1.0 TITLE

Determination of Ammonia Nitrogen by Flow Injection Analysis Colorimetry

2.0 LOCATION

Wet Chemistry Instrument Lab

3.0 SCOPE & APPLICATION

Ammonia is a biochemically inter-convertible form of nitrogen and is a component of the nitrogen cycle. It is produced largely by the deamination of organic nitrogen-containing compounds and by hydrolysis of urea. It is naturally present at varying concentrations in surface and wastewaters. Concentration of ammonia is generally low in groundwater because it adsorbs to soil particles and clays and is not leached readily from soils.

4.0 SUMMARY

4.1 Ammonia is separated from the sample matrix using gas diffusion. When ammonia is heated with salicylate and hypochlorite in an alkaline phosphate buffer, an emerald green color is produced which is proportional to the ammonia concentration. The color is intensified by the addition of sodium nitroprusside.

4.2 This procedure is applicable to drinking, ground, surface, and saline water; domestic and industrial wastes; sludge; leachates; and extracts. The working range is 0.2 to 20mg/L NH_3 as N. Higher concentrations may be determined by sample dilution.

4.3 Method 350.1 has been modified within the flexibility in 40 CFR 136.6. The method modifications are as follows:

4.3.1 Gas diffusion is used in lieu of distillation as allowed by 40 CFR 136.3 Table IB.

4.3.2 EPA method 350.1 instructs the use of 0.05% Sodium Nitroprusside. This SOP instructs the use of a stronger concentration per the instrument manufacturer's recommendation.

4.3.3 The instrument is calibrated at least once each day it is used, and the C1 standard is prepared at the upper limit. Because samples with a concentration greater than C1 must be appropriated diluted and retested, verification of the LCR every 6 months does not provide a quality control measurement pertinent to laboratory activities.

4.3.4 Salicylate is substituted for phenate in the Berthelot reaction. This is an allowed modification per 40 CFR 136.3 Table IB.

NOTE: ACZ has extended this procedure to the analysis of sludge and leachates. Although ACZ believes this procedure to be technically sound, it should not be employed for regulatory compliance monitoring of such matrices in lieu of an EPA approved method.

5.0 REFERENCES

5.1 Method 350.1, "Nitrogen, Ammonia (Colorimetric, Automated Phenate),"Methods for the Chemical Analysis of Water and Wastes, EPA-600/R-93-100-August 1993.

5.2 QuickChem Method 10-107-06-5-J, "Determination of Ammonia by Flow Injection Analysis Gas Diffusion Separation Method" Lynn Egan, Lachat Instruments, January 2015.

5.3 "QuickChem QC8500 Automated Ion Analyzer User Manual", Lachat Instruments, Hach Company 2004

6.0 SAMPLE COLLECTION, HANDLING AND PRESERVATION

6.1 Samples are preserved with sulfuric acid (H_2SO_4) to pH < 2 and are stored in bottles designated with a **yellow** dot. Samples are kept refrigerated at 0-6.0°C in the South inorganic walk-in cooler.

6.2 No hold time is specified for soils extracts from date of sampling to start of extraction. The hold time for preserved soil extracts is 28 days from date of extraction completion. Non-preserved extracts should be analyzed as soon as possible after extraction. Use un-prepped, "wet" soil or sludge samples. Inaccurate data will result if dried samples are digested (ammonia can be lost during the drying process). Samples are stored in the organic walk-in cooler on soils extract shelves, or soils sample shelves if using an as received soil at 0-6.0°C**.** Sludges are located downstairs in the sludge fridge, stored at 0-6.0°C. If prepped sample must be used, it will be located downstairs on the soils sample tables or shelving in organic walk-in cooler.

6.3 Hold Times

Parameter description	Matrix	Hold Time Starts – Ends With
N-NH3	Liquid	28 days from collection to analysis
	Sludge without extraction	
	Sludge and Soil with extraction	28 days from extraction to analysis

7.0 APPARATUS & SUPPLIES

7.1 Analytical balance, accurate to 0.0001g. Verify calibration before use (refer to SOPAD013).
7.2 Class A volumetric flasks and pipettes
7.3 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
7.4 Sampler
- 7.4.1 Multi-channel proportioning pump
- 7.4.2 Reaction unit or manifold (See table 18.1 and 18.2)
- 7.4.3 Colorimetric detector
- 7.4.4 Data system

7.5 Adjustable or fixed volume mechanical pipettes. Verify accurate delivery (refer to SOPAD013).
7.6 Heater Module (Lachat Part No. A85132)
7.7 Stir plate and magnetic stir bars
7.8 PVC PUMP TUBES MUST BE USED FOR THIS METHOD
7.9 15mL plastic centrifuge tubes
7.10 Centrifuge
7.11 Transfer pipettes
7.12 Disposable culture tubes

8.0 REAGENTS & STANDARDS[1]

NOTE: All standards and reagents must be stored separately from client samples.

8.1 Reagents Used for Lachat

NOTE: Prepare using Class A volumetric glassware and Type I H_2O, unless otherwise specified. Clean all reagent/standard glassware with copious amounts of Type II H_2O. If glassware contamination is suspected rinse glassware with 1:1 HCl solution followed by Type II H_2O rinse. If air spikes become a problem, degas all reagents with helium. .Use sonicator located in Wet-Chem manul lab by turning on pump and pressing degas button. Make sure rubber stop-cap is firmly in place on erlmeyer flask.

