Standard Operating Procedure for:

Silica

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1 Identification of the method

1.1 A Measurement of silica via a colorimetric, flow injection analysis (APHA Method 4500 Si E).

2 Applicable matrix or matrices

2.1 This method is suitable for the analysis of environmental samples.

3 Detection limit

3.1 Method Detection Limit: 0.7 mg Si/L.

3.2 This Method Detection Limit was determined by taking the calculated concentrations of the lowest calibration standard from multiple analytical runs (using seven or more individual values taken from at least three analytical runs) and calculating a standard deviation from those concentrations. This standard deviation is then multiplied by the one-sided t-statistic at the 99 % confidence level for the appropriate degrees of freedom (n – 1).

4 Scope of the method

4.1 This standard operating procedure is intended to provide MU Limnology operators, technicians, and analysts with guidance on the analysis of silica with the Lachat Quikchem 8500. This document is not intended to replace individual training in this method by an experienced MU Limnology technician.

5 Summary of the method

5.1 Filtered water samples are drawn into the Lachat Quickchem flow injection system by a peristaltic pump. Silica quantitatively reacts with molybdate and the resulting yellow acid is reduced to form a molybdenum blue product. The solution's absorbance is measured at 820 nm and peak areas are converted to concentrations based on a 1st order calibration curve.

5.2

- Operating Range: 0.7 to 15 mg/L
- Sample Volume: 5 ml
- Sample Injection Volume: 1–2 ml
- Run Time: 1.5 hrs
- Samples per run: 40Definitions

6 Interferences

6.1 Phosphate may interfere, but this interference is reduced by the addition of Oxalic Acid. If interference due to phosphate is encountered, replace the 12 cm reaction coil located after Oxalic Acid addition on manifold with 550 cm of 0.8 mm I.d. tubing on a 22 cm coil.

6.2 Tannin and large amounts of iron or sulfide are interferences. Sulfides can be removed by boiling an acidified sample. Addition of disodium EDTA will eliminate the interference due to iron.

6.3 Silica contamination may be avoided by storing samples, standards, and reagents in plastic.

7 Health and Safety

7.1 This method involves handling freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing hands frequently.

7.2 Wear protective gloves, lab coats, and other appropriate PPE when handling all chemical substances used in this method. All operators and technicians preforming this method should review the MSDS for additional information and safety concerns regarding the chemical substances used throughout these procedures.

7.3 The following chemicals used in this method are considered especially hazardous and should be handled with extra care:

- Sulfuric Acid
- Oxalic Acid
- Sodium Bisulfite
- 4-amino-3-hydroxy-1-napthalenesulfonic acid

8 Personnel qualifications

8.1 This method is considered advanced. Lab personnel should be trained to the technician level in a number of other lab protocols before being trained in this method. They must also be familiar with all standard MU Limnology sampling handling and labeling procedures and appropriate SOPs. There is no operator designation for this method. The lowest level of certification is as a technician.

8.2 New technicians learning to operate this method should perform 4 runs before being certified.

- Run 1, the trainee should watch an experienced technician carry out all parts of a run (including reagent preparation, data analysis, etc.)
- Run 2, the trainee should carry out a run with close supervision by an experienced technician.

- Run 3, the trainee should carry out a run independently with occasional check-ins from an experienced technician.
- Run 4, the trainee should carry out a run fully independently. An experienced technician should check the results of this run after its finished.

If a trainee completes all 4 runs without significant issues (poor sample replication, bad calibration, drifting base lines), they will be certified as a technician.

9 Equipment and supplies

9.1 Glycerin, Fisher Scientific, G33-500
9.2 Sodium Sulfite, Fisher Scientific, S447-500
9.3 Sodium Bisulfite, Fisher Scientific, S654-500
9.4 Oxalic Acid Dihydrate, Fisher Scientific, A219-500
9.5 Ammonium Molybdate Tetrahydrate, Fisher Scientific, A674-500
9.6 ANSA (4-amino-3-hydroxy-1-napthalenesulfonic acid), Aldrich, 109754
9.7 Silica Standard, 1000 ppm SiO2 (467 ppm Si), RICCA, 6750-4
9.8 Concentrated Sulfuric Acid
9.9 Volumetric Glassware
9.10 Whatman GF/F Glass Microfiber Filters, 47mm, 0.7µm, CAT No. 1825-047
9.11 47 mm Magnetic Filter Flask, PALL
9.12 Internal Vacuum System or Portable Vacuum Pump
9.13 1 ml and 10 ml micropipettes

10 Reagents and standards

10.1 Calibration Standards

Prepare standards as shown below in Table 1 in clean volumetric flasks. All stock solutions should be added quantitatively using a calibrated micropipette. Rinse all glassware three times with UPDI and then fill ~75 % of the way to the line. Then add appropriate stock solution, fill to the line with UPDI, cover with parafilm and invert three times to mix. Standards may be stored in plastic at 4 °C for up to 100 hours after preparation.

