Standard Operating Procedure for the Analysis of Ammonia in Fresh Waters
CCAL 30C.2

Cooperative Chemical Analytical Laboratory
College of Forestry
Oregon State University
3015 Western Blvd
Corvallis, Oregon

Prepared by Kathryn Motter
And Laura Hartley
Revised September 2023

Standard Operating Procedure for the Analysis of Ammonia In Fresh Waters
CCAL 30C.2

Table of Contents

Standard Operating Procedure for the Analysis of Ammonia In Fresh Waters
CCAL 30C.2

1.0 Scope and Application

1.1 This method details the determination of ammonia in fresh waters by automated colorimetric analysis. The practical range of determination for this method is 0.005 to 0.25 mg/L Ammonia as N. Method detection limit for this analysis is 0.003 mg/L as N.

2.0 Summary of Method

2.1 An automated analysis method is used for the colorimetric determination of ammonia nitrogen in fresh water. Ammonia reacts with hypochlorite generated by hydrolysis of sodium dichloroisocyanurate. The monochloramine formed reacts with salicylate to form indophenol blue that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroprusside to form a colored complex that absorbs at 660 nm. Concentration of ammonia is determined by comparison of absorbance signal with calibration results obtained from prepared standards of varying concentrations.

3.0 Definitions

3.1 DI water: Water that has been through a deionization system to produce water similar to ASTM Type I reagent with 16.7 Mohms resistivity (ASTM) (Reference 16.3).

3.2 Method Detection Limit (MDL): The minimum concentration of an analyte that can be measured and reported with 99% confidence, based on a one-sided 99% confidence interval (*t*-value at a significance level of 0.003 and *n-1* degrees of freedom) from at least seven repeated measurements of a low concentration standard measured within an analysis run.

$\text{MDL} = ts$

Where,

t = Student's t value at a significance level of 0.003 and $n-1$ degrees of freedom
s = standard deviation of at least seven repeated measurements of a low level standard

4.0 Interferences

4.1 Magnesium forms a precipitate at the high pH required for full color development (pH>12), which can cause interference. Addition of trisodium citrate, a complexing agent, reduces the problem.

4.2 Color development is pH dependent, therefore variability in sample/reagent pH must be avoided. If samples are preserved with acid, the calibration standards, blanks and quality assurance samples must be preserved in the same manner.

4.2 Turbidity and color that persists in the sample after filtration may absorb at the 660 nm. Highly colored or turbid samples must be reanalyzed without the color reagent in-line. Any response from the turbidity/color blanks must then be subtracted from the initial analysis response.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent has not been precisely determined; however, each chemical should be regarded as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. Cautions are included for known extremely hazardous materials.

5.2 The following chemicals have the potential to be highly toxic or hazardous. For detailed explanations, consult the MSDS.

5.2.1 Sodium hydroxide
5.2.2 Sodium salicylate
5.2.3 Sodium nitroferricyanide

6.0 Equipment and Supplies

Note: *Brand names, suppliers and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Balance measuring to at least 0.1 mg

6.2 Glassware: including volumetric flasks and pipettes as required

6.3 Lachat QuikChem 8500 Flow Injection Analyzer

  6.3.1 Multichannel proportioning pump (peristaltic)
  6.3.2 XYZ Autosampler
  6.3.3 Reaction Manifold
  6.3.4 Colorimetric Detector
  6.3.5 Data System
  6.3.6 Heating Unit

6.4 Safety glasses

6.5 Nitrile gloves

6.6 Lab coat or apron

6.7 Laboratory Exhaust Fume Hood

6.8 High Density Polyethylene (HDPE) bottles

7.0 Reagents and Standards

7.1 Preparation of Reagents

All reagents need to be filtered and degassed with He

7.1.1 *Salicylate/Citrate Mixed Reagent:*
Dissolve 63.0 g sodium salicylate and 114.o g trisodium citrate in approximately 700 mL DI water in a 1 L volumetric flask. When dissolution complete, add 0.740 g sodium nitroprusside. Swirl to dissolve, and dilute to the mark. Filter. Degas with He. Transfer to amber glass bottle. Stable two weeks.

7.1.2 *Sodium Dichloroisocyanurate Reagent:*
Dissolve 14.0 g sodium hydroxide in approxiamately 500 mL DI water in a 1 L volumetric flask. Cool to room temperature, then add 1.07 g sodium dichloroisocyanurate. When dissolution complete, fill to the mark with DI water. Filter. Degas with He. Transfer to amber glass bottle. Store at 4ºC. Stable one week.