8.2.1 Alkaline Donor

To a 2L volumetric flask, add approximately 1600 mL Type I H_2O and 60.0 g ethylenediaminetetraacetic acid disodium salt (Na_2EDTA). Mix with a magnetic stirrer. Add 24.8 g boric acid (HBO_3). While mixing, add 80 g of sodium hydroxide (NaOH). Dilute to the mark with Type I H_2O. The pH of the solution will be approximately 13. Prepare monthly and store in glass bottles in the instrument lab at room temperature.

8.2.2 DCIC Reagent (hypochlorite generator)

In a 500 mL volumetric flask, dissolve 2.5 g of sodium hydroxide (NaOH) and 2.5 g sodium dichloroisocyanurate [dichloro-triazine 2,4,6,(1H,3H,H)-trione sodium salt] in about 300 mL of DI water. Stir to mix and dilute to volume. Prepare fresh daily. Dispose of with phenol waste. Degas with sonicator located in Wet-Chem manual lab.

8.2.3 Buffer

[1] ACZ has found inconsistency between different manufacturer's storage recommendations of standards and has decided that storing them at 0-6.0˚C in the reagent cooler is sound practice. Samples that are tested for these parameters are also stored at 0-6.0˚C. (CAR 1211)

In a 2L flask, dissolve 30 g sodium hydroxide (NaOH), 25 g ethyenediaminetetraaccetic acid disodium salt (EDTA) and 67 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$) in about 1L Type I H_2O. Dilute to the mark and stir to mix. Prepare monthly and store in glass bottles at room temperature in the instrument lab.

8.2.4 **Salycilate-Nitroprusside Color Reagent:**

In a 1L flask, dissolve 350 g sodium salycilate and 3.5g sodium nitroprusside (Sodium Nitroferricyanide [$Na_2Fe(CN)5NO \cdot 2H_2O$]) and dilute to the mark. Stir to mix. Prepare weekly or if solution turns blue and store in amber glass bottles at room temperature in the instrument lab.

8.2.5 **Carrier and Diluent**

In a 2L volumetric flask, dilute 8 mL concentrated sulfuric acid (H_2SO_4). Dilute to the mark and stir to mix. Prepare annually and store in glass bottles at room temperature in the instrument lab.

8.2.6 **1000mg/L NH_3 Calibration and Spike stock:** A 1000mg/L stock solution is purchased pre-made from a vendor. Manufacturer's expiration date applies. Store at 0-6.0°C in the **reagent** cooler.

NOTE: If purchased solution is not available, then prepare from solid. In a 1L volumetric flask, dissolve 3.8195g Ammonium Chloride (NH_4Cl) in ~500mL Type I H_2O. Add 5mL 25% H_2SO_4 and dilute to the mark. Shelf-life = 1 year; store at 0-6.0 °C in reagent cooler.

NOTE: Oven-dry the Ammonia salt at ~110ºC and cool to room temperature in desiccator.

8.2.7 **1000mg/L NH_3 ICV Stock**: A 1000mg/L stock solution is purchased pre-made from a vendor. Manufacturer's expiration date applies. Store at 0-6.0°C in the reagent cooler.

NOTE: If purchased solution is not available then prepare from solid. In a 1L volumetric flask, dissolve 4.71464g Ammonium Sulfate [$(NH_4)_2SO_4$] in ~500mL Type I H_2O. Add 5mL 25% H_2SO_4 and dilute to the mark. Shelf-life = 1 year; store 0-6.0 °C in the reagent cooler.

NOTE: Oven-dry the Ammonia salt at ~110ºC and cool to room temperature in desiccator.

8.2.8 **Working Calibration Standards**.

Ammonia standards are preserved with 250µL concentrated H_2SO_4 per 200mL. Shelf life = 3 months. Store at 0-6.0°C in the reagent cooler.

STD	C1	C2	C3	C4	C5	C6++
Total Vol. (mL)	500	500	500	*200	*200	*200
mL of Stock added	10.0	5.0	2.5	*20.0 (of C1)	*20.0 (of C3)	*2.0 (of C1)

Final Conc. (mg/L)	20	10	5	2	0.5	0.2
*** C4, C5** and **C6** may be prepared as dilutions. Dilutions must be performed manually with a Class A pipette or mechanical pipette.						
C7 (calibration blank) is Type I H_2O + 250µL concentrated H_2SO_4 per 200mL						
++ There must be a calibration standard with the same concentration as the PQL.						

8.2.9 **QC Standards.**

Instrument standards are preserved with 125µL concentrated H_2SO_4 per 100mL. ICB/CCB is Type I H2O with 125µL concentrated H_2SO_4 per 100mL. Shelf life = 3 months. Store at 0-6.0°C in the reagent cooler.

QC STD	Final Conc. (mg/L)	Stock Conc. (mg/L)	mL stock used	Final Vol. (mL)
ICV	12	1000	2.4	200
CCV (C2)	10	1000	5.0	500
Spike/LFB	10	1000	0.1	10

9.0 SAFETY

9.1 HAZARDS

This procedure does not propose to address all safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous.

9.2 SAFETY TECHNIQUE

9.2.1 Safety glasses are required and the use of gloves and lab coat is strongly recommended. Shorts and open-toed shoes are not allowed in the lab.

9.2.2 Use care when pouring and pipetting reagents. Always add acid to water. Use the proper method when washing glassware.

