

Standard Operating Procedure for:

Ammonium

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1 Identification of the method

- 1.1 Measurement of ammonium via a phenol-based, colorimetric, flow injection analysis (APHA Method 4500-NH₃ G).

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](#): 0.01 mg N/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 ammonium 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 Quikchem flow injection system by a peristaltic pump. Dissolved ammonium quantitatively reacts with phenol and sodium hypochlorite producing a blue indophenol. This reaction is facilitated via heating and the resulting solution is carried to an optical sensor with an EDTA based buffer and sodium nitroprusside in order to reduce interference and enhance sensitivity. Solution absorbance is measured at 630 nm and peak areas are converted to concentrations based on a 1st order [calibration curve](#).

5.2

Operating Range: 0.01 to 1.0 mg N/L
Sample Volume: 5 ml
Sample Injection Volume: 1-2 ml
Run Time: 2.5 hours
Samples per run: 40

6 Interferences

- 6.1 Ammonium is not a stable species in water. Samples must be filtered within 12 hours of collection and should be frozen immediately. Leaving samples thawed for extended periods may result in the transformation of nitrate in the sample.
- 6.2 Ammonium based reagents utilized in other lab protocols may be volatile and produce fumes which can contaminate samples or standards used in this method.
- 6.3 Extreme sample color or turbidity may produce interference.
- 6.4 Sulfide concentrations greater than 2 mg H₂S/L will produce interference.
- 6.5 Color response is proportional to sample pH. Standards and samples should match in the range of their pH.

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 and lab coats when handling all chemical substances used in this method. All [operators](#) and [technicians](#) performing 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:
 - Sodium Hydroxide
 - Sodium Nitroferricyanide
 - Sodium Phenolate

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 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 [operator](#) 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.
 - Run 3: The trainee should carry out a run independently with occasional check-ins.

- Run 4: The trainee should carry out a run fully independently. An experienced [technician](#) should check the results of this run after it is finished.
- If a trainee completes all 4 runs without significant issues (poor sample replication, bad calibration, drifting base lines), they may be certified as a [technician](#).

9 Equipment and supplies

- 9.1 Sodium Hydroxide, Fisher Chemical, S318-500
- 9.2 Phenol, Fisher Chemical, A931I-1
- 9.3 Disodium Ethylenediamine Tetraacetic Acid Dihydrate, Fisher Chemical, S311-500
- 9.4 Sodium Nitroferricyanide Dihydrate, Fisher Chemical, S350-100
- 9.5 Ammonium Standard (250 [ppm](#) NH₄, 194 [ppm](#) N), RICCA, 691-4
- 9.6 Sodium Hypochlorite 5%
- 9.7 Volumetric Glassware
- 9.8 Whatman GF/F Glass Microfiber Filters, 47 mm, CAT No. 1825-047
- 9.9 47 mm Magnetic Filter Flask, PALL
- 9.10 Internal Vacuum System or Portable Vacuum Pump
- 9.11 1 ml and 10 ml micropipette

10 Reagents and standards

10.1 [Calibration Standards](#)

Prepare standards as shown below in Table 1 in clean volumetric flask. 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 deg C for up to 100 hours after preparation.

10.2 Phenol

Phenol is an inhalation and skin contact hazard. Prepare this reagent in a fume hood with all possible [PPE](#) (e.g., lab coat, double gloves, goggles). All glassware should be rinsed multiple times with [DI](#) after use and these [DI](#) rinses should be treated as waste. All waste should remain in a fume hood until it has been transferred to an appropriate sealed container.

Fill a 1 L volumetric flask with ~600 ml of [UPDI](#). To this solution add 88 ml of liquid phenol. Slowly and while magnetically stirring, add 32 g of sodium hydroxide to the solution. Stir until solution becomes clear and cools to room temperature. Dilute to the mark, cover with parafilm, and invert three times to mix. This solution should be transferred to a brown glass container and may be stored at 4 deg C for up to 3 days. Check the solution before use for a light brown color as at this point the reagent is no longer usable.

