Determination of Silicate from 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 "molybdenum 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 0.01 mg Si/L was determined using the Student's *t* value (3.14) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.
1.3. The Quantitation Limit for Si was set at 0.03 mg Si/L.
1.4. The method is suitable for Si concentrations 0.03 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 conforms to EPA Method 366.0. (1997).

2. SUMMARY

2.1. Filtered samples are mixed with oxalic acid, ammonium molybdate, and sulfuric acid. The resulting silicomolybdate is reduced to 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 0.03 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 0.2 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. A **preparation**

batch is composed of one to 200 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 8 hours. 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 20 field sample analysis.

3.13. **Certified Reference Material -** 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. **Instrument Detection Limit (IDL)** - The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

3.23. **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.

3.24. **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 apparatus.

3.25. **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)

3.26. **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. (ACS)

3.27. **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.

3.28. **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).

3.29. **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.

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

3.31. **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

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

3.33. **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.

3.34. **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)

3.35. **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.

3.36. **Quality Control Sample (QCS)** - A sample of analytes 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.

3.37. **Run** - One sample analysis from start to finish, including printout.

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

3.39. **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.

3.40. **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.

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

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

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

3.44. **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.

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

3.46. **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 and safety glasses and enclosed shoes must always be worn. In certain situations it may also be necessary to use gloves and goggles. 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 CBL Business Manager of the incident. Contact the CBL Business Manager 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.

Chemical	Health	Flammability	Reactivity	Contact	Storage
Sulfuric acid	4	0	2	4	White
Oxalic acid	2	1	1	3	White
Ascorbic acid	1	1	1	1	Green
Ammonium molybdate	2	0	1	2	Green
Potassium phosphate	0	0	0	1	Green
Sodium silicofluoride	2	0	0	2	Green
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)					
STORAGE Red - Flammability Hazard. Store in a flammable liquid storage area. Blue - Health Hazard. Store in a secure poison area. Yellow - Reactivity Hazard. Keep separate from flammable and combustible materials. White - Contact Hazard. Store in a corrosion-proof area. Green - Use general chemical storage (On older labels, this category was orange). Striped - Incompatible materials of the same color class have striped labels. These Products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.					

6. EQUIPMENT AND SUPPLIES

6.1. Aquakem 250 multi-wavelength automated discrete photometric analyzer. Aquakem 250 control software operates on a computer running Microsoft Windows NT or XP operating system.
6.2. Freezer, capable of maintaining -20 ± 5° C.
6.3. Refrigerator, capable of maintaining 4 +/- 2^{o}C.
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 deionized 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
Deionized water	up to 1000mL

In a 1000mL plastic volumetric flask, dissolve 100g of oxalic acid in ~900mL deionized water and dilute to 1000mL with deionized water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot 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 ($H_2C_2O_4 \cdot 2H_2O$)	5g
Ascorbic acid ($C_6H_8O_6$), U.S.P. quality	100g
Deionized water	up to 1000mL

In a 1000mL plastic volumetric flask, dissolve 5g of oxalic acid in ~800mL of deionized water. Add 100g of ascorbic acid and mix until dissolved. Dilute to 1000mL with deionized water. Write name of preparer, preparation date, reagent manufacturers, manufacturers' lot numbers in the Analytical Reagent log book. Divide into 4-6 plastic bottles and freeze until needed. Thawed bottles should be stored at 4^{o}C and are stable for 6 months.

7.5. Ammonium Molybdate Solution -

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

In a 100mL plastic volumetric flask, dissolve 3.0g ammonium molybdate in ~80mL of deionized water. Dilute to 100mL with deionized water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot number in the Analytical Reagent log book. Store in the dark at room temperature. Make every other day.

7.6. Stock Phosphate Solution –

Potassium phosphate (KH_2PO_4), dried at 45^{o}C	0.4394g
Deionized water	up to 1000mL

In a 1000mL volumetric flask, dissolve 0.4394g of potassium phosphate in ~600mL of deionized water. Dilute to 1000mL with deionized water. Write name of preparer, preparation date, reagent manufacturer, manufacturer's lot number in the Analytical Reagent log book. Store the flask at room temperature. Prepare fresh when making 0.7 N sulfuric acid solution.

7.7. Sulfuric Acid Solution –

Sulfuric acid (H_2SO_4), concentrated (sp. Gr. 1.84)	4.06mL
Stock phosphate solution	21.4 mL
Deionized water	up to 1000mL

In a 1000mL plastic volumetric flask, add 4.06mL of concentrated sulfuric acid and 21.4mL of stock phosphate solution to ~600mL of deionized water. Dilute to 1000mL with deionized 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 6-9 months.