9.2.3 Do not eat or use tobacco products in unauthorized areas.

9.2.4 Wipe up ALL spills immediately. Implement the Emergency Response Plan if necessary.

9.2.5 Do not wear gloves or lab coat outside of the laboratory. Remove gloves before using a computer, telephone, etc.

9.2.6 Do not conduct “experiments” unrelated to the analysis.

9.3 PROTECTIVE EQUIPMENT

9.3.1 Use a fume hood when there is a potential for strong fumes.

9.3.2 A fire extinguisher is located near each analytical laboratory.

9.3.3 The emergency shower and eye wash station are located in the cyanide prep lab.

10.0 INTERFERENCES

10.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA is added to the sample in-line to prevent this problem.

10.2 Lauryl sulfate and detergents can cause low ammonia recoveries by wetting the membrane. Oil and grease will also wet the membrane.

10.3 Method interference may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

11.0 PROCEDURES

11.1 Preparation of Sludge Samples

NOTE: The spike is prepared at the instrument. Do not add spiking solution during sample prep.

11.1.1 Acquire the sludge sample from north inorganic walk in cooler or soils sludge fridge. Inspect the sludge sample to make sure it is not too high in solid content for analysis. If it is, sample needs to be re-logged for extraction in the soils department. Notify supervisor, soils supervisor and PM that the sample must be extracted.

11.1.2 Tare a labeled 50mL centrifuge tube and weigh 1g of sample into the tube. Document the exact weight on the bench sheet.

NOTE: For each client sample, verify that the sample Log-in number on the workgroup matches the sample number on the bottle. This ensures that samples are not inadvertently switched when they are poured off.

11.1.3 Dilute the contents to 50mL with 0.4% H_2SO_4.

11.1.4 Prepare a PBS (0.4% H_2SO_4).

11.1.5 Cap each tube tightly and shake the sample to mix and break apart the sludge mass. If the sample does not break after centrifuging it must be extracted by the soils department.

11.1.6 Centrifuge the tubes for 5 minutes at 3000 rpm.

11.1.7 Analyze the supernatant according to procedure in §11.2

11.2 Instrument Set-up

11.2.1 Collect samples or extracts.

11.2.2 Turn on the instrument and start the computer and then the Lachat Omnion software by clicking on the Omnion 3.0 icon.

11.2.3 Refer to Figure 18.1 to set up each reagent on the manifold in the proper location. Connect ammonia sample loop (13 cm) to positions 1 and 4 in the six port valve on the FIA (Figure 18.1). Insert the 660 nm interference filter in the FIA (see FIA 8500 series manual).

11.2.4 Check the date the manifold was last inspected. If more than one month has passed since the last inspection, then visually check and verify that all connectors and unions are clean; replace dirty o-rings, pump tube adaptors, and tee fittings; inspect reagent tubing for wear. If the reagent tubing appears flat when twisted then replace it with new tubing. Record inspection and replacements in the logbook. Write the date of inspection and initials on the manifold.

11.2.5 Connect the manifold to the unit.

11.2.5.1 Remove the tube from the tee position marked "from valve" (the furthermost left connection on the manifold).

11.2.5.2 Connect this tube to position 2 on the six port valve.

11.2.5.3 Connect the tube from position 3 on the six port valve to the tee position marked "from valve."

11.2.5.4 Connect both the 175 cm and the 650 cm heater tubing to the manifold (Refer to Figure 18.1).

11.2.6 Fit pump tubes carefully in the platons, making sure that the tabs are placed in the slots on either side to prevent tubes from twisting. Place the tubes in Type I H_2O and run H_2O through the manifold for several minutes. NOTE: Place salycilate tube in separate flask with Type I H_2O to prevent contamination.

11.2.7 Make sure the diffusion block has a membrane in it or change if necessary.

11.2.8 Place the waste line from the diffusion block into a waste container labeled "non-hazardous waste" located on the benchtop even with the instrument. At the end of the analysis or when container is full, dump contents down the sink drain.

11.2.9 Connect the FIA1/FIA2 waste line to the phenol hazardous waste container. Open the fume cabinet and remove the small cap from the phenol waste container. Place the phenol waste line into the phenol waste container.

11.2.10 Collect all reagents from the instrument lab and/or make as necessary.

11.2.11 Place pump tubing in the appropriate reagents and pump for several minutes before starting analysis to allow system to equilibrate. Allow all reagents except for salycilate to flow through manifold. After 5 minutes place the salycilate line in the reagent and allow the baseline to stabilize before starting analysis.

11.3 **Importing Workgroup**

11.3.1 In the software under the file heading "Run" click on "Open." Choose "ACZ Methods" and then "Ammonia."

11.3.2 Open "NH3-GD Template."

11.3.3 The Auto Data Quality Management (DQM) should already be set up in the existing template to run required instrument QC. To modify, highlight the DQM rows in the template and right click on the mouse. Select "Clear DQM Set," "yes." Modifications can then be made in the "properties box" to the right. Once changed, set the DQM by highlighting the DQM rows, right click and select "Define DQM set." For ICV/ICB set choose "once" under "Scheduling Options." For CCV/CCB set choose "After every N samples" and type in 10 in Enter # box and "enter." Make sure "End of Run" box is checked to automatically insert a CCV and CCB after every 10 samples and at the end of the run.

11.3.4 Omnion will display the following prompt "Do you want to change the set points of the relevant heater." Select yes.

11.3.5 Open "Ammonia Blank Template."

11.3.6 To import workgroup into the template, go under "Run" and choose "Import Worksheet Data." Cut and paste samples into the "NH3-GD Template".

11.3.7 The template begins with a calibration followed by ICV and ICB measurement before analyzing the workgroup samples. A CCV and CCB are measured after every 10 samples and at the end of each run. This procedure allows the use of a continuing calibration, whereby a calibration curve generated in a previous workgroup is used for subsequent workgroups so long as instrument QC results remain acceptable. Two consecutive attempts are allowed for a continuing calibration – if both attempts fail then the instrument must be recalibrated.

