Health and Environmental Application Laboratory

Standard Operating Procedure For the Determination of Ammonia (Phenolate) by Flow Injection Analysis

SOP Number AN.HEAL.IN.NH4.15.1 (AN-0014)

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NOTE THE HEALTH & SAFETY WARNINGS IN SECTION 4.0

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1.0 Scope & Applicability

This method is applicable to the automated colorimetric determination of ammonium in wet deposition samples by reaction with phenate. The reactions are specific for the ammonium (NH_4^+) ion. The applicable calibration range is 0.025 - 2.576 mg NH_4^+/L with a method detection limit of 0.005 mg NH_4^+/L.

2.0 Summary of Method

This method is based on the Berthelot reaction. Sodium EDTA is added as a complexing reagent to reduce formation of hydroxide precipitates. Ammonium in the samples reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitro ferricyanide) is added to enhance color sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonium concentration in the sample (EPA Method 350.1, Lachat Method # 10-107-06-1B). Ammonium and orthophosphate analyses are done simultaneously on the FIA.

3.0 Definitions

ACS	American Chemical Society
DI	Deionized (water) at 18.0 Mohms-cm or higher
FIA	Flow Injection Analysis
FR50	An in-house prepared quality control synthetic sample targeting the 50th percentile concentration of the precipitation samples analyzed for the NADP/NTN
FL	An in-house prepared quality control sample targeting the low end of the calibration curve
FH	An in-house prepared quality control sample targeting the high end of the calibration curve
IDT	Instrument Data Tool
MDL	Method Detection Limit
MSDS	Material Safety Data Sheet
HDPE	High Density Polyethylene
LIMS	Laboratory Information Management System
QA	Quality Assurance
QC	Quality Control
R^2	Coefficient of Determination displayed as a percentage reflecting the deviation of the measured data points from the calibration curve
QCS	Quality Control Standard
SOP	Standard Operating Procedure
Wet Deposition Samples	Rain, snow, dew, sleet, and hail

4.0 Health & Safety Warnings

4.1 Caution should be exercised when handling liquid phenol–wear protective gloves and a lab coat. Avoid prolonged inhalation of vapors. The sodium phenol reagent should be prepared in an exhaust hood, and the reagent container should be kept tightly covered during analysis. Waste reagents are flushed with copious amounts of water down the drain in hood. Store concentrated phenol in an exhausted storage cabinet.

4.2 Handle sodium nitroprusside with caution. This chemical has the potential to be highly toxic.

4.3 Prepare sodium hypochlorite reagent daily with caution. When pouring, avoid any splashing or spilling of reagent. Degas daily in designated green-taped Erlenmeyer flask with side arm.

4.4 Waste should be collected in a 5 gallon Jeri can. Snap the waste line tube into the can's opening to avoid the release of vapors. Once full, the waste should be neutralized by reducing the pH to at least 9 (it will most likely be around pH 11 when it comes out of the instrument.). This neutralization is accomplished by adding ~ 15-20 mL concentrated hydrochloric acid per one liter of waste. This should then be mixed and the pH should be tested using standard pH test paper. Once the pH is below 9, the waste can be disposed of down the sanitary sink drain located in the fume hood. Waste jugs can be reused.

4.5 Protective eyewear is required in the laboratory at all times.

4.6 No food or drinks are allowed in the sample processing and equipment area to protect the sample integrity.

4.7 The waste disposal method used has been suggested and approved by Landon Hill from the Division of Research Safety at the University of Illinois.

4.8 Safety Data Sheets (SDS) applicable to this SOP can be found by searching the University of Illinois SDS page at: https://www.drs.illinois.edu/Programs/SafetyDataSheets

4.9 The Illinois State Water Survey Chemical Hygiene Plan covers the ISWS laboratory safety program, including, but not limited to, personal protective equipment used, control equipment inventory and operations (such as vented hoods), employee training programs, medical programs, and safety. The ISWS Chemical Hygiene Plan is available at http://isws.illinois.edu/staffonly/resources/manuals.asp. Procedural notes are included in test methods used (e.g. ASTM International, United States Environmental Protection Agency (USEPA), or Standard Methods for the Examination of Water and Wastewater).

4.10 The University of Illinois Division of Research and Safety requirements for chemical safety can be found at http://www.drs.illinois.edu.

4.11 The HEAL has listed known health and safety warnings for this SOP, but this list should not be assumed to comprise all health and safety issues.

5.0 Cautions

5.1 Working knowledge of proper operation of the component instrumentation is vital. Sampler, pump, and reaction manifold all work together to attain smooth sampling and detection. Each component must perform at peak efficiency, or the timing of the analysis will be compromised. Refer to the Lachat System Operation Manual and/or Method Manual for specific details.

5.2 Potential sample contamination is a concern. Care must be taken whenever handling glassware, sample bottles and vials. A clean lab environment and NH_4^+ free DI water must be maintained. Only use DI water with a final point of use filter (0.2 µm) attached for making reagents and standards. Caution must be taken when pouring samples; avoid cross contamination of one sample to the next.

5.3 Maintain good working quality of pump tubing. Replace all pump tubes monthly or when they become flat or a breakage occurs. Record when tubing has been changed in the instrument log book.