7.2 Preparation of Standards

7.2.1 *Calibration Standards*
Standards are prepared by dilution of a single element standard purchased from a vendor that provides traceability to NIST standards. Working standards in concentrations of 0.200, 0.100, 0.050 and 0.010 mg/L ammonia nitrogen are prepared by serial dilution of the stock standard.

7.2.2 *Second Source Check Standard:*
Standards are prepared by dilution of a standard purchased from a vendor that provides traceability to NIST standards; this reagent is from a source other than that of the calibration standards. A mixed stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. Check standard concentrations are the same as calibration standard 3.

8.0 Sample Handling and Storage

8.1 Unfiltered samples are filtered upon receipt through glass fiber filters into clean HDPE bottles and stored at 4°C in the dark. Samples are analyzed within 48 hours to ensure sample integrity. If samples must be held prior to analysis, they are stored frozen at -15ºC.

9.0 Quality Control

9.1 Preparation of stock standards is recorded on worksheets and documented by weight of standard added to a given flask before dilution to volume with DI water. All records of certification are kept on file at CCAL Laboratory.

9.2 Blank: DI water run after the calibration and before and after each check standard.

9.3 Quality Control Check Standard: Calibration standards run in rotation every ten samples to monitor stability and validate the calibration.

9.4 Method Detection Limit (MDL): Established for each analyte. Based on a one-sided 99% confidence interval (t-value) from at least seven repeated measurements of a low concentration standard. The t-distribution value is multiplied by the standard deviation of the population (n-1) to obtain the MDL.

9.5 Analytical Duplicate: Separate analysis from the same sample aliquot. Run a minimum of once every ten samples.

10.0 Calibration and Standardization

10.1 Balances: calibrated yearly by external vendor.

10.2 Pipette delivery checked by weight to within 2% of theoretical weight of aliquot volume.

10.3 Calibration curve with $r^2 \geq 0.995$. (See 17.2 for calibration data set-up.)

10.4 Calibration verification with check standards, monitored throughout the run. If measurement exceeds +/- 10% of the theoretical value, the analysis should be terminated and the instrument recalibrated. The calibration must be verified before continuing analysis.

11.0 Procedure

11.1 Calibration and Analysis Procedure

11.1.1 Prepare reagents and standards as outlined in Section 7.
11.1.2 Set up manifolds as shown in Section 17.1.
11.1.3 Samples are injected into the reaction path at a fixed time interval, determined by timing set in the software. Setup or confirm data system parameters as detailed in Section 17.2.
11.1.4 Pump DI water through all reagent and sample lines. Check for leaks, even flow and monitor for stable baseline. Pump reagents through until the system equilibrates. Monitor until baseline is stable.
11.1.5 Record sample id’s in the data template.
11.1.6 Calibrate the instrument with standards. Calibration regression equations must have $r^2 \geq 0.995$.

11.2 System Notes

11.2.1 Allow time for the ammonia channel heating unit to warm up to 60ºC.
11.2.2 If the baseline is excessively noisy, clean the manifold using the following procedure:
  - Place all reagent and carrier lines in rinse water and pump to clear reagents.

- Place all lines in 1M HCl solution or Contrad NF for several minutes. Follow with thorough DI water rinse.
- Place lines in DI water and pump until thoroughly rinsed.

11.2.3 If baseline has a large number of air spikes, check pump tubes for excessive wear and replace as necessary.

11.2.4 Excessively noisy baseline may be the result of old reagents. Prepare fresh and flush system thoroughly.

11.2.5 Turbidity and color that persists in the sample after filtration may absorb at the 660 nm. Reanalyze samples without the color reagent as turbidity/color blanks. Any response from the turbidity/color blanks must then be subtracted from the initial analysis response.

12.0 Data Analysis and Calculations

12.1 The data system prepares a calibration curve by plotting response of injected standards versus known standard concentration. The resulting regression equation is used to calculate the sample concentration.

12.2 All results and sample information are filed in the analysis data system by analysis run. Details specific to the instrumental analysis are noted in the Instrument Run Log created and maintained for the Lachat. Analytical results are entered into electronic format and entries are verified by a second person.

13.0 Method Performance

13.1 This method was validated through inter-laboratory studies. The CCAL Water Analysis Laboratory participates in the USGS Standard Reference Water QA program and the National Water Research Institute’s (NWRI) Environment Canada Proficiency Testing (PT) Program.