NOTE: Any workgroups in sequence following the calibration workgroup must begin with a CCV/CCB DQM set. The data set containing the valid calibration curve must be identified by its workgroup number on the data review checklist and in LIMS at AREV. The DQM template can be modified by deleting the blue calibration section and the green ICV and ICB DQM sections and copy/pasting the CCV/CCB DQM set in place of the ICV/ICB DQM set. The raw data and workgroup bench sheet must indicate which verification standard was used.

11.3.8 If a sample dilution is required, in the "Manual Dilution" column find the correct row where the sample is located and type in the correct dilution factor. Document the dilution factor on the bench sheet (include reason for dilution).

11.4 **Sample Organization & Analysis Set-up**

11.4.1 Pour off the samples according to the order on the workgroup bench sheet. A copy of the samples and their corresponding cup numbers can be printed by going to "Run" heading and choose "export worksheet data."

NOTE: For each sample, verify that the sample Log-in number on the workgroup matches the sample number on the container. This ensures that samples are not inadvertently switched when they are poured off.

Pour samples/extracts into sample tube. Perform dilutions as necessary using calibrated pipettes and **carrier.** Document dilutions and reason on the bench sheet. Samples with high sediment can be centrifuged if necessary.

11.4.2 Set up the cup tray with the samples in their correct locations.

11.4.3 Review current settings in the "Run Properties Display" box. Click on "Analytes" tab, Analyte name under Channel # to display the analyte properties in the box on the right. Make sure "Calibration Fit Type" is second order (if not then select "second order") this will apply a second order quadratic equation to fit the calibration curve generated. Make sure the weighting is set to "1/x" (if not then select "1/x").

11.4.4 Start the analysis by clicking the green **Start** button.

11.5 **Calibration Verification**

Calibrate the instrument by analyzing the standards in order of decreasing concentration, ending with the calibration blank. Check the calibration under the calibration icon. "R" must be $\geq$ 0.995. If r <0.995 then try again. There are several factors that can lead to a failing calibration. If none of the following apply then remake the standards. Refer also to §11.6 for troubleshooting.

- Are the correct standards in their corresponding cups?
- Is the autosampler sampling the correct cups?
- Is the tray set up properly for the calibration?
- Are the sample tubes flowing correctly?
- Reagent contamination?
- Replace the Type I H_2O carrier with fresh, degassed Type I H_2O.
- Is the heater set at the correct temperature and functioning properly?

11.6 **Troubleshooting**

Indications of impaired analytical capability should be inspected immediately and resolved prior to continuing analysis. Effective troubleshooting employs a logical, systematic approach. Common problems encountered with this procedure include the following. If problems continue after going through these possible scenarios call LACHAT. Any general maintenance, troubleshooting, repairs, calls to Lachat technical support, etc. must be documented in the instrument and/or maintenance logbook(s).

- Are the platons crimped down on the pump tubes? Peristaltic pump turned on?
- Is the sample tray sitting correctly on the autosampler tray?
- Are the standards placed correctly on the auto sampler rack (labeled 1-16)?
- Are leaks occurring anywhere on the manifold? Cracks or breaks in manifold tubing?
- Do the pump tubes need to be changed?
- Is there a sufficient amount of rinse water in the carboy for the auto sampler?
- Is the drain carboy for the auto sampler full and needing to be emptied?
- Is the heating module set to the correct temperature for the analyte?
- Is the correct manifold and filter being used for the analyte?
- Does the analyte method require a backpressure loop?
- If contamination present, check the auto rinse carboy.
- Are the pump tubes in the correct reagents?
- Is the correct "Method, DQM, and Channel" defined within the software?
- Are clogs anywhere on the manifold or in pump tubes? Drain lines crimped or plugged?
- If baseline is OK, then computer is not communicating with the instrument. Restart.
- If the software is incorrectly marking peaks then valve timing, threshold and or inject to peak start may need to be changed in the Analyte table.
- If there is no reading, check the bulb and channel.

- If the baseline is jumpy (huge peaks or noisy) then check for air bubbles passing through the manifold and make sure the flow cell is properly set within the detector.
- Noisy baselines can also be attributed to a bad detector, bad fluidics, etc. If the detector is suspected, exchange with another upon the instrument. Fluidics could be pinpointed to pump tubes, clogs, or a degenerating pump.
- If the manifold tubing around the heater is discolored, a 10% HCl solution can be pumped through the manifold to clean the stained tubing followed by H20 then an NaOH solution to recondition the lines. Always run H20 through manifold for several minutes after cleaning procedure.
- Does the gas diffusion membrane need to be changed?
- The waste (leading to the phenol waste container) should be at a pH of ~11. If not, proper mixing ratios of the reagents is suspect.
- If salicylate precipates into manifold lines, run NaOH through manifold to bring it back into solution. Follow with extensive H20 rinse.

11.7 **Failing QC and "R" Groups**

If any non-conformance occurs and the final CCV/CCB have been sampled then an "R" group must be run. An "R" group includes the samples which need to be retested and required instrument QC. Create an "R" group by editing the current tray delete the calibration and ICV/ICB DQM sets. As with any continuing calibration group, the group should begin with a CCV/CCB, deleting any samples that won't be included in the R group and only the desired samples remain and the tray. Start the run by clicking the green arrow. Save the run by adding an R to the end (i.e. save WG145889 as WG145889R).