5.4 Keep reagent bottles filled to above the intake tubing line. Never allow air to get into the transmission lines, onto the manifold, or through the flow cell during analysis. This will cause a false reading of the sample values.

5.5 Standards should be made on a bi-weekly basis. Reagents should be made as needed.

6.0 Interferences

6.1 High levels of both Ca^{2+} and Mg^{2+} ions will form a precipitate during analysis and interfere with proper detection of NH_4^+. The EDTA buffer reagent is added to samples through a sample line to prevent this occurrence.

6.2 Sample color and turbidity absorbing at 630 nm can also cause a false positive reading.

6.3 Sample lines from the sampler through the pump tubing and onto the manifold need to be maintained free of debris and air bubbles at all times. Avoid introduction of any material that could impede smooth flow of reagents and samples through the system, especially at the point of detection. False readings can occur if air spikes are detected in the flow cell.

6.4 Reagent bottle tops are covered during analysis to eliminate negative impacts of exposure.

7.0 Personnel Qualifications

Analysts should be trained either by an experienced lab analyst and/or by participation in a vendor's training course on an instrument similar to the one being used in the lab. Basic colorimetry theory, methods of operation, and working knowledge of software is vital. Proven capability should be verified by comparison of precipitation sample values and quality control checks to known concentrations. Minimum of a Bachelor's Degree in biology, chemistry, environmental studies, or another related field is preferred.

8.0 Apparatus & Materials

8.1 Equipment

8.1.1 Flow Injection Analyzer - Lachat Instruments (HACH), Quik Chem 8500 Series 2, Ser. # 100800001237. 6645 West Mill Rd., Milwaukee, WI 53218. (414) 358-4200

8.1.2 AutoSampler ASX-520 Series, 90 positions/rack, 4 racks/sampler, with customized plexiglass cover, Ser. # 100506A520.

8.1.3 Multichannel proportioning Watson Marlow pump, 12 positions, speed regulated at 35 rpm, Ser. # 0577361-1.

8.1.4 Reaction unit or manifold, includes 6 port rotary valve, 75 cm sample loop (0.8 mm i.d.), several sized mixing coils, a heating block regulated at 60 ˚C, and 80 μL volume flow cell (10 mm in length). Detection occurs within flow cell fitted with an interference filter of 630 nm. Reaction model # 10-107-06-1B. Refer to Appendix B for specific details.

8.1.5 Pump tubing–Tygon® precision rated tubing with color coded collars indicating flow rates. See Appendix B for appropriate sizes of tubing. Purchased through Lachat Instruments (HACH) or Fisher Scientific, but they are also available through other chromatography suppliers.

8.1.6 Transmission tubing and connectors used on manifold 0.8 mm (0.032" i.d.), Lachat Instruments (HACH) purchased.

8.1.7 Halogen lamp–light source to flow cell for detection – Lachat Instruments (HACH) purchased

8.1.8 Data collection and processing system. Omnion software version 4.0 controls operation of the instrument and data collection. Refer to Omnion 3.0 Software User Manual or Omnion 4.0 software help section for specific details.

8.2 Chemicals

8.2.1 Sodium Nitroprusside

Dissolve 3.5 g of sodium nitroferricianide dihydrate in 1 L of ammonium-free DI water. Mix well. Store in a tightly capped 1L HDPE bottle. Prepare biweekly or more frequently as needed. Use ACS reagent grade chemical purchased from Fisher Scientific (100 g) (catalog # S-13829). The reagent is also available at Lachat Instrument (HACH) – part # 50006. Caution: potential health hazard.

8.2.2 Sodium Phenolate

In a 1 L volumetric flask, dissolve 88 mL of 88% liquefied phenol in approximately 600 mL DI water. While stirring, slowly add 32 g of sodium hydroxide (NaOH). Cool. Add DI water to mark. Mix well. Store in a tightly capped 1L HDPE bottle. Prepare weekly or more frequently as needed. Use 88% liquid phenol (catalog # A9311-500) and NaOH pellets (ACS reagent grade) (catalog # S318-500) purchased from Fisher Scientific. Prepare daily. The reagent is also available at Lachat Instrument (HACH) – part # 50005. Caution: phenol causes severe burns and is easily absorbed through the skin.

8.2.3 Sodium Hypochlorite

Dilute 250 mL Fisher Scientific brand sodium hypochlorite (4-6% NaOCl) (Fisher Scientific catalog # SS290-1) to 500 mL with DI water. Degas in 0.5 L side arm flask. Store solution in tightly capped 1L HDPE bottle. Prepare daily.

8.2.4 Buffer reagent

In a 1 L volumetric flask, dissolve 50.0 g Na_2 EDTA·$2H_2O$ (Fisher Scientific catalog # S311-500) and 5.5 g of sodium hydroxide (NaOH) (Fisher Scientific catalog # S318-500) in about 900 mL DI water. Dilute to the mark. Mix well. Degas in a 1 L side arm flask. Store solution in a tightly capped 1 L HDPE bottle. Prepare monthly or more frequently as needed. Use ACS reagent grade.

