DETERMINATION OF AMMONIA IN WATER USING THE AA3 GAS DIFFUSION SEGMENTED FLOW ANALYZER

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1. SCOPE AND APPLICATION

1.1. This SOP is applicable to the determination of ammonia in surface, ground, effluents, influents, and saline water. Tests covered by this SOP are W-NH3 and W-NH3-F.

Calibration range	0 – 0.20 mg N/L
Minimum Detection Limit (MDL)	0.002 mg N/L
Practical Quantitation Limit (PQL)	0.005 mg N/L

The calibration range may be extended by dilution.

2. SUMMARY OF THE METHOD

2.1. This SOP is based upon EPA Method 350.1, Rev. 2.0 (1993) and SEAL Auto Analyzer Method G-427-14 Rev. 3.

2.2. The sample is air-segmented and made alkaline in the donor stream. The ammonia molecules generated at this pH flow into the dialysis block holding the gas diffusion membrane. On the other side of the gas diffusion membrane is an acidic acceptor stream that the ammonia gas diffuses into. The ammonia reacts with salicylate and dichloro-isocyanuric acid at 37˚C to produce a blue-green color proportional to the ammonia concentration. Sodium nitroferricyanide is used as a catalyst. The absorbance is measured at 660 nm.

2.3. Interferences: EDTA is added to eliminate the precipitation of heavy metals. Samples with background absorbance do not interfere because interferents with color do not diffuse. Samples with particulates are filtered prior to analysis. Interferences may occur if the dialyzer donor stream does not reach a pH of at least 12.6.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – SEAL Analytical AA3 HR Segmented Flow Analyzer System ("Bran Luebbe"):

Component	Manufacturer	Model	Application Notes
Autosampler	SEAL Analytical	XY-3	Probe speed: Fast
Pump	SEAL Analytical	AA3 Pump 4	Pump speed: Normal
Manifold	SEAL Analytical	AA3 HR	6" Dialyzer Heater Assembly at 37°C
Colorimetric Detector	SEAL Analytical	AA3 HR	Flowcell: 10 mm Filter: 660 nm
Software	SEAL Analytical	AACE 7.10	

3.2. Equipment:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Analytical Balance	Mettler Toledo	XSR105
Analytical Balance	Denver Instrument	XS-410
Stirrer/Hot Plate	Corning	PC-420

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Stir bar	Fisher (SR)	14-513-61
Drying Oven	ThermoScientific	Lindberg Blue M
Desiccator Cabinet	Fisherbrand	08-645-6
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher	Research Plus, 3123000080
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-212B; 10-210-5D; 10-212E; 10-212-5L; 10-208M
Graduated Cylinder, Class A, Assorted	Fisher (SR)	08-553A; 08-548D
Glass Beaker, Assorted	Fisher (SR)	02-540E; 02-540G; 02-540K; 02-555-20E
Or Equivalent [1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom		

3.3. Consumable and frequently replaced supplies:

Supplies/Consumables	Vendor[1]	Catalog Number
Pump Tubing, ORN/WHT (0.64mm ID)	Oregon Manufacturing Support	170-0596-06
Pump Tubing, BLK/BLK (0.32 mL/min)	SEAL Analytical	116-0549-07
Pump Tubing, GRY/GRY (1.00 mL/min)	SEAL Analytical	116-0549-11
Pump Tubing, GRN/GRN (2.00 mL/min)	SEAL Analytical	116-0549-14
Pump Tubing, Airbar	SEAL Analytical	117+0540-07
Membrane, PTFE, 6”	SEAL Analytical	170+B810-01
Pump Lubricant and Oil	SEAL Analytical	590-0161-01; 538+9000-01
Pump and Autosampler Cleaning Swabs	SEAL Analytical	AQCSW-20
Pipette tips, Eppendorf, 200 µL	Fisher (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
pH Test Paper, MColorpHAst, 11.0 – 13.0	Fisher	M1095450001
pH Test Paper, Fisherbrand, 12.5 – 14.0	Fisher	13-640-505
pH Test Paper, Hydrion, 0.0 – 13.0	Fisher (SR)	14-850-2
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Sample Tubes, 13 x 100 mm	Fisher (SR)	14-961-27
Standard Rack Tubes, 16 x 100 mm	Fisher	14-962-10D
Centrifuge Tubes, Polypropylene, 15 mL	Fisher (SR)	05-538-59A
Syringe, Polypropylene, Assorted	Fisher (SR)	22-204-500; B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Reagent Bottles, Polyethylene, Assorted	Fisher (SR)	05-716-151; 05-719-146; 05-719-145; 05-719-88
Reagent Bottles, Amber Glass, Assorted	Fisher (SR)	05-719-75; 05-719-183
Parafilm	Fisher (SR)	13-374-12
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