NOTE: Dilute samples with a concentration greater than the high standard using a mechanical pipette and **carrier**. The concentration of the diluted sample must be > PQL or the reason for reporting a concentration < PQL must be documented on the bench sheet and the data must be appropriately qualified.

11.8 **Run Completion and Data Export**

11.8.1 After the analysis has ended save the data file by selecting "Run" (upper left hand corner of display) "Save File As," type the workgroup number for the file name.

11.8.2 Under "Tools" choose "Custom Report." Click on the arrows to ensure that all required information is included. Make sure all samples appear on the chromatogram. To alter the format go under "Report" and choose "Format." The # of peaks per chart can be changed under "Charts." All chromatograms should be roughly the same size. To zoom in or out on the chromatogram, alter the view under the channel view on the main page. This will change the view in the Custom Report. When satisfied with the chromatogram, select "Custom Report" and printer icon.

11.8.3 Close the run file by selecting "Run" and then "Close."

11.8.4 Reopen the file and click the "Run" tab in the "Run Properties" display box. Click on "Export Data to File" box. The WG will be automatically exported to U:\Waitload\FIA1 or FIA2.

NOTE: To attach an "R" group, open the "R" group in waitload, highlight all data (ignoring the heading columns) and copy. Open the original WG in waitload, paste the copied "R" group data at the end of the original WG data and save.

11.8.5 If the workgroup is ready for review (AREV) then go to Waitload and drag or copy the WG into U:\Autoload\FIA1 or U:\Autoload\FIA2. An upload file will be created and the WG will be available for AREV in LIMS.

11.8.6 If a workgroup needs additional editing ("R" group, dilution factors must be entered, or soil data need to be entered) those changes can be made while the WG is in waitload. If changes are not made before the WG is sent to Autoload, make changes in the Excel version of the Uploads file, open the WG at AREV, select errors and re-upload. This will upload the most current changes made in the Upload file.

11.8.7 If the file does not export then verify the WG number is correct.

11.8.8 Review the workgroup in LIMS (AREV), completing a data review checklist (FRMWC006). Note any QC failures, sample o-cals, etc. on the review form and document the corrective action in the appropriate column. Attach all raw data, the data review form/checklist, and a current standard/reagent form to the bench sheet, and turn in data package for SREV.

11.8.9 Record all pertinent workgroup analysis information in Lachat instrument logbook. Also record any changes made to the instrument manifold and maintenance operations performed.

11.9 **Lachat Cleaning Procedure**

11.9.1 Place the Salycilate line in a separate flask containing type I water and allow the water to pump through the manifold for ~5min. Then take pump tubes out of reagents and place them in a separate flask containing Type I water. Allow the instrument to pump water through for at least 5-10 minutes.

11.9.2 Take pump tubes out of water and allow air to pump through the entire manifold. After air has passed completely through the manifold, unclamp the platons. Clean the surfaces and rods/moving parts of the sampler. Clean the pump surfaces and rinse cartridges. Clean and dry system unit.

11.9.3 **Open the fume cabinet and remove the phenol waste line from the phenol waste container and place tubes in secondary container in case of any dripping. Replace cap on phenol waste container.**

11.9.4 Reseal reagents and return to correct storage location.

11.9.5 Routine maintenance must be performed monthly, semianually and yearly as prescribed by the LACHAT FIA User Manual. Refer to the manual for maintenance procedures. All maintenance or inspections must be documented in instrument logbook and Lachat Maintenance Log FRMWC017.

12.0 QUALITY CONTROL

To ensure data validity and quality, a series of QC samples are analyzed with each analytical run. These are required by the methods published by EPA and other regulatory agencies. QC limits and required corrective actions are stated in Table 12.1

12.1 Calibrate the instrument each day prior to analysis of the first workgroup.

12.2 Analyze the ICV and then ICB immediately following calibration.

12.3 Analyze one LFB and one LRB (or PBS) for every 20 or less client samples.

12.4 Analyze one matrix duplicate (DUP) for every 10 or less H_2O client samples.

12.5 Analyze one matrix duplicate (DUP) for every 20 or less solid or extract samples.

12.6 Analyze one spike (LFM) for every 10 or less H_2O client samples (20 or less extract samples).

12.7 Analyze one spiked sample (MS) for every 20 or less soil or sludge samples.

12.8 Analyze a CCV and then a CCB after every 10 samples and at the end of the analysis.

Table 12.1

Standard	Limits	Corrective action if QC fails
Calibration	r ≥ 0.995	Recalibrate.
ICV	90-110%	1 retest OK then recalibrate. Redo samples associated with a failed ICV.
CCV	90-110%	Re-analyze associated samples. Client samples < MDL can be accepted and reported with the appropriate qualifier if bracketing CCV fails high.
ICB	3X - MDL ≤ sx < MDL	1 retest OK then recalibrate. Redo all samples < 10X recovered ICB value.
CCB	3X - MDL ≤ sx < MDL	Re-analyze all associated samples. Client samples < MDL can be accepted and reported with the appropriate qualifier if bracketing CCB fails high.
LFB	90-110%	1 retest OK then REDO all associated samples. Client samples < MDL can be accepted and reported with the appropriate qualifier if the LFB fails high.
LFM/AS (H_2O)	90-110%	Flag associated data due to matrix interference if all other QC passes.
MS/AS (soils)	75-125%	Flag associated data if all instrument QC passes.
DUP	RPD ≤ 20	1 retest OK then REDO associated samples (H_2O) if [sx] > 10x MDL. Flag data for soils.
LRB (H_2O)	3X - MDL ≤ sx < MDL	REDO all samples < 10 x LRB. Client samples < MDL can be accepted and reported with the appropriate qualifier if the LRB fails high.
PBS (solids)	3X - MDL ≤ sx < MDL	REDO all samples < 10 x PBS. Client samples < MDL can be accepted and reported with the appropriate qualifier if the PBS fails high.