8.2.5 Carrier water

In a designated 2 L side arm flask, degas about 1600ml of 18.0 Mohm-cm resistance, 0.2 µm point of use filtered DI water for about 1 hour. Repeat this procedure throughout run as more carrier water is needed.

8.2.6 NH_4^+ Stock Standard

1000 mg NH_4^+/L (as N) stock made from Ammonium Chloride, (Fisher Scientific catalog # A661-500), dried approximately 2 hours at 104˚C. In a 1 L volumetric flask, dissolve 3.819 g Ammonium Chloride in about 900 mL DI water. Dilute to the mark. Mix well. Keep refrigerated in a 1L HDPE bottle. Stable for twelve months.

8.2.7 NH_4^+ Working Standards

Prepare NH_4^+ working standards from a stock standard of 1000 ppm NH_4^+ (as N) as follows: A) 2.576 mg NH_4^+/L, B) 1.288 mg NH_4^+/L, C) 0.6440 mg NH_4^+/L, D) 0.2580 mg NH_4^+/L, E) 0.090 mg NH_4^+/L, F) 0.0515 mg NH_4^+/L, and G) 0.0258 mg NH_4^+/L.

	NH_4^+ Target {mg NH_4^+/L}	Actual amount of Stock added
Std. A	2.576	200 µL/100 mL
Std. B	1.288	250 µL/250 mL
Std. C	0.644	250 µL/500 mL
Std. D	0.258	200 µL/1000 mL
Std. E	0.090	70.0 µL/1000 mL
Std. F	0.0515	40.0 µL/1000 mL
Std. G	0.0258	20.0 µL/1000 mL

8.2.8 Blank Standard (DI water) is also used as one of the calibration standards (Std. H). Standards are prepared every two weeks at a minimum. Keep working standards refrigerated in HDPE storage bottles when not in use. Use clean bottles and ammonium-free DI water.

8.2.9 Quality control check solution FR50 is a simulated rain solution prepared in house targeting the 50th percentile concentration of the NADP/NTN network precipitation values.

- FH and FL QC Standards: these standards are prepared every two weeks, at a minimum, using NH_4^+ stock prepared with $(NH_4)_2SO_4$ (ammonium sulfate) from Fisher Scientific, catalog # A702-500, and PO_4^{3-} standard solution 100.0 ± 1.0 mg/L as PO_4^{3-} (Fisher Scientific, catalog # NC9657400).
- To prepare NH_4^+ stock: dry some amount of $(NH_4)_2SO_4$ at 104°C for two hours. Let the salt reach room temperature in desiccator. Weigh 3.6628 g of the salt and dissolve it in a 1L volumetric flask (do not use stir bar). The concentration of this stock is 1000 mg NH_4^+/L.
- To prepare FHXXXXXX: add 0.75 mL of 1000 mg/L NH_4^+stock and 0.5 mL of 100 mg/L PO_4^{3-} stock to 0.5 L volumetric flask with ~ 400 mL DI water and dilute to 500 mL. The target concentration of FHXXXX is NH_4^+ = 1.500 mg/L; PO_4^{3-} = 0.100 mg/L. The solution is stable for two weeks.
- To prepare FLXXXXXX: add 0.025 mL of 1000 mg/L NH_4 stock and 0.075 mL of 100 mg/L PO_4^{3-} stock to 0.5 L volumetric flask with ~ 400 mL DI water and dilute to 500 mL. The target concentration of FLXXXX is NH_4^+ = 0.050 mg/L; PO_4^{3-} = 0.015 mg/L. The solution is stable for two weeks.

8.2.11 HCl (hydrochloric acid) rinse – 83 mL concentrated HCl from Fisher Scientific (catalog # A508-P500) per liter of DI water. Used to clean the system tubing after analysis. Use caution when pouring HCl. Store in a tightly capped 1 L HDPE bottle.

8.3 Supplies

8.3.1 Vacuum Supply - used for degassing reagents before placing them on-line.

8.3.2 Magnetic Stir Plates and Stir Bars.

8.3.3 Polystyrene Sample Vials: 12 x 75 mm, 5 mL vial, 1000/pkg, Fisher Scientific catalog # 14-961-10; 22 x 90 mm and 50 mL polystyrene capped vials (Evergreen) for holding standards and quality control checks.

8.3.4 Volumetric Flasks–various sizes for reagent and standard preparation–Class A (Fisher Scientific).

8.3.5 Side arm filtering flasks – 500 mL, 1 L, and 2 L, for degassing reagents (Fisher Scientific).

8.3.6 Pipettes – Eppendorf or Rainin adjustable 10-100 µL and 100-1000 µL with corresponding sized tips (Fisher Scientific).

8.3.7 Reagent Storage Bottles–HDPE 1 L, cleaned and labeled.

8.3.8 Standard Storage Bottles–HDPE 250 mL, square, wide mouth, cleaned and labeled.

8.3.9 Sample Vial Holders–72 spaces/rack, plastic, spaces and rows numbered.

8.3.10 Parafilm® – for sample, reagent, and glassware coverage & storage.

8.3.11 Graduated cylinders of various sizes for measuring liquid reagents.

8.3.12 Laboratory Wipes – Kimwipes®, small and large (Fisher Scientific).

8.3.13 Millipore Quantum® cartridges–for final point-of-use DI water unit.

9.0 Instrument and Quantum Method Calibration

Refer to Quik Chem System Operation Manual and Method Manual for specific details for Instrument and Method Calibration.