14.0 Pollution Prevention

14.1 The chemicals used in this method pose little threat to the environment when properly managed.

14.2 All standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of disposable waste.

14.3 For further information on pollution prevention, consult *Less is Better: Guide to Minimizing Waste in Laboratories*, available from the American Chemical Society at www.acs.org.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, and to protect the environment by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is required.

15.2 For further information on waste management, consult *Less is Better: Guide to Minimizing Waste in Laboratories*, available from the American Chemical Society at www.acs.org, and *Environmental Management Guide For Small Laboratories* (233B00001) from the US Environmental Protection Agency at https://nepis.epa.gov.

16.0 References

16.1 Lachat Instruments Methods Manual. Ammonia Nitrogen 10-107-06-3-D. Lachat Instruments, 5600 Lindburgh Drive, Loveland CO 80539.

16.2 Standard Methods For The Examination of Water and Wastewater, Method 4500-NH3 H, Flow Injection Analysis Method. American Public Health Association. 22nd Edition, 2012.

16.3 Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen, Kjeldahl, Total", Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII), STORET NO. Total 00625.

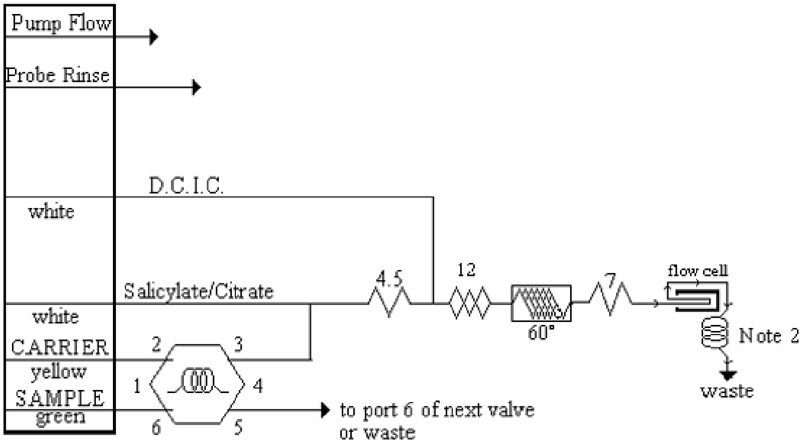
16.3 Code of Federal Regulations. Protection of Environment. Section 40, Appendix B to Part 136. Definition and procedure for the determination of the method detection limit. Revision 1.11. Revised July 1, 1990. Office of the Federal Register, National Archives and Records.

16.4 ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, PA, 1991.

16.5 Ammonia in Water 1981. Methods for Examination of Waters and Associated Materials. London, Her Majesty's Stationary Office.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Ammonia Nitrogen Reaction Manifold



Carrier: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 150 cm
QC8000 Sample Loop: 150 cm
Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The ▨ shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: **70** cm of tubing on a 4.5 cm coil support
7: **135** cm of tubing on a 7 cm coil support
12: **255** cm of tubing on a 12 cm alternating coil support

Note 1: PVC PUMP TUBES MUST BE USED FOR THIS METHOD
Note 2: Back pressure loop is 200 cm x 0.022" i.d.

18.0 Document Revision History

Original Document: March 2006
Version: 30A.0
Title: Standard Operating Procedure for the Analysis of Ammonia in Fresh Waters

Edit Date: February 2010
New Version: 30A.1
Address update

Section 7.2: change preparation of standards from raw materials, to serial dilution of purchased standards. Change standard concentrations. Add Second Source Check Standard.
Section 13.1: add Environment Canada Proficiency Testing Program participation
Section 17.2: change standard concentrations and order of standards.

Edit Date: January 2019
New Version: 30B.1
Address update
Update document to salicylate method and Astoria Pacific Analyzer
Updates throughout to reflect new chemistry and instrumentation settings
Change from phenol method on the Technicon to the salicylate method on the Astoria Pacific Analyzer effective August 4, 2017.

Edit Date: June 2019
New Version: 30C.1
Update to sodium dichloroisocyanurate and sodium salicylate method on the Lachat QuikChem 8500 Flow Injection Analyzer.
Updates throughout to reflect new chemistry and instrumentation.
Change from the salicylate and hypochlorite method on the Astoria Pacific Analyzer to the Lachat method effective 1/28/19.

Edit Date: September 2023
New Version: 30C.2
Update method detection limit to 0.003 mg/L in Sections 1.0 and 3.2.