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4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Prior to using the chemicals in this analysis, read their MSDS and the safety notes found in section 10 of this SOP.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

Note: Reagent and standard preparations are documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the standard and reagent sheets submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent, standard, and standard reference material is maintained in the following folder: \\floridadep\data\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Ammonia\CertificatesofAnalysis\. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

4.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Ammonium Chloride	NH_4Cl	Fisher Chemical	A661-500	ACS, ≥99.5%	See CoA	Room Temp
4.1.2.	Dichloroisocyanuric acid sodium salt, DIC	$C_3Cl_2N_3NaO_3$ *$2H_2O$	Acros Organics	436450500	≥97%	N/A	Room Temp
4.1.3.	Potassium sodium tartrate tetrahydrate	$C_4H_4KNaO_6$ *$4H_2O$	Fisher Chemical (SR)	S387-500	ACS, ≥99%	See CoA	Room Temp
4.1.4.	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	ACS, ≥97%	See CoA	Room Temp
4.1.5.	di-Sodium hydrogen phosphate-12-hydrate	Na_2HPO_4 *$12H_2O$	Acros Organics	448160010	≥99%	N/A	Room Temp
4.1.6.	Sodium nitroferricyanide	$Na_2[Fe(CN)_5NO]$*$2H_2O$	Fisher Chemical	S350-100	ACS, ≥99%	See CoA	Room Temp
4.1.7.	Sodium salicylate	$NaC_7H_5O_3$	Sigma Aldrich	S3007-500G	≥99.5%	See CoA	Room Temp
4.1.8.	Sulfuric acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus, ≥95%	See CoA	Room Temp
4.1.9.	Tetrasodium EDTA dihydrate	$Na_3C_{10}H_{12}O_8N_2$*$2H_2O$	Acros Organics	46085000	≥99%	N/A	Room Temp
4.1.10.	Triton X-100	Surfactant	Fisher Bioreagents	BP151-500	Electrophoresis	See CoA	Room Temp
4.1.11.	Simple Nutrients, WasteWatR	ALTQC, ERA	ERA	505	See CoA	See CoA	Room Temp
4.1.12.	Hydrochloric Acid	HCl, For cleaning	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	See CoA	Room Temp
4.1.13.	Bleach, Generic	NaOCl, For cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	See CoA	Fridge
4.1.14.	DI Water	H_2O	In House	N/A	N/A	Daily	N/A
Or Equivalent **[1]Note: (SR) designates supply is typically ordered through the in-house stockroom**							

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Note: Wash all glassware and bottles with 1:1 HCl and rinse with DI water sufficiently to remove all traces of hydrochloric acid before preparing reagents and standards. Make sure any containers that are used to store prepared reagents and standards are clean. Neutralize the 1:1 HCl with sodium bicarbonate. Do not discard down the sink.

4.2. Reagents:

4.2.1. Triton X-100, 30% Solution –

DI water (4.1.14.)	70 mL
Triton X-100 (4.1.10.)	30 mL

In a 100mL beaker with a stir bar, add 70 mL of DI water. Use a graduated cylinder to measure 30 mL of Triton-X-100. Place the beaker on a stir plate then add the Triton-X to the DI water very slowly while stirring. Continue stirring overnight covered with parafilm to dissolve.