9.1 Instrument Power Up

Turn on the computer and monitor to the FIA system. The computer will automatically connect to the network, check for viruses, and ask for log on name and user password. Switch on power strip to the FIA. Note the movement of sampler arm. Also note the sound of the valves switching on the manifold as it rotates to check its positioning. Turn the power on to the instrument at least 30 minutes prior to analysis to allow the heating unit in the chemistry module to reach 60˚C.

9.1.1 Rinse carrier water and pump tubing bottles three times before filling with DI and starting pump.

9.1.2 Once the reagents are prepared and degassed, check the pump tubing for flattening and cracks, then turn on the pump on by pressing the **Manual Run** button. Place the pump tubing into the cartridges on the sample pump and snap them into the holder. (Do not allow tubes to be clamped down without pump being on!). Pump DI water through the system. Keep checking for leaks, reagent surges, or bubbles hanging up in any transmission lines or at tubing connections.

9.2 Omnion Tray Creation

Load the software by clicking on the **Omnion icon** or "C:\ACER (C:)\Users\Public\Public Documents\FIA Files\YYYY\Template (where YYYY equals current year). Click **Yes** in the pop-up window to change temperature settings. The login ID on the computer will be the login Omnion recognizes. All the methods, analytical tables, and instrument configurations were preset and tested when the instrument was first installed and calibrated. Known working standards, quality control check solutions, and system timing have been saved in Omnion. Review specific files in the software or in the Lachat Methods Manual for details on general methodology. Refer to Appendix A for specific details.

9.3 Worksheet Edits:

9.3.1 DI check standards (FB) are inserted at the beginning and end of a run.

9.3.2 Check standards (FL, FH and FR50) are rotated randomly throughout the run after every 12^{th} sample.

9.3.3 Check the previous trays to make sure you're not repeating already ran samples.

9.3.4 Create run sequence. Scan sample bar codes into the template to create the run sequence.

9.3.5 Delete any extra rows between last sample and the last set of QCS.

9.3.6 After all edits are complete, highlight the **Cup No.** column and select **Auto Number Cups**, if needed.

It is recommended to keep run to a maximum of 75 samples to avoid drift. Create multiple runs, if needed.

9.4 Instrument Stabilization

9.4.1 Initiate the autosampler by clicking Configuration, Autosampler, Initiate Autosampler. Click **Preview** and check baseline.

9.4.2 Label all samples plus all the QCS. Load sample tubes into the rack(s).

9.4.3 Place reagent transmission lines into appropriate reagent containers in order in which they are introduced on the manifold. Check that the reagents are being drawn into the system at a smooth and continuous rate. Make sure tubing is resting on bottom of reagent bottle for proper reagent uptake. Reagent line waste is collected in a separate waste container that must be in a holding container of its own.

9.4.4 Thoroughly mix each of the calibration standards before pouring them into individually labeled standard cup holders on the sampler. Each standard cup has a specific position on the sampler module which has been predetermined and recorded in the Omnion software.

9.4.5 Once the system has equilibrated with reagents (at least 15 minutes); heating element has reached appropriate temperature (60˚C); all standards are loaded into the sampler, and the first rack of sample tubes has been poured, then analyses can begin.

10.0 Sample Collection

Wet precipitation samples to be analyzed on the FIA for NH4+ are stored in DI-water-cleaned, 60 mL HDPE bottles on trays at 4˚C ± 2˚C in the refrigerator in room 301, or in the walk-in cooler (room 214). Samples are retrieved by each analyst prior to analysis and checked for proper sequence of numbers to be analyzed.

11.0 Handling & Preservation

11.1 All samples are to be handled with care, avoiding any direct hand/body contact with the sample or interior of the bottle and lid.

11.2 Keep sample bottles tightly sealed when not being poured. Keep samples and standards covered as much as possible prior to analysis to reduce airborne contamination.

11.3 When pouring samples into the sample tubes, avoid splashing or spillage of sample into an adjoining sample tube. Once samples have been poured, make a red dot on the sample bottle cap with a red marker to indicate that the sample has been analyzed.

12.0 Sample Preparation and Analysis

12.1 Sample Preparation

No sample preparation by the analyst is necessary.

12.2 Final Instrument Check

Make final checks of all the equipment before starting a run. Check that:

- Calibration standards and QCS are poured and loaded in proper positions on the sampler and capped until analysis begins.
- Samples are poured and loaded onto the sampler in the correct positions as listed in the tray file.
- Reagents are flowing smoothly through transmission lines, onto the chemistry module, and through the flow cell to waste (no air bubbles or debris hanging up in lines).
- Valves on chemistry module are functioning correctly
- No leaks or drips are evident throughout the system
- Heating controller is displaying correct temperature (60˚ C)
- Lamp source is ON

12.3 Sample Analysis

12.3.1 Initiate the run by clicking **Start**. The file name will automatically be generated by Omnion. Example: OM_9-1-2018_01-04-18PM.OMN, where the month, day, and year are the first set of numbers followed by the hour, minute, and second.