13.0 CALCULATIONS, DATA REPORTING & ARCHIVING

13.1 QUADRATIC CALIBRATION REGRESSION EQUATION WITH 1/X WEIGHTING FACTOR:

$y=ax^2+bx+c$

WHERE:
y=sample concentration (C_x) in mg/L Chloride
x= peak area (A_x) in Vs
c=y-intercept

13.1.1 x^2 Coefficient (a) $= \frac{(Sx^2y * Sxx) - (Sxy * Sxx^2)}{(Sxx * Sx^2x^2) - (Sxx^2)^2}$

13.1.1.1 $Sx^2y = \left(\sum_i^n w_i x_i^2 y_i\right) - \frac{\left(\sum_i^n w_i x_i^2 \times \sum_i^n w_i y_i\right)}{n}$

WHERE:

w_i = weighting factor for the i^{th} calibration standard; equals 1/xi for all but C7, which equals 1/C6.
x_i=peak area for the i^{th} calibration standard
y_i= expected concentration for the i^{th} calibration standard
n= response factor count
i→n = calibration points in order of decreasing concentration

13.1.1.2 $Sxx = \left(\sum_i^n w_i x_i^2\right) - \frac{\left(\sum_i^n w_i x_i^2\right)^2}{n}$

13.1.1.3 $Sx^2x^2 = \left(\sum_i^n w_i x_i^4\right) - \frac{\left(\sum_i^n w_i x_i^2\right)^2}{n}$

13.1.1.4 $Sxx^2 = \left(\sum_i^n w_i x_i^3\right) - \frac{\left(\sum_i^n w_i x_i \times \sum_i^n w_i x_i^2\right)}{n}$

13.1.2 x Coefficient (b) $= \frac{(Sxy * Sx^2x^2) - (Sx^2y * Sxx^2)}{(Sxx * Sx^2x^2) - (Sxx^2)^2}$

$$Sxy = \left(\sum_i^n w_i x_i y_i\right) - \frac{\left(\sum_i^n w_i x_i \times \sum_i^n w_i y_i\right)}{n}$$

13.1.3 Intercept (c) $= \frac{\sum_i^n y_i}{n} - b\left(\frac{\sum_i^n x_i}{n}\right) - a\left(\frac{\sum_i^n x_i^2}{n}\right)$

13.1.4 Concentration (x) $= \frac{\sqrt{(b^2 - 4a(c - y_i))} - b}{2a}$

13.1.5 Calibration Coefficient (R) $= \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \Sigma(y - \bar{y})^2}}$

where:
y= theoretical concentration for the i^{th} calibration standard
$\bar{y}$= mean theoretical concentration for calibration standards
x= observed concentration for the i^{th} calibration standard
$\bar{x}$= mean observed concentration for calibration standards

Note: FRMQA049 may be used to calculate calibration coefficients and convert signal response to concentration. Use the worksheet titled "ESTD IO FIA". This worksheet was customized for the Omnion software and flips the X and Y axes (Y values are concentration and X values are response). Additionally, the coefficient of determination (R) and quadratic on-column concentration equations were customized to match Omnion's equation. Enter the known concentration in the Y-values column and the corresponding peak areas in the X-values column. The weighting factor is 1/x where x equals the known concentration for the corresponding calibration point (e.g. if the standard is 20 ppb the weighting factor would be 1/20, or 0.05). For the zero point calibration, enter the same weighting factor used for the low standard. The calculated coefficients will match those specified in the Omnion calibration report concentration equation.

13.2 Method Detection Limit (MDL) & Practical Quantitation Limit (PQL):

NH3	MDL = 0.05mg/L	PQL = 0.2mg/L
NH3-KCL	MDL = 0.1 mg/Kg	PQL = 0.5 mg/Kg

13.3 Report aqueous (including leachates) results in mg/L NH_3 as N. Sludge results are reported as % mass. The MDL and PQL are calculated for each sample; variation in % solid significantly impacts the MDL and PQL reported to the client.

13.4 Report only those values between the lowest and highest calibration standards. LIMS will automatically qualify all data with a concentration less than the MDL with a "U" and all data with a concentration less than the PQL with a "B."

13.5 Samples exceeding the highest standard must be diluted and reanalyzed. Samples analyzed on a dilution that upload with a "U" or "B" qualifier must be reanalyzed on a smaller dilution unless the dilution is necessitated by the sample matrix or volume.

13.6 Calculate the prep dilution factor for soil/sludge samples using the following equation. Be sure to use the proper dry weight value for the type of sample used (see NOTE below):

$$Dilution = \frac{V_t}{(w)(\%solids)}$$

Where: V_t = total volume in mL (must equal the initial volume of standard used before prep)
w = weight in grams
%solids = the **fraction** of the sample matrix composed of solids (always ≤ 1.00)

NOTE: If the sample is a sludge (unprepped by soils), then use the total solids value from Seedpak. If the sample used is a soil (prepared by the soils dept.) then use the air dry value available from the soils dept. If the soil sample is not prepped by the soils dept., use the percent solids value from Seedpak.