Do not apply heat to dissolve.

Store at room temperature. Holding time, 90 days.

4.2.2. System Wash Solution –

Triton X-100, 30% (4.2.1.)	2.5 mL
DI water (4.1.14.)	to 1000 mL

In a 1000 mL volumetric flask containing approx. 800 mL of DI water, add 2.5 mL of Triton X-100, 30% solution. Invert to mix, then bring to volume with DI water.

Store at room temperature. Holding time, 7 days.

4.2.3. Diluent –

Sulfuric acid (4.1.8.)	4 mL
DI water (4.1.14.)	to 2000 mL

In a 2 L volumetric flask containing approx. 1.8 L of DI water, add 4 mL of concentrated Sulfuric acid. Dilute to mark and invert to mix.

Store at room temperature. Prepare fresh daily.

Note: Diluent is used for CARs, BLKs, MBLKs, CCBs, the Cal 1 Standard, and to prepare dilutions of samples. (See 4.3.3.)

4.2.4. EDTA/NaOH Reagent –

Tetrasodium EDTA dihydrate (4.1.9.)	28 g
Sodium Hydroxide (4.1.4.)	11.75 g
DI water (4.1.14.)	to 500 mL
Triton X-100, 30% (4.2.1.)	2.75 mL

In a 500 mL volumetric flask containing approx. 200 mL of DI water, dissolve 28 g of tetrasodium EDTA dihydrate and 11.75 g of NaOH. Bring to volume with DI water. Next, add 2.75 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

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4.2.5. Sulfuric Acid Reagent –

Sulfuric acid (4.1.8.)	6 mL
DI water (4.1.14.)	to 500 mL
Triton X-100, 30% (4.2.1.)	2.75 mL

In a 500 mL volumetric flask containing approx. 400 mL of DI water, add 6 mL of sulfuric acid. Bring to volume with DI water. Next, add 2.75 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

4.2.6. Buffer Reagent –

di-Sodium hydrogen phosphate-12-hydrate (4.1.5.)	8 g
Sodium hydroxide (4.1.4.)	19.5 g
Sodium potassium tartrate tetrahydrate (4.1.3.)	12 g
DI water (4.1.14.)	to 500 mL
Triton X-100, 30% (4.2.1.)	0.75 mL

In a 500 mL volumetric flask containing approx. 100 mL DI water, dissolve 8 g of di-sodium hydrogen phosphate-12-hydrate, 19.5 g of NaOH, and 12 g of potassium sodium tartrate tetrahydrate. Bring to volume with DI water. Next, add 0.75 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

4.2.7. Salicylate Reagent –

Sodium salicylate (4.1.7.)	32 g
Sodium nitroferricyanide (4.1.6.)	0.065 g
DI water (4.1.14.)	to 500 mL

In a 500 mL volumetric flask containing approx. 200 mL of DI water, dissolve 32 g of sodium salicylate and 0.065 g of sodium nitroferricyanide. Bring to volume with DI water and invert to mix thoroughly.

Transfer to dark bottle. Store in the refrigerator. Holding time, 7 days.

Caution: Can produce cyanide gas if in contact with acid. Refer to safety section for handling and disposal (10.6.).

4.2.8. DIC Reagent –

Dichloroisocyanuric acid sodium salt (4.1.2.)	0.15 g
DI water (4.1.14.)	to 200 mL

In a 200 mL volumetric flask containing approx. 150 mL of DI water, dissolve 0.15 g of DIC using a stir bar and stir plate. Bring to volume with DI water and invert to mix thoroughly.

Store at room temperature. Prepare fresh daily.