7.8. Stock Silicate Standard, 10,000uM

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

In a 1000mL plastic volumetric flask, dissolve 1.88g of sodium silicofluoride in ~900mL of deionized water. Dilute to 1000mL with deionized water (1ml contains 10umoles Si). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Store in a plastic container. Make fresh every 6 months.

7.9 Aquakem Cleaning Solution –

Clorox	75.0 mL

In a 100 mL volumetric flask, dilute 75.0 mL of Clorox to volume with deionized water to yield a concentration of 75% Clorox. Recent (2012) trends in commercially available Clorox, have necessitated altering this formula to 55.0 mL Clorox in 100 mL flask. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Reagent is stable for six 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 (DOC**) – 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 or middle and end of the run, 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)** – MDLs should be established for Si using a low level estuarine water sample, typically three to five times higher than the estimated MDL. The same procedure should be followed for sediments or other weighed samples. To determine the MDL values, analyze seven replicate aliquots of water and process through the entire analytical procedure. Perform all calculations defined in the procedure (Section 12) and report the concentration values in the appropriate units. Calculate the MDL as follows:

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

Where,

S = Standard deviation of the replicate analyses.
n=number of replicates
$t_{(n-1,1-\alpha=0.99)}$ = Student's *t* value for the 99% confidence level with n-1 degrees of freedom (*t*=3.14 for 7 replicates.)

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9.2.4. MDLs should be determined annually, whenever there is a significant change in instrumental response, change of operator, or a new matrix is encountered.

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 Nanopure 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. LRB above the lowest standard requires that the source of the problem must be identified and corrected before proceeding with analyses.

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 and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ 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 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. Set up an accuracy chart by using percent recovery since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5. **Continuing Calibration Verification (CCV)** – Following every 18-23 samples, one CCV of 50 µM Si/L (1.4 mg Si/L) for Regular Si, 250 µM Si/L (7.0 mg Si/L) for Si HIGH is analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (Na_2SiF_6), and are to be within TV ± 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.

9.3.6. **Reagent Blank** – The Reagent Blank Control Chart for Reagent Blank samples is constructed from the average and standard deviation of the 20 most recent Reagent Blank measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter Reagent Blank results on the chart each time the Reagent Blank is analyzed.

9.4. **Assessing Analyte Recovery**

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

9.4.2 % Recovery = (Actual value/expected value) X 100

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

10. CALIBRATION AND STANDARDIZATION

10.1. **Calibration** - Daily calibration must be performed before sample analysis may begin. Five point calibrations are specified for silicate calibration with the AquaKem 250 analyzer.

10.2. **Working Silicate Standards** – For the low curve (SILCBL), dilute 0.75 mL and 0.5 mL of Stock Silicate Standard to 100 mL with deionized water to yield concentrations of 75 µM Si (2.1 mg Si/L) for working calibration standard and 50 µM Si (1.4 mg Si/L) for working CCV, respectively. For the high curve (SILCBLHI), dilute 3.75 mL and 2.5 mL of Stock Silicate Standard to 100 mL with deionized water to yield concentrations of 375 µM Si (10.5 mg Si/L) for working calibration standard and 250 µM Si (7.0 mg Si/L) for working CCV, respectively. Write name of preparer, preparation date, Stock Standard preparation date in the Analytical Standard log book. Make fresh every month. The AquaKem 250 uses the working standard for each calibration curve to produce the five defined dilutions for the calibration curve.

10.3. **Silicate Calibrators**:

Test Name	Working Standard	Dilution Factor	Concentration mg Si/L
SILCBL	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	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 (Person'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 (Person's r value) for the calibration curve must be greater than 0.980.

11. PROCEDURE – DAILY OPERATIONS AND QUALITY CONTROL

11.1. Turn on 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. Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh deionozed water.

11.3. Remove from refrigerator samples that will be analyzed that day. Begin daily bench sheet documentation. Remove SRM from refrigerator as well.

11.4. Once water reservoir is full, "perform washes" – complete five wash cycles and then initiate "start-up" at main menu.

11.5. Gather working standards and reagents from refrigerator during startup. Assess standards and reagents. Remake anything that has exceeded the time over which it is considered stable. Molybdate reagent is made every other day.

11.6. Once startup is complete, check that the instrument water blank has performed within acceptance limits. If any of the instrument functions 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.

11.7. Load reagents into reagent carousel and place into refrigerated reagent compartment.

11.8. 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.