12.3.2 Analysis begins with sampling of the calibration standards. The calibration curve will pass if a correlation of 0.999 or better is achieved. The FR50 check standard, DI water, and High and Low QC standards are then analyzed, and if values within the preset QCS limits specified by the Quality Assurance chemist are met (set in the Omnion), analysis begins.

12.3.3 Each sample takes about 1 minute from the time the probe goes into the sample cup until the report of concentration.

12.3.4 Sampling continues until all samples have been analyzed that were loaded into the “sample list” file.

12.3.5 At the end of a run, before the instrument has finished sampling, any sample values that were outside the calibration range must be manually diluted by the analyst.

12.3.6 Run a complete set of QCS at the very end of the analyses.

12.4 System Shut-down

12.4.1 Once a set of samples has been analyzed and all final QCS pass, the system will stop collecting values and original color of template will appear on the screen indicating that the run is complete.

12.4.2 Make a final check of all data collected before discarding samples. If the collection appears complete, proceed to remove reagent lines in reverse order from start. Place them in the 1M HCl cleaning solution, and close the reagent bottle lids tightly. Rinse system for 45 minutes with 1 M HCl solution (see section 8.2.11) then place the reagent tubes in DI water and rinse at least 5 minutes.

12.4.3 Unclick and release tension on pump tube cartridges prior to shutting off the pump.

12.4.4 Close Omnion - do not save files.

12.4.5 Shut down the system: post cleaning and data processing (close all files), close Omnion program, and turn off power strip to the chemistry system and data collection system.

13.0 Troubleshooting

13.1 The most common problem is occlusions in the reagent and/or sampling line. Check all “T” and straight connections on the instrument and sampler. This can be done with the pump tubes off or with DI water flowing through the system.

13.2 Check that the pump tubes are not flat or cracked. Replace all tubes if there is any doubt.

13.3 Re-pour calibration and check standards to ensure they are not contaminated and in the correct position. Remake standards if counts are low and pump tubes, reagents, and connections are good.

13.4 With the pump tubes on, check all lines for air bubbles. Tighten the connections closest to the source of the air bubbles. Check that a sufficient volume of liquid is present in the reagent bottles.

13.5 Replace Standard A in S1 cup with dye in green capped tube. Start the run. Watch the sample flow to check the timing of the valves. Check for plugs in the valve and the lines. If the valve is malfunctioning, it must be sent in for repair to Lachat. Arrangement can be made for a replacement valve while the original is being repaired. To ensure valve timing is correct, refer to Quik Chem FIA User Manual Valve Timing, p. 151.

13.6 If the sampler probe should malfunction or “jam,” analysis will automatically stop. After the problem is solved, you must recalibrate with beginning calibration standards. To complete a run, delete samples already run from the tray table and restart the run. You must recalibrate with calibration standards every time you start a new run. A new file will be generated by Omnion. Refer to Operator’s System Manual for details on restarting or repair.

13.7 Once a month, the sample probe and DI carrier lines are cleaned with 1:1 sodium hypochlorite for 5 minutes then DI rinse for 5 minutes.

14.0 Data Acquisition, Calculations & Data Reduction

14.1 Calibration Curve Formation

Once analysis begins, data is collected and calculated continuously – first with the calibration standards then the samples. The data system will prepare a calibration curve by plotting absorption responses versus standard concentrations. The sample concentrations are then calculated from the regression equation.

14.2 Data Collection

To access information on the calibration and correlation coefficient, click the **Calibration Results** box for Channel 1.This icon is on the left side of the peak table and looks like a linear graph. The run time report will automatically be in the upper right hand side of the computer screen. Sample concentrations in mg/L are reported in the order they are analyzed. QCS checks will appear in this report Check all samples for air spikes. If there are some, note these samples. The

analysis of them should be repeated. All information for the run will be saved as a file generated by Omnion.

14.3 Data Calculation

A first order polynomial regression curve is used to determine sample concentrations after an R^2 value is calculated (see Appendix C).

14.4 Data Processing

14.4.1 Close the Omnion file, then open it again. Export data by clicking on **Export data to file**. The Excel file will be created in C:\User\Public\Documents\FIAFiles\Month and Year.

14.4.1 Check the QCS data

14.4.1.1 Open LIMS Instrumental Chemistry Tool (can be found at \\pri-fs1\HEAL\HEAL-IT\Program Install Files\Lims\Instrumental Chemistry). Select **Load/Review**, **FIA Data**. A new screen will open. Select **FIA Review**, **Data IO**, **Load Review Table**, then choose the appropriate Excel file to open.

14.4.1.2 Select **FIA Review**, **Filter Data**, **Filter by Type**.

14.4.1.3 Check filter by **Type** box, from the drop down menu next to it, choose **QC** and click **Apply**. A list of the QCS will open in a new window.

- In this QCS window, click the number header to sort the QCS in order
- Left-click the actual number value collected from the run. When this cell is highlighted, right-click in it to bring up pull-down and select **Graph**. This will make a graph of all of that QCS points. All points must fall in a specific range for the QCS tested.