4.2.9. 1:10 HCl Cleaning Solution–

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Hydrochloric acid, conc. (4.1.12.)	100 mL
DI water (4.1.14.)	to 1000 mL

In a 1 L volumetric flask containing approx. 800 mL of DI water, add 100 mL of concentrated hydrochloric acid. Bring to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

4.2.10. 1 N NaOH Cleaning Solution–

Sodium hydroxide (4.1.4.)	40 g
DI water (4.1.14.)	to 1000 mL

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 40 g of NaOH. Bring to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

4.2.11. 10% Bleach Cleaning Solution–

Bleach (4.1.13)	20 mL
DI water (4.1.14.)	to 200 mL

In a 200 mL volumetric flask containing approx. 150 mL of DI water, add 20 mL of bleach. Bring to volume with DI water and invert to mix.

Do NOT use bleach with additives (incl. Clorox brand).

Prepare **fresh** each use. Used for cleaning only.

4.3. Standards:

4.3.1. NH_3 stock, 1000 mg N/L –

Ammonium chloride (4.1.1.)	3.819 g
DI water (4.1.14.)	to 1000 mL

Before preparation, dry at least 4 g of NH_4Cl for 12-24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 3.819 g of dried NH_4Cl. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

4.3.2. NH_3 intermediate and spiking solution, 10 mg N/L –

1000 mg N/L NH_3 stock (4.3.1.)	1 mL
DI water (4.1.14.)	to 100 mL

In a 100 mL volumetric flask containing approx. 50 mL of DI water, add 1 mL of the 1000 mg N/L NH_3 stock standard. **Do not acidify**. Bring to volume with DI water and invert to mix.

Store in the refrigerator. Holding time, 30 days.

4.3.3. NH_3 working calibration and QC standards –

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10 mg N/L NH_3 intermediate solution (4.3.2.)	See table
Sulfuric acid (4.1.8.)	See table
DI water (4.1.14.)	See table

Using the table volumes below, prepare standards by pipetting the specified amounts of the 10 mg N/L NH_3 intermediate standard into volumetric flasks partially filled with DI water. Acidify with sulfuric acid to 2%. Bring standards to volume with DI water. Invert 12 times to mix thoroughly.

Store in the refrigerator. Holding time, 30 days.

Standard Concentration (mg N/L)		QC Standard, Alias	Final Volume	Vol of 10 mg N/L standard added	Vol of Sulfuric Acid added
Cal 1 (4.2.3.)	0	MBLK, CCB, BLK, CAR	2000 mL	None	4 mL
Cal 2	0.005	PQCK	500 mL	0.25 mL	1.0 mL
Cal 3	0.010		500 mL	0.50 mL	1.0 mL
Cal 4	0.020		500 mL	1.0 mL	1.0 mL
Cal 5	0.050		500 mL	2.5 mL	1.0
Cal 6	0.10	CCV	500 mL	5.0 mL	1.0 mL
Cal 7	0.20	Primer, Drift	500 mL	10 mL	1.0 mL

4.3.4. ALTQC stock standard (NHX ERA Stock) –

The ALTQC stock standard is used as a second source reference material and must have a different supplier from the calibration standards. Currently, the second source supplier used is Environmental Resource Associates.

Simple Nutrients WasteWatR (4.1.11.)	see ERA's instructions
DI water (4.1.14.)	see ERA's instructions

The Simple Nutrients, WasteWatR is purchased as a concentrated ampule that must be diluted according to the manufacturer's instructions prior to analysis. The resulting prepared standard will be the certified value according to the manufacturer's certificate of analysis (COA). **Do not acidify.**

Store in the refrigerator. Holding time, 180 days.

4.3.5. ALTQC Working Standard (NHXERA) –

ALTQC Stock Standard (4.3.4.)	refer to certified value
Sulfuric acid (4.1.8.)	0.2 mL
DI water (4.1.14.)	to 100 mL

In a 100 mL volumetric flask, add the appropriate amount of the ALTQC stock standard solution so that the working concentration is diluted within the calibration range. **Acidify with Sulfuric acid**. Use DI water to bring standard to volume and invert 12 times to mix thoroughly.