10.2 Ammonium Molybdate

To a 500 ml volumetric flask, add 400 ml of UPDI followed by 20 g of ammonium molybdate. Using a calibrated pipette, add 8 ml of sulfuric acid to the solution. Magnetically stir until all reagents are dissolved. This may take up to 2 hours. Dilute to the mark, cover with parafilm, and invert three times to mix. This solution should be prepared daily.

10.3 Oxalic Acid

To a 500 ml volumetric flask, add 400 ml of UPDI followed by 50 g of oxalic acid. Magnetically stir until all reagents are dissolved. Dilute to the mark, cover with parafilm, and invert three times to mix. This solution should be stored in plastic for up to 28 days.

10.4 ANSA Reducing Agent

In a 100 ml volumetric flask, dissolve 2.0 g of sodium sulfite in 80 ml of UPDI. Add 0.25 g ANSA, dilute to the mark, cover with parafilm, and invert three times to mix. In a separate container, dissolve 15 g of sodium bisulfite in 300 ml of UPDI. Mix the solutions in a third container and add 4 g of glycerol. Cover the solution with parafilm and invert three times to mix. This solution should be stored in plastic at 4 °C for up to 28 days or when the solution turns dark.

10.5 Table 1: Nominal Concentration and Preparation of Silica Standards

Standard Concentration (mg Si/L)	15	10	5	2.5	0
Total Standard Volume (ml)	100	100	100	100	100
Volume 467 mg /L Ammonium Standard	3.21	2.14	1.07	0.535	-

11 Quality Control

11.1 Reagent Coloring

Prior to beginning analysis, all reagents should be visually expected for discoloring.

11.2 Baseline Check

Before beginning the run, technicians should observe the instrument's baseline for 5 minutes and record it in the log. Significant movement upwards or downwards in the baseline may be a sign of reagent contamination. If the issue persists, reagents should be remade.

11.3 Check Standards

For every 20 sample measurements (10 sets of 2 duplicates) a check standard must be analyzed. Check standards will be identical to the calibration standards used in the run. Check standards should be run in descending concentration starting from the 15 mg Si/L standard down to the 0 mg Si/L standard. The full range of standards used in the calibration must be run as check standards before ending a run, even if the total number of samples analyzed is less than 10*Number of standards.

11.4 Secondary QC Standards

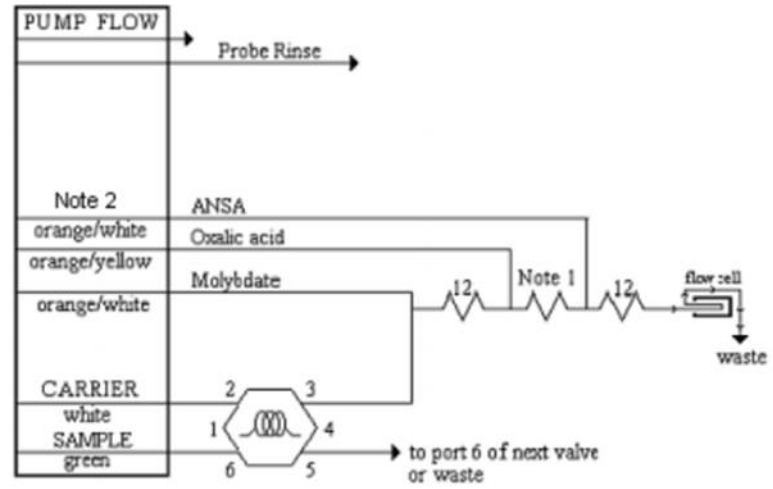
For every 20 sample measurements (10 sets of 2 duplicates) a secondary QC standard must be analyzed. Secondary standards should be identical to the calibration standards in concentration but prepared from a different stock solution. Secondary standards should be run in descending concentration starting from the 15 mg Si/L standard down to the 0 mg Si/L standard. The full range of standards must be run before ending a run, even if the total number of samples analyzed is less than 10*Number of standards.

12 Analysis

12.1 Set Up

- 30 minutes prior to analysis, all reagents and standards should be placed at room temperature and allowed to sit until they are no longer cold to the touch.
- Pour standards and sample into appropriate tubes.
- Connect optical filters and reagent lines as shown below (Figure 1).
- Place all reagent lines in the UPDI container and hit "run" on the peristaltic pump.
- Let run for 10 minutes, then place reagent lines in their appropriate containers and let run for 5 minutes. Also place the rinse line is a separate container of UPDI.
- Open the silica template in the Omnion software and select "preview".
- Enter standards and sample IDs into the Omnion software.
- Monitor the baseline for significant upward or downward drift for 10 minutes.