14.4.1.4 Follow steps 14.4.1.2 through 14.4.1.3 for every QCS checked.

14.4.1.5 After all QCS are checked, transfer the results to LIMS. Select **FIA Review**, **Data IO**, **Transfer Results to LIMS Results Table**. Close the graph window.

14.4.2 Check the sample data.

14.4.2.1 Select **FIA Review**, **Data IO**, **FIA**. This will load the samples tested from that same run.

- Left-click the grey number header cell to sort the values in numerical/alphabetical order.
- Make sure values appear for all samples and diluted sample values are correct, by multiplying the number of times diluted by the collected value for every diluted sample.

14.4.2.2 After checking, select **FIA Review**, **Data IO**, **Transfer Results to LIMS Results Table**. The data will be sent to LIMS.

14.5 Data Export

14.5.1 Open LIMS, select **LIMS Query**. Click the **LIMS Query** tab. Enter the first and last numbers of the sample range to be exported. This feature can be used to check multiple types of samples. Make sure to click on the correct tab for whichever sample type you would like to query. Samples can also be checked by number, client, or project.

14.5.2 After a few seconds the sample list will be on the screen. Scroll through the data and check for missing samples or values.

15.0 Computer Hardware & Software

Omnion software version 4.0

16.0 Data Management & Records Management

16.1 A daily log book is kept of instrument repair and maintenance, troubleshooting, reagent and standard preparations and sample analysis. This log is shared by all FIA analysts. All log books are kept permanently.

16.2 Current SOP's and instrument manuals are maintained in the lab where the instrument is located.

16.3 Data files are kept on the PC for one year from the date data is sent to the Program Office and then transferred to CD ROM for archival. See data and computer support personnel for backup and computer program assistance.

16.4 Instrument programs and files are backed up weekly through the network by computer support personnel (see *Computer Backup and Recovery*, SOP # AD-0011).

17.0 Quality Control and Quality Assurance

See Quality Assurance Plan 2019.

17.1 A calibration curve is always used at the start of each run followed by a blank. Coefficient of Determination of ≥ 99.9% for NH_4^+ is required (see section 18.5).

17.2 One solution of internally prepared simulated rain containing NH_4^+ concentrations approximating network 50^{th} percentile level is used for NH_4^+ to verify the calibration curve (FR50). Control limits and warning limits are established at the time of preparation by measuring new QC standards at least 10 times and calculating the standard deviation. Warning limits = target value ± 2σ. Control limits = target value ± 3σ. Control limits and warning limits for FLYYXXX and FHYYXXX are calculated the same way.

17.3 A DI water blank (FB) is analyzed after the calibration curve as a system check and at the end of each run. If NH_4^+ is found at or above the Method Detection Limit (MDL), another DI water blank is analyzed. If a high value still exists, DI water from another source should be analyzed. If the DI water from the separate source shows greater than the MDL concentration, further review of the system will be conducted.

17.4 Blind internal blanks, splits, replicates, and QCS are analyzed. Values are compiled and checked in LIMS by the quality assurance chemist.

17.5 QCS (FR50, FL, FH) are analyzed every 12 samples in any combination to monitor the system and verify the accuracy of data.

17.6 QCS control charts are maintained by the analyst and may be checked in LIMS upon data transfer to observe fluctuation of these samples over time. The bench analyst can observe real time data generated and make adjustments to the instrument according to the QCS results.

17.7 Accuracy is determined on a weekly schedule by reanalyzing selected samples. Report results in LIMS. If any major differences are determined between the original and the reanalysis values, then comments should be included to explain the reasons and make suggestions for any changes.

17.8 The Quality Assurance chemist oversees all QCS data collected and makes suggestions for improvement or correction.

17.9 External quality control procedures include participation in several national and international quality assurance programs. The United States Geological Survey Interlaboratory Comparison Study, World Meteorological Organization, the Norwegian Institute for Air Research (NILU), and the National Water Research Institute of Canada are 4 programs that we regularly participate in. Results are published annually/biannually.

18.0 Reference

18.1 Quik Chem Automated Ion Analyzer, Methods Manual, Lachat Instruments.

18.2 Quik Chem 8500, Automated Ion Analyzer User Manual, Lachat Instruments.

18.3 Omnion 3.0 Software User Manual, 2007. A manual is no longer produced for Omnion software. Relevant information is in the Omnion help section.

18.4 Domestic Parts and Price List, Lachat Instruments, 2005.

18.5 QuickChem Method 10-107-06-1-B. Determination of Ammonia (Phenolate) by Flow Injection Analysis Colorimetry, Lachat Instruments.