13.7 Percent Recovery (%R) is determined using the following equation:

% R = [(measured value) / (true value)] * 100

13.8 Relative Percent Difference (RPD) is determined using the following equation:

$$RPD = \frac{(S - D)}{[(S + D) / 2]} \times 100$$

Where: S = Sample Value and D = Duplicate Value

13.9 Refer to SOPAD044 for details of instrument data backup and archiving.

13.10 Retrieval of archived data files:

13.10.1 Go to Labweb.
13.10.2 Change the internet address to 'http://saloon'.
13.10.3 Log onto the internet by typing user name and password.
13.10.4 Click on 'Instrument', 'FIA1' or 'FIA2', 'Program Files', 'Lachat', 'Omnion', 'Data', 'ACZ Methods', 'Ammonia'.
13.10.5 Right click on workgroup filename (WG number), select 'Save Target As…' and save file to local desk top computer in C://Program file/Lachat/Omnion/Data/ACZ Methods/Ammonia directory.
13.10.6 Click 'Close' after saving file.
13.10.7 Open Omnion software and open saved archive file in the Ammonia directory.

14.0 METHOD PERFORMANCE / DETECTION LIMITS

14.1 Method Detection Limit Study: A MDL must be established as part of the initial method development, **every 6 months**, each time there is a change in the method that affects how the test is performed, and when a change in instrumentation occurs that affects the sensitivity of the analysis.

Spike at least seven aliquots of reagent blank at 1-5x MDL.. The analysis must include a passing calibration (a continuing calibration is permitted). Enter the data into form FRMAD031 in LabWeb. Do not include outliers or non-detect values. Print the form and sign it. Attach all raw data and supporting documentation and submit the data package to the department supervisor for review. Refer to SOPAD001 for more detail.

14.2 Demonstration of Capability (DOC): A successful initial DOC (IDOC) must be completed and approved by the QA/QC department for each analyst prior to independent generation or review of client data. Continued proficiency must be demonstrated and documented annually (CDOC) for analysts who routinely perform the procedure.

Prepare and analyze four individual aliquots of the ICV. Include a passing calibration, ICV/ICB and CCV/CCB. Enter the results on the DOC form (FRMAD023), attach all raw data, any supporting documentation, and turn in to the department supervisor for review. For CDOCs analysts may also use four consecutive passing LFBs from client workgroups or a passing PT sample.

14.3 A linear calibration range (LCR) study must be performed during initial method development, whenever the calibration range is extended, or whenever a significant change in instrument response is observed or expected.

NOTE: For initial development of a calibration range refer to Method EPA 350.1.

14.3.1 Calibrate the instrument. R must be $\geq$ 0.995 or the instrument must be recalibrated.

14.3.2 Verify the existing calibration range by analyzing a calibration blank and at least three standards (one prepared at the upper calibration limit). Include a passing ICV/ICB and CCV/CCB.

14.3.3 Compare the observed concentration to the actual concentration. The values must agree within +/- 10%. If this is not the case, then either determine the cause of the discrepancy and perform another study at the same concentrations or analyze additional standards prepared at lower concentrations until the observed concentration agrees within 10% of the true value. The upper limit cannot be greater than the highest concentration that meets this criterion.

14.3.4 The calibration range may be extended if a higher concentration is within +/- 10%.

14.3.5 Enter results in the appropriate section of FRMQA029. Attach all raw data and supporting documentation and turn in to the department supervisor for review.

14.3.6 C1 concentration must reflect any change to the upper limit of the calibration range.

15.0 DOCUMENTATION

15.1 Record the pertinent information for all prepared standards and reagents in LIMS or the Wet Chem Standards/Reagent Logbook. Include analyst's initials, prep date, expiration date, and the PCN or SCN of any stock standard or reagent used.

15.2 Label each standard or reagent prepared in §8.0 with the following information:

- standard name
- SCN (or other unique ID)
- preparer's initials
- expiration date
- prep date

15.3 Make sure the following information is included on the Workgroup:

- Dilution factors, including dilutions made at prep.
- Analysis date, start time and completion time.
- Analyst's initials.
- Any remarks about analysis or samples.
- ANY OTHER PERTINENT INFORMATION about samples preparation or analysis.

15.4 Record all pertinent information for each workgroup analysis in the instrument logbook.

15.5 Attach a completed data review form and a current standard/reagent form to each workgroup.

16.0 WASTE MANAGEMENT/POLLUTION PREVENTION

16.1 Auto-sampler rinse waste and instrument waste are collected in receptacles next to the instrument and is emptied into the ACZ neutralization tank.

16.2 Phenol (sodium salicylate) waste is disposed of as hazardous waste. Contact ACZ's CHO for disposal when phenol waste container is ~ 3/4 full.

16.3 DCIC reagent must be disposed of along with phenol waste (hypochlorite waste) as it contains high concentrations of hypochlorite that is extremely toxic to aquatic life and has longstanding effects to environment.

17.0 DEFINITIONS

17.1 Calibration Standards –Calibrate the instrument at the beginning of each working day using at least three standards and one calibration blank. The calibration blank is a reagent blank. The calibration curve must be linear and r must be ≥ 0.995.

17.2 Initial Calibration Verification Standard (ICV) –A solution of method analyte(s) of known concentration(s) intended to determine the validity of the instrument calibration. The ICV must be analyzed immediately after each calibration and must be prepared from a source independent of the calibration standards, preferably from a different manufacturer. The ICV is equivalent to the QCS referenced in the method.

17.3 Initial Calibration Blank (ICB) –This solution is the same as the calibration blank and confirms the absence of background contamination in the calibration blank. The ICB is analyzed immediately

after the ICV.