1.1 If there is no baseline drift, hit start.



Carrier: **DI water**
Manifold Tubing: **0.5 mm (0.022 in) i.d. This is 2.5 µL/cm.**
AE Sample Loop: 7 cm
QC8000 Sample Loop: 13 cm
Interference Filter: 820 nm

Figure 1: Silica Manifold Diagram

12.2 Calibration and Standardization

- All standards should be measured twice.
- Omnion will automatically generate a calibration curve as standards are measured based on a first order calibration curve.
- Technicians should ensure that standards replicate well and that the curve generates an r^2 > 0.99. Technicians should also particularly check the low end of the calibration curve and ensure that there is not a significant deviation from the calibration curve by low standards.
- If any of these criteria are not met, halt and restart the run.
- Sample analysis
- The Lachat may be left to operate unattended for the remainder of the run. However, the technician should check the instrument at least once between every check/secondary standard. Halt the run if a standard does the not meet QC criteria.
- Between check standards, technicians should check the concentrations of the newly analyzed samples for duplicates with poor replication and samples which exceed the high standard (15 mg Si/L). These samples may be rerun or run with dilution respectively at the end of the run. Dilutions may be prepared in sample tubes using UPDI and a calibrated micropipette.

- Technicians should regularly monitor the flow injection system during the run and check for leaks or plugs.
- Technicians should regularly check that reagent containers are sufficiently full and that reagent lines are fully submerged.
- When sample analysis is finished, transfer reagent lines to UPDI. Let run for 10 minutes, then remove from UPDI and allow the lines to pull air for another 10 minutes.
- If another run is being immediately performed, the preceding steps may be ignored and the run can be started with the instrument as is.

13 Data acquisition, calculations, and reporting

13.1 At the completion of the run, the Omnion software will automatically export an Excel file with the run data.

13.2 This data includes determined concentrations. These concentrations are automatically calculated by the Omnion program using the first order calibration curve created at the start of the run.

13.3 Open the silica template and paste the run export into the tab labeled “Raw Export.”

13.4 Open the calculations tab and enter appropriate sample information (site, date, dilution factor, etc.).

13.5 All calculations including averaging of duplicate measurement and calculation of QC parameters will be performed automatically.

13.6 Check that all standards meet QC requirements (Section 17.3). Standards which fail the QC requirements will be automatically indicated by the Excel sheet. If any standards do not meet the requirements, mark the preceding and following 10 samples as reruns.

13.7 Samples which fail to meet QC requirements (Section 17.4) will be indicated by the sheet and should be marked as reruns.

13.8 All sample results will be consolidated in the “summary” tab.

14 Computer hardware and software

14.1 Windows 7 Enterprise

14.2 Omnion Software 4.0, Lachat Instruments, Hach

15 Method performance

15.1 Desired Performance Criteria

- Method Detection Limit: 0.7 mg Si/L
- Precision: Duplicate CV < 5 or duplicate range < 0.2 mg N/L
- Calibration $r^2 > 0.99$

16 Pollution prevention

16.1 All reagents and standards will be prepared in appropriate volumes to reduce waste.

16.2 All sample and reagents will be handled according to MU EHS policies to ensure proper disposal.

17 Data assessment, acceptable criteria for quality control measures and corrective actions for out-of-control or unacceptable data

17.1 Excel Cell References and Data Entry

Poor cell references or improperly entered data will lead to erroneous results. If a problem is noticed with a run, the data entry and the cell references should first be checked by a supervisor and pointed out to a technician if found.

17.2 r^2 Value

The mandatory r^2 for valid results is a minimum of 0.99. In the event that a technician proceeds with a run which has a calibration r^2 less than 0.99, all samples from this rerun should be rerun.

17.3 Standard QC criteria

Both check and secondary standards must meet at least one of the following QC criteria:

- The CV of standard's concentration and its known concentration is less than 5 %.
- The absolute difference of the standard's concentration and its known concentration is less than 0.2 mg/L.

If neither of these criteria are met the standard is not considered valid.

17.4 Sample QC criteria

Sample duplicates must meet least one of the following QC criteria:

- The CV of the two duplicates is less than 5 %.
- The absolute difference of the two duplicates is less than 0.2 mg/L.

If neither of these criteria are met, the sample result cannot be considered valid and should be rerun.

18 Waste management

18.1 All waste generated is considered hazardous.

18.2 All analyzed standards, and reagents should be treated as waste upon completion of the run.

18.3 Waste should be kept in an approved MU Environmental Health and Safety (EHS) container with proper labeling.

18.4 Waste will not be held for longer than 6 months and MU Environmental Health and Safety (EHS) will be notified an appropriate time before this point so that waste can be collected and disposed of.

19 References

19.1 Standard Methods for the Examination of Water and Wastewater, 23rd Edition. 2017. Method 4500 Si E. American Public Health Association. Washington, DC.