Store in the refrigerator. Holding time, 30 days.

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5. SAMPLE PREPARATION PROCEDURE (NU-033)

5.1. Samples are preserved in the field to a final concentration of 0.2 % (v/v) with H_2SO_4, shipped on ice, and stored ≤ 6 ºC (not frozen). Samples analyzed with improper preservation are reported with the Y qualifier and appropriate comment.

5.2. Samples must be analyzed within 28 days of collection date for the data to be reported unqualified. Samples analyzed more than 28 days after collection date are reported with the Q qualifier.

5.3. Remove samples from the Receiving Room refrigerator and allow them to come to room temperature.

5.4. Samples with conductivities greater than 5000 µmhos/cm are batched separately. If no conductivity value is given, batch according to the matrix identification in LIMS. Effluents are batched separately.

5.5. For each batch (maximum of 10 samples), prepare:

5.5.1. One method blank (MBLK) (4.3.3.)

5.5.2. A sample matrix spike to calculate accuracy and a sample or matrix spike in duplicate to calculate precision.

- Make sure the selected sample is representative of the samples in the batch as the QC sample.
- Matrix spikes are prepared by adding 50 µL of the spiking solution (4.3.2.) to 10 mL portions of a selected sample for a 0.050 mg N/L spike (spike code: LO).
- If the selected sample has an NH3 level above the spiking level (or if the combined sample and spike concentration is outside the calibration range), another sample from the same batch can be selected to spike instead.
- Alternatively, if all samples are high, the entire batch may be diluted.

5.5.3. One lab fortified blank (LFB) by adding 50 µL of the spiking solution (4.3.2.) to a 10 mL portion of diluent (4.2.3.) for a 0.050 mg N/L spike (spike code: LO).

5.6. Samples with particulates are filtered prior to analysis with a 0.45 µm filter (indicated with an "_F"). To check for positive bias, the batch's MBLK must also be filtered. To check for negative bias, an LFB or check standard is filtered per analytical run.

6. SAMPLE ANALYSIS AND QUANTITATION

6.1. Instrument Startup:

6.1.1. Turn on the power to the sampler, pump, and colorimeter. The heater is kept plugged in. The colorimeter is ready for operation when the status light on the back is flashes green.

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6.1.2. Check the condition of the tubing and make sure all connections are made according to the following:

Path into the top of the dialyzer (donor stream):
BLK/BLK – Air
GRY/GRY – Sample line
ORN/WHT – EDTA/NaOH Reagent* (4.2.4.)

Path into the bottom of the dialyzer (acceptor stream):
BLK/BLK – Air
ORN/WHT – Sulfuric Acid Reagent* (4.2.5.)

Path out of the dialyzer:
ORN/WHT – Buffer Reagent* (4.2.6.)
ORN/WHT – Salicylate Reagent (4.2.7.)
ORN/WHT – DIC Reagent (4.2.8.)

To autosampler wash receptacle:
GRN/GRN – Diluent (4.2.3.)

6.1.3. Flush the instrument by placing reagent tubing for reagents containing surfactant (designated with an '*' above) in the System Wash solution (4.2.2.) and reagent tubing without surfactant in DI water. Secure pump platen and turn pump switches to 'Run' and 'Normal.'

Note: Parafilm all open reagent bottles to keep reagents from absorbing NH3 from the air.

6.1.4. Open AACE software. Username is "Superuser" with no password.

6.1.5. Click Charting in the System window, make sure <<Ammonia GD>> analysis is selected, and click OK. The sample probe should move down into sampler wash receptacle.

6.1.6. Right-click the chart and click "Set Base/Offset" (normally 5%) once the DI water and system wash have reached the detector.

6.1.7. After 15 minutes, check that a good bubble pattern is established and that the water baseline is stable.

6.1.8. Place the reagent tubing for the EDTA/NaOH, Sulfuric Acid, Buffer, and DIC reagents in the respective reagent bottles. Place the autosampler wash tubing into the Diluent.