10.3 Sodium Nitroprusside (Sodium Nitroferricyanide)

Fill a 250 ml volumetric flask with ~200 ml of [UPDI](#) and add to it 0.875 g of sodium nitroferricyanide. Magnetically stir until fully dissolved. Dilute to the mark, cover with parafilm, and invert three times to mix.

10.4 Table 2: Preparation of Ammonium Standards

Standard Concentration (mg N/L)	1.0	0.5	0.25	0.1	0.05	0
Total Standard Volume (ml)	100	250	500	1000	1000	100
Volume 194 mg N/L Ammonium Standard	0.1	0.125	0.125	0.1	.05	-

10.5 Sodium Hypochlorite

Fill a 500 ml volumetric flask with 250 ml of 5% sodium hypochlorite. Dilute to the mark, cover with parafilm, and invert three times to mix.

10.6 Buffer

Fill a 1000 ml volumetric flask with ~600 ml of [UPDI](#) and add to it 50 g of disodium ethylenediamine tetraacetate and 5.5 g of sodium hydroxide. Magnetically stir until fully dissolved. Dilute to the mark, cover with parafilm, and invert three times to mix. This solution should be stored in plastic at room temperature for up to one week.

11 Quality Control

11.1 Reagent Coloring

Prior to beginning analysis, all reagents should be visually expected for discoloring. In particular, [operators](#) should check for a slight brown discoloration of the phenol reagent as this can be a sign that it is no longer usable. If discoloration is observed, new reagents should be reprepared.

11.2 Baseline Check:

Before beginning the run, [operators](#) should observe the instrument's baseline for 5 minutes and record it in the log. [Operators](#) should ensure that the baseline remains stable in the range of 1 V during this time period. Significant movement upwards or downwards in the baseline, or a baseline significantly above 1 V 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 1 mg N/L standard down to the 0 mg N/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 1 mg N/L standard down to the 0 mg N/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.
- Thaw samples in room temperature water until fully thawed. Partial thawing is not acceptable. Pour standards and sample into appropriate tubes.
- Connect optical filters and reagent lines.
- Place all reagent lines in the [UPDI](#) container and set 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 in a separate container of [UPDI](#).
- Open the ammonium 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.
- If there is no baseline drift, hit start.

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 second order [calibration curve](#).
- [Operators](#) should ensure that standards replicate well and that the curve generates an $r^2 > 0.99$. [Operators](#) 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 during the run, halt and restart the run.

12.3 Sample Analysis

- The Lachat may be left to operate unattended for the remainder of the run. However, the [operator](#) should check the instrument at least once between every check/secondary standard. Halt the run if a standard does not meet [QC](#) criteria.
- Between [check standards](#), [operators](#) should check the concentrations of the newly analyzed samples for duplicates with poor replication and samples which exceed the high standard (1 mg N/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.
- [Operators](#) should regularly monitor the flow injection system during the run and check for leaks or plugs.
- [Operators](#) should regularly check that reagent containers are sufficiently full and that reagent lines are fully submerged.
- When sample analysis is finished, turn the nitrate column to “off” and 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 last step 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 ammonium template and paste the run export into the tab labeled "Raw Export".
- 13.4 Open the calculations tab and enter appropriate sample information (e.g., 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. 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 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
- 15.2 [Method Detection Limit](#): 0.01 mg N/L
- 15.3 Precision: Duplicate [CV](#) <5 or duplicate range < 0.01 mg N/L
- 15.4 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 an [operator](#) if found.

17.2 r^2 Value

The mandatory r^2 for valid results is a minimum of 0.99. In the event that an [operator](#) proceeds with a run which has a calibration r^2 less than 0.99, all samples from this run 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 calculated concentration and its known concentration is less than 0.01 mg N/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 0.01 mg N/L

If neither of these criteria are met, the sample result cannot be considered valid and should be rerun. If other nitrogen data was analyzed for the sample, nitrate results should also be compared to this data before data reports are finalized. [Analysts](#) should check that the following conditions are met.

- $([\text{NH}_4] + [\text{NO}_3]) \leq [\text{TDN}] \leq [\text{TN}]$

If the above conditions are not met, the issue should be investigated by [analysts](#).

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 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-NH₃ G. American Public Health Association. Washington, DC.