17.4 Continuing Calibration Verification Standard (CCV). A solution of method analytes of known concentrations used to confirm the continued calibration of the instrument.

17.5 Continuing Calibration Blank (CCB) –The same solution as the calibration blank, it detects baseline drift in the calibration of the instrument.

17.6 Laboratory Reagent Blank or Prep Blank Soil (LRB or PBS) –A reagent blank carried through the entire preparation scheme. It is treated exactly as a sample, including exposure to all glassware, equipment, and reagents, and confirms the absence of contamination in the prep stages (not required for non-digested analysis).

17.7 Laboratory Fortified Blank (LFB) – An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and determines whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

17.8 Matrix Spike (LFM or MS) –A sample spiked with a known amount of analyte and carried through the preparation steps along with the samples. The matrix spike confirms the appropriateness of the sample prep scheme to the sample and estimates the accuracy of the method. MS nomenclature is used for solid/sludge samples; LFM nomenclature is used for aqueous samples.

17.9 Analytical Spike (AS) –The AS is required for samples that are not prepped prior to analysis. It is prepared exactly the same way as the LFB, only spiking into sample instead of reagent blank. The AS demonstrates the absence or presence of interfering elements in the matrix.

17.10 Duplicate (DUP) –A second aliquot of a client sample prepared and analyzed like all other client samples in the same workgroup. The DUP demonstrates the precision of the method.

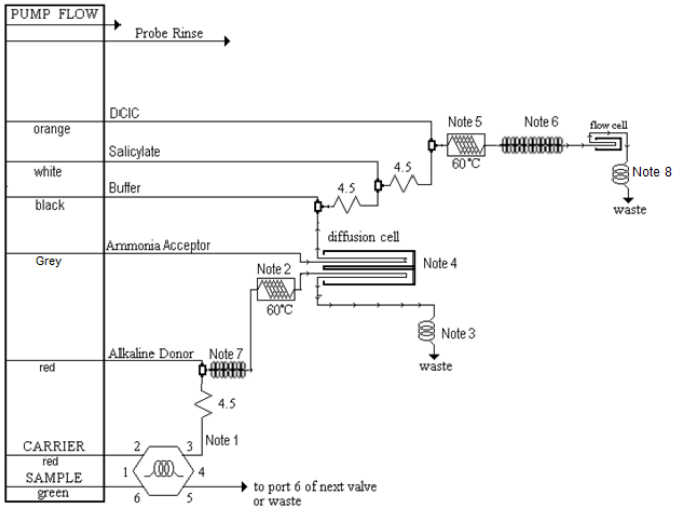
17.11 Relative Percent Difference (RPD)–The difference between two replicates (a sample and its duplicate) divided by the average of those two replicates times 100.

17.12 Method Detection Limit (MDL) –The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

17.13 PQL-Practical Quantitation Limit—The lowest level that can be reliably reported within method specified limits during routine operations.

18.0 TABLES & DIAGRAMS

Figure 18.1 – Ammonia Manifold Diagram



Carrier: **DI water for ammonia or Reagent 5 for TKN**
Acceptor: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
QC8000/8500 Sample Loop: 350 cm Low Range Ammonia
80 cm High Range Ammonia and TKN
Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 or 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

tubing wrapped in a figure 8 around 2, 7 or 22 cm coil supports (see below)

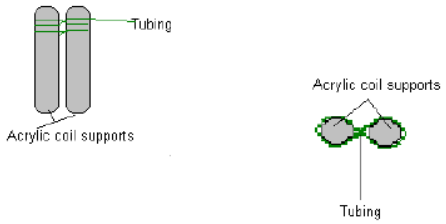
PVC PUMP TUBES MUST BE USED WITH THIS METHOD.

Note 1: 30 cm of manifold tubing is used to connect Port 3 to the first tee.
Note 2: 175 cm of tubing on the heater
Note 3 400 cm of 0.022" i.d. tubing backpressure loop, then to waste. **Waste container for the diffusion block should be on the bench with the instrument.** Placing this on the floor can lead to poor precision due to cavitation/vapor lock.
Note 4: Diffusion block with membrane PN 5033101-10 (Pack of 10 3.5 x 8.6 mm). Flow is **concurrent.** Be sure to place a membrane in the block before beginning. **Run water in place of all reagents to be sure there are no leaks.**

Note 5: 650 cm of tubing on the heater

Note 6: **510** cm of tubing wrapped in a figure 8 around 2, 22 cm coil supports

Note 7: **200** cm of tubing wrapped in a figure 8 around 2, 7 cm coil supports



Note 8: 50 cm of 0.022" i.d. tubing backpressure loop, then to waste

19.0 CORRECTIVE ACTION

19.1 For QC samples that do not meet the method acceptance criteria, refer to Table 12.1. For retests that will occur past the method hold time, check first with the department supervisor to determine if the reanalysis should be conducted.

19.2 For any SOP/method deviation fill out section 1 of a corrective action report (FRMQA001). If necessary, the department supervisor and/or project manager may provide additional information in the appropriate sections; however, QA does not need to close a minor corrective action. Attach a copy of the minor corrective action report to all workgroups affected. A minor corrective action is for documentation purposes – any SOP or method deviation may be noted on the data review checklist or on the workgroup bench sheet in lieu of using FRMQA001.

19.3 For any system failure a major corrective must be opened and the problem investigated. A department supervisor or the QA Officer can open a major corrective action. The corrective action will be assigned a unique tracking number by the QA Officer or delegate and will be closed by the QA Officer or delegate once the failure has been resolved. Use FRMQA001 (in Labweb).