6.1.9. After 15 minutes, place the reagent tubing for the Salicylate reagent (that has been brought to room temperature) in the respective bottle.

Note: Salicylate should never be added at the same time as the other reagents. Otherwise, precipitation will occur in the system.

6.1.10. Allow reagents to pump for an additional 15 minutes, then check that a good bubble pattern is established, and the heater is at 37˚C.

6.1.11. Set the base once again and let the reagent baseline stabilize.

6.1.12. Check that the pH of the colorimeter waste line is 12.6-12.9 once a week

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prior to analysis.

Note: If the final pH is too high, the amount of NaOH in the Buffer reagent can be decreased (4.2.6.). If the final pH is too low, the amount of NaOH in the Buffer reagent should be increased.

6.2. Create a new run sequence:

6.2.1. Click Set Up → Analysis/Run → Ammonia GD. Select "Ammonia GD.anl" so that it is highlighted, then select New Run.

6.2.2. Rename the run with the NHYYMMDDX format, where NH stands for ammonia, YY is the year, MM is the month, DD is the day, and X indicates the instrument used ('C' for Bran Luebbe C and 'D' for Bran Luebbe D). Postscripts 2, 3, etc. are used to denote the second, third, etc. runs of the day if necessary.

6.2.3. Fill out the Tray Protocol, click Print in the "Main Page" tab, and then OK to save the sequence. This will print out the run sequence with the analysis parameters.

An example run sequence includes:

Primer (0.20 mg N/L, 4.3.3.)
Drift (0.20 mg N/L)
RINSE (Diluent, 4.2.3.)
CAL1-7 (Calibration Standards, 4.3.3.)
High (0.20 mg N/L, to calculate carryover)
Low (Diluent)
Low (Diluent)
Baseline (Diluent)
PQCK1 (CAL2)
NHXERA (ALTQC, 4.3.5.)
CCV_F
CCB (BLK, 4.3.3.)
CCV (CAL6)
Drift (0.20 mg N/L)
Baseline (Diluent)
Batched samples with **MBLK(_F)**, **LFB**, and **spike(s)** (see 5.5.)
CAR (BLK)
CCB
CCV
Drift (0.20 mg N/L)
Baseline
End

6.3. Load the sampler tray with calibration standards (4.3.3.) and batches (5.5.) according to the corresponding positions in the Tray Protocol.

Note: When filling calibration standard tubes and sample tubes, only fill below the level of the wash pot. Otherwise, contamination between tubes may occur.

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6.4. Once the baseline is stabilized, start the run by going to Run → Start → Ammonia GD → Double click the sequence → OK.

6.5. The pump and autosampler cannot be paused while running, but the sequence can be changed in the Tray Protocol.

Note: If the autosampler arm is hit while running, the autosampler error will flash and will no longer move. If the autosampler is quickly turned off then on, it may resume sampling according to its position in the system window. If not, the run will need to be restarted. All samples and QC affected by the autosampler error must be reran accordingly.

Note: Before accepting changes made to the Tray Protocol, check the system window for the autosampler's peak number position ("No: #"). Any changes must be at least two peaks numbers **after** the number on the system window before pressing "OK." Ensure that changes are made according to peak numbers and not cup numbers!

6.6. When ready to stop the run, go to the Tray Protocol and add the "End" function to the cup where the run should stop. There should be a CAR, CCB, CCV, Drift, and baseline prior to the "End."

6.7. **Delete all unused lines after the "End" and click "OK" to save the changes.**

6.8. Once analysis is complete, the software will automatically print the final report containing the calibration and the chart of the peaks.

6.9. Print the raw data file in Retrieve → Raw Data. Run files are automatically exported to the Nutrients drive (see 7.4.).

6.10. Instrument Daily Shutdown:

6.10.1. Place the reagent tubing for the Salicylate reagent and autosampler wash into DI water. Pump on fast for at least 10 minutes to flush the salicylate out of the system.

