, 2017 would be named 010117. Raw results for each run are copied into a Microsoft Excel spreadsheet. Data are sorted by sample name and time of analysis so that all samples will be displayed by number and results for each sample will be displayed consecutively.

12.2. Dilution by the instrument is noted by software as analysis ensues and, also, documented in the data report spreadsheet. The analyst examines each row of data. Results are eliminated that are outside the limits of the calibration range, or have an unrepeated high blank response greater than 0.001 absorbance units.

12.3. The analyst examines salinity data for each sample. For all samples with salinity above 0.1 ppt, CBL Nutrient Analytical Services Laboratory’s empirically derived salinity correction is applied to the original undiluted reported concentration.

Salinity Corrected mg Si/L= (((100-((0.0103* (salinity*salinity)) + (-0.9113*salinity) +98.434))/100) +1)* Uncorrected mg Si/L

12.4. Report analyte concentrations in mg Si/L

12.5. Report analyte concentrations to two decimal places.

13. POLLUTION PREVENTION

13.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity of toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the agency recommends recycling as the next best option.

13.2. For information about pollution prevention that may be applicable to laboratories and research institutions, consult “Less is Better: Laboratory Chemical Management for Waste Reduction”, available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N. W., Washington, D.C. 20036.

14. WASTE MANAGEMENT

14.1. The reagents used in this procedure are minimal and are not hazardous with the exception of the sulfuric acid. Due to the small quantity used, the sulfuric acid and other reagents can be flushed down the drain with running water.

14.2. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society.

15. REFERENCES

15.1. U.S. Environmental Protection Agency, 1997. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples. Method 366.0. U.S. Environmental Protection Agency. Washington, D.C.

15.2. Grasshoff, K., M. Ehrhardt and K. Kremlin (eds). 1983. Methods of Seawater Analysis. Verlag Chemie. Weinheim, Germany.

15.3. Frank, J. M., C.F. Zimmermann and C. W. Keefe (2006). Comparison of results from Konelab Aquakem 250 and existing nutrient analyzers. UMCES CBL Nutrient Analytical Services Laboratory, Dec. 2006.