Appendix A Method Printout - Settings and Timing of NH_4^+ Analysis

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	60 samples/h, 60 s/sample
Pump Speed:	35
Cycle Period:	60

Analyte Data:

Concentration Units:	mg N as NH_3 / L
Peak Base Width:	29 s
% Width Tolerance:	100
Threshold:	10000
Inject to Peak Start:	30 s
Chemistry:	Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg N/L as NH_3	5.00	2.50	1.25	0.50	0.10	0.05	0.00

Calibration Rep Handling:	Average
Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None
Force through zero:	No

Sampler Timing:

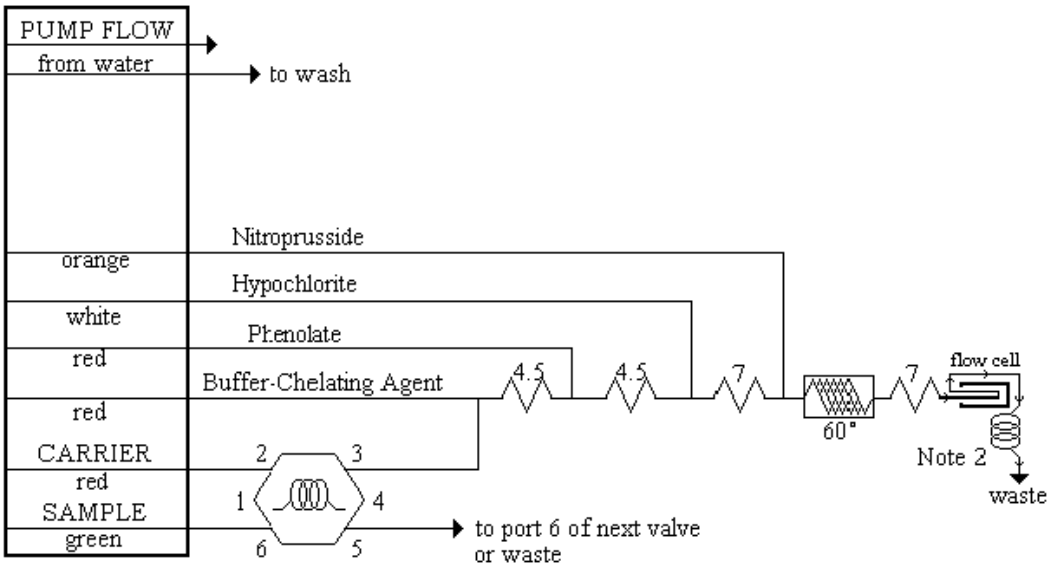
Min. Probe in Wash Period:	5.0 s
Probe in Sample Period:	24 s

Valve Timing:

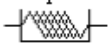
Load Period:	15 s
Inject Period:	45 s

Appendix B Manifold Diagram for NH_4^+ Analysis Method #10-107-06-1-B

17.3. AMMONIA MANIFOLD DIAGRAM



Carrier: **Reagent 5**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 75 cm
QC8000 Sample Loop: 75 cm
Interference Filter: 630 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

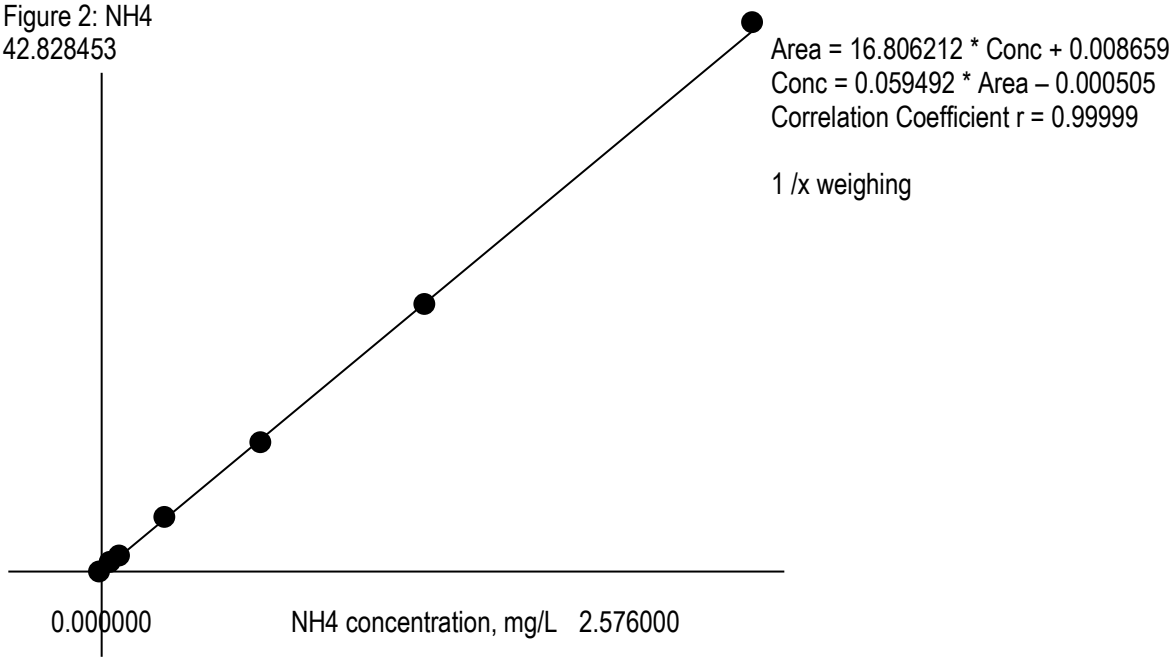
Note 1: PVC PUMP TUBES MUST BE USED FOR THIS METHOD
Note 2: 200 cm x 0.022" i.d. backpressure loop.