11.9. Select the methods to be calibrated. Two methods will be calibrated – SILCBL, and SILCBLHI.

11.10. 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 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.

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

11.12. 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.

11.13. Once calibration curves are accepted, samples are loaded into the sample segments and loaded into the instrument for analysis. 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 the usual ICV samples for each curve: 1.4 mg Si/L for SILCBL, and 7.0 mg Si/L for SILCBLHI.

11.14. Samples are loaded into the segments and analyzed. CCV (Continuing Calibration Verification) samples (one for each of the two calibration ranges) follow every 18-23 samples. Standard Reference Material (SRM) samples as well as Laboratory Reagent Blanks (LRB) are scattered throughout the analytical batch. 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 or greater to ten percent of the total number of samples in the analytical batch.

11.15. 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 calibration range it was run within, 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 that range. If the result is such that it will fall within a lower calibration range, it should be reanalyzed within that range.

11.16. Upon completion of all analysis, results should be saved to a daily report file. The file is named by the run date. The daily report file for analytical batch of January 2, 2005 would be named 010205. The file is converted to Microsoft Excel for data work up and copied to a removable flash drive. The sample results are printed in order to maintain a hard copy. Remaining samples are discarded.

11.17. All reagents are removed from the reagent chamber and returned to the refrigerator. Reagents that have exceeded their stability period are discarded.

11.18. AquaKem Cleaning Solution is inserted into the instrument and shut down procedures are initiated. Daily files are cleared from the instrument software, the software is exited and the instrument is shut down. The computer is shut down. The instrument is shut off. The waste is flushed down the drain with

copious amounts of tap water. The waste cuvette box is moved to the fume hood. 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, 2005 would be named 010105. Raw results for each run are copied into a Lotus123 or Microsoft Exel 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. Analyst edits results taking into account dilutions and scale, and discarding values with unrepeated high blank response greater than 0.001 absorbance units.

12.3. The analyst examines salinity data for each sample. For all samples with a 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. Example of sorted and edited spreadsheet of results:

	A	B	B	C	D
1	AquaKem v. 6.5 AQ1				
2	JANE DOE:				
3	Fri Feb 27 09:11:41 2009				
4	SAMPLE BATCH NAME				
5	SAMPLE BATCH DATE				
6					
7	SAMPLE	MG Si/L			
8	1	2.11			
9	2	3.50			
10	3	1.42			
10	4	0.43			
11	5	1.32			
12	6	0.81			
13	7	1.58			
14	8	3.79			
15	9	2.91			
16	10	5.10			
17	Si CRM 11/2008	0.93			
18	DHOH	0.01			
19	1.4 Si CCV	1.41			
20	7.0 Si CCV	7.02			

21

12.4.1. Cell 1A – Instrument and software version
12.4.2. Cell 2A – Analyst's name
12.4.3. Cell 3A – Date and time of start-up
12.4.4. Cell 4A – Sample batch name
12.4.5. Cell 5A – Sample batch date
12.4.6. Cell 7A – Column heading for sample
12.3.7. Cell 7B - Column heading for Si concentration in units of mg Si/L
12.3.8. Cells 8A to 16B – Sample Results table.
12.3.9. Cell 17A – SRM/CRM name and date
12.3.10. Cell 17B – SRM/CRM concentration, mg Si/L
12.3.11. Cell 18A – Deionized water blank name (DHOH)
12.3.12. Cell 18B – DHOH concentration, mg Si/L
12.3.13. Cells19A and Cell 20A – CCV name
12.3.14. Cells 19B and 20B – CCV concentration, mg Si/L

12.5. Report analyte concentrations to two significant figures.

13. METHOD PERFORMANCE

13.1. The procedure validation MDL, based on seven filtrations of an estuarine sample, was found to be 0.01 mg Si/L for silicate.

13.2. Twenty-seven analyses on separate dates of the Silicate SRM, from 1/2008 to 3/2009, produced an average value of 0.88 +/- 0.02 mg Si/L. The true value for the QC is 0.94 mg Si/L. This is an average recovery of 93.4%.

13.3. Twenty-seven analyses on separate dates of the 1.40 mg Si/L and 7.0 mg Si/L CCVs from 1/2008 to 3/2009, produced the following values respectively: 1.42+/- 0.04 mg Si/L and 7.12+/-0.09 mg Si/L. This is an average recovery of 101.4% for the low curve, and 101.7% for the high curve.

14. POLLUTION PREVENTION

14.1. 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.

14.2. 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.

15. WASTE MANAGEMENT

15.1. 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.

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

16. REFERENCES

16.1. 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.

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

16.3. 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.