6.10.2. Place the reagent tubing for the EDTA/NaOH, Sulfuric Acid, Buffer reagents into the system wash solution. Place the reagent tubing for the autosampler wash and DIC reagent in DI water. Pump on fast for 10-15 minutes.

6.10.3. Place all reagent tubing that was in the System Wash Solution into DI water and continue pumping on fast for 15 minutes.

6.10.4. Turn off autosampler and raise probe out of the wash receptacle. Remove all reagent tubing from the DI water. Pump air through the system on fast for 10-15 minutes to dry off the membrane.

6.10.5. Release the pump platen and pump tubing then turn off the pump and colorimeter.

6.11. Quantitation/Calculations:

6.11.1. The instrument reports results for Ammonia analysis in mg N/L.

6.11.2. The QC Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and

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spikes. For general calculations including RPDs and spike recoveries, see the FDEP Quality Manual.

6.11.3. The individual calibration standards are prepared according to section 4.3.3. The linear calibration and percent error of the standards are calculated by the instrument software and are part of the analysis report. It includes the correlation coefficient to evaluate the fit (see 8.2.).

7. DATA ARCHIVAL/VALIDATION

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test(s) (W-NH3(-F)), filename/s (NHYYMMDDX), and initials of the analyst.

7.2. Each report folder should contain the following items –

- Result report (6.8.)
- Calibration curve (6.8.)
- Chart of the peaks (6.8.)
- Bench sheet with analysis parameters (6.2.3.)
- Raw data printout (6.9.)
- Backlog with batching (5.4.)
- Reagent and standard logsheet (see Section 4)
- Pipette Logsheet (NU-PIPETTE)
- QC assessments uploaded from QC Manager to LIMS (test authorization)

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. SEAL Bran Luebbe instrument run and text files for upload to QC manager are stored in electronic format in the current year's subfolder in the instrument's subfolder (BranLuebbeC or BranLuebbeD) located at \\floridadep\data\dear\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\SegmentedFlowAnalyzers\.

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\floridadep\data\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. QUALITY CONTROL/DATA ASSESSMENT (NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures and corrective action for handling out-of-control or unacceptable data refer to DEP SOPs NU-038 and NU-043.

8.2. Linear Calibration Range (LCR) - See Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'.

- The correlation coefficient of the linear calibration (R2) must be ≥ 0.995.

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- Acceptance limits of the blank level calibration standard calculated results are <0.002 and >-0.002 mg N/L.
- Acceptance limits for the percent error of the PQL level standard is ±30%.
- Acceptance limits for the percent errors of calibration standards above the PQL is ±10 %.
- A new calibration is performed every day samples are analyzed at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

8.3. A continuing calibration verification, CCV (4.3.3.) and a CCB (4.3.3.) are run every 10 samples and at the beginning and end of each run. Acceptance limits for the recoveries of the check standard are 90-110 %. Acceptance limits for all BLK/CCB/CAR's are < 0.002 and > -0.002 mg N/L.

8.3. One certified standard reference material, ALTQC (4.3.5.), is analyzed on every analytical run. The supplier for the QC stock standard differs from the supplier of the calibration standards. Acceptance limits for the recovery of the ALTQC are 90-110 %.

8.4. One practical quantitation limit level standard, PQCK (4.3.3.), is analyzed on every analytical run. Acceptance limits for the recovery of the PQCK standard are 70-130 %. The PQCK is also used as a spike for the ongoing MDL and PQL verification.

8.5. A MBLK, LFB, matrix spike, and a matrix spike duplicate or a sample in duplicate must be analyzed for every batch of 10 samples (see Section 5.5.).

- The MBLK's are filtered if the samples in the batch are filtered in house (indicated with an "_F").
- Acceptance limits for the MBLK's are between < 0.002 and > -0.002 mg N/L.
- Acceptance limits for the recoveries of the LFB's and matrix spikes are 90-110%.
- The sample/matrix spike reproducibility should be less than 20% RPD for results above the practical quantitation limit (PQL).