Appendix C Calibration Curve and Statistics

NH_4^+

	Conc. (mg/L)	Rep	Peak Area (Volt-s)	Peak Height (Volts)	% Residual	Detection Date	Detection Time
1	2.576000	1	42.828453	2.527909	1.1	3/26/2008	12:12:16 PM
2	1.288000	1	21.869013	1.298175	-1.0	3/26/2008	12:13:27 PM
3	0.644000	1	10.986296	0.653663	-1.4	3/26/2008	12:14:39 PM
4	0.258000	1	4.428546	0.261379	-1.9	3/26/2008	12:15:50 PM
5	0.090000	1	1.535005	0.089585	-.0.9	3/26/2008	12:17:03 PM
6	0.051520	1	0.885506	0.049239	-1.3	3/26/2008	12:18:16 PM
7	0.025760	1	0.437545	0.025323	0.9	3/26/2008	12:19:28 PM
8	0.000000	1	-0.010841	-0.000559		3/26/2008	12:20:42: PM

Figure 2: NH4



Appendix D FIA Ordering Guide

Vendor	Catalog No.	Description
Fisher Scientific	A931I-500	Phenol Liquid Certified, 500 mL
Fisher Scientific	S311-500	Disodium EDTA, Certified ACS, 500 g
Fisher Scientific	S318-500	Sodium Hydroxide (Pellets), Certified A.C.S., 500 g
Fisher Scientific	S350-100	Sodium Nitroprusside, Certified ACS, 100 g
Fisher Scientific	O2674-25	Sodium Dodecyl Sulfate Certified, 25 g
Fisher Scientific	BP351-500	L-Ascorbic Acid, Tissue Culture Grade, 500 g
Fisher Scientific	SS290-1	Sodium Hypochloride, Purified 4-6%, 6/case
Fisher Scientific	AC22380-1000	Potassium Antimony Tartrate C4H2KO6Sb·1.5H2O, 100 g
Fisher Scientific	A510-500	Sulfuric Acid Trace Metal Grade, 500 mL, 6/case
Fisher Scientific	A508-500	Hydrochloric Acid Trace Metal Grade, 500 mL, 6/case
Fisher Scientific	08-732-112	Polystyrene Antistatic Weighing Dishes **1 5/8 x 1 5/8 x 5/16"** *Pack of 500*
Fisher Scientific	08-732-113	Polystyrene Antistatic Weighing Dishes **3 1/2 x 3 1/2 x 1"** *Case of 500*
Fisher Scientific	06-666C	Kimwipes Delicate Task Wipers 15x17" *Case of 15 pkg*
Fisher Scientific	19-041-190D	Powder Free Vinyl Exam Gloves *Pack of 100, Case of 10 Pk*
Fisher Scientific	13-374-16	Parafilm 2" x 250'
Fisher Scientific	02-923F	Nalgene HDPE Narrow mouth bottles, 1000 mL, 6/pk, case of 4 pk
Fisher Scientific	14-961-10	Sample Tubes 1000/pk 4pk/case
Fisher Scientific	14-190-509	Yellow-Yellow manifold tubing, pack of 12
Fisher Scientific	14-190-512	Green-Green manifold tubing, pack of 12
Fisher Scientific	14-190-508	Gray-Gray manifold tubing, pack of 12
Fisher Scientific	14-190-507	Red-Red manifold tubing, pack of 12
Fisher Scientific	14-190-505	Orange-Orange manifold tubing, pack of 12
Fisher Scientific	14-190-513	Purple-Purple manifold tubing, pack of 12
Fisher Scientific	05-403-57	Eppendorf Pipet Tips (2 - 200µL). 1000/case
Fisher Scientific	05-403-59	Eppendorf Pipet Tips (50 - 1000µL). Bulk, 2 bags of 500
Fisher Scientific	05-403-59	Eppendorf Pipet Tips (50 - 1000µL). Bulk, 2 bags of 500
Fisher Scientific	50-110-6572	HACH Phosphate Standard Solution 100 mg/L as PO4, 100 mL
Fisher Scientific	A661-500	Ammonium Chloride
Fisher Scientific	A702-500	Ammonium Sulfate
HACH	50928	PTFE Manifold Tubing 15.2 m x 0.8mm id
HACH	54414	Green-Green Duraprene Pump Tubing
HACH	53410	Red-Red PVC Pump Tubing
HACH	54408	Orange-Orange Duraprene Pump Tubing
HACH	53408	Orange-Orange PVC Pump Tubing
HACH	54412	Yellow-Yellow Duraprene Pump Tubing
HACH	54415	Purple-Purple Duraprene Pump Tubing
HACH	50015	1/16" x 1/16" barbed straight fitting 6/pkg
HACH	50059	Universal food dye, 1000 mL
HACH	31069	Tungsten Halogen Lamp
HACH	50013	O-rings, 25/pkg
HACH	31201	Temperature controller for QuikChem FIA+ 8000 Series (low temperature use <=100 ° C)
HACH	52005	Sodium Phenolate Reagent
HACH	52006	Sodium Nitroprusside Reagent
HACH	52002	Molybdate Color Reagent
VHG Labs	QWSONUT – 15	Water Supply Check Sample (o-Phosphate Nutrients)
ERA	994	Phosphate as Phosphate (PO4) QC Standard 1000mg/L, 500 mL

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