9. MAINTENANCE/TROUBLESHOOTING

9.1. Write down all maintenance, troubleshooting, and service visits in the instrument's maintenance logbook.

9.2. Remove the PTFE membrane from the dialyzer block if the system will not be in use for a long time. Otherwise, the membrane can stick to block and damage dialyzer channels causing leaks.

9.3. Weekly Cleaning Procedure –

The following protocol should be done at the **minimum once a week** after samples are analyzed during the instrument shut down procedure.

It should also be done more frequently when more difficult sample matrices are

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analyzed to prevent contamination from building up in the system. This will help prevent from having to do a full system deep clean more often.

During the instrument daily shut down, once all reagents have been flushed from the system (6.10.2.) and with the pump set to "fast," place all reagent tubing in:

- 1:10 HCl (4.2.9.) (incl. wash line and sample probe/line) – 10 minutes
- DI water – 10 minutes
- 1 N NaOH (4.2.10.) – 10 minutes
- DI water – 5 minutes
- 10% Bleach (4.2.11.) (Only the wash line (NOT incl. sample probe or reagent lines)) – 15 minutes
- DI water – 30 minutes

With the probe raised, clean the autosampler wash receptable with 10% bleach and a cleaning swab. Rinse thoroughly with DI water.

Continue following the instrument shut down procedure (6.10.4.).

9.4. Monthly Cleaning Procedure –

The following protocol should be done at the **minimum once a month** as preventative maintenance.

It should also be done if instrument has contamination issues that were not resolved through the weekly cleaning procedure (9.3.).

9.4.1. With the pump set to "fast," place all reagent tubing in:

- 1:10 HCl (incl. wash line and sample probe/line) – 15 minutes
- DI water – 15 minutes
- 1 N NaOH – 15 minutes
- DI water – 15 minutes
- 10% Bleach (4.2.11.) (**NOT** incl. sample probe/line) – 15 minutes
- DI water (incl. sample probe/line) – 30+ minutes

Note: **Do not allow bleach to go through the sample probe!** Bleach will aggressively strip the probe leading to sample breaking and carryover issues. This will lead to the probe needing to be replaced.

9.4.2. With the probe raised, clean the autosampler wash receptable with 10% bleach and a cleaning swab. Rinse thoroughly with DI water.

9.4.3. Change pump tubing and airbar tubing.

9.4.4. Replace the dialyzer membrane.

9.5. Troubleshooting Tips –

9.5.1. Drifting or noisy baseline:

- Make sure waste lines from dialyzer, autosampler, and colorimeter are not submerged. Submerged waste lines can cause a drifting baseline or air spikes. Replace dialyzer and colorimeter waste lines if necessary.

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- Make sure bubble pattern is even and all bubbles are symmetrical. Be sure reagent lines are submerged.
- When switching from wash solutions to reagents, the air introduced may get stuck before the glass injection joints and between the tubing joints. Remove these bubbles and check if baseline has improved.
- Baseline noise may also be caused by the air flow tubing (black-black). Check to be sure the flexible tubing is in the center of the air flow regulators and properly clamped on both sides.
- A noisy baseline with fresh reagents and diluent can indicate a contaminated autosampler wash receptacle or sampling probe. Clean the reagent tubing/line of the autosampler wash receptable with 10% bleach, the sampling probe with acid and base, and replace the sample line and sample probe if necessary.

9.5.2. An increase of air spikes in the run usually indicates pump tubing needs to be changed. Glass tubing joints may also need to be replaced if there is a white band and/or bubbles that are getting caught in the joint's gap.

9.5.3. If the tops of peaks are noisy or take a long time to come to a plateau, the membrane most likely needs to be changed.

9.5.4. Carryover should be below 2%. A higher carryover indicates the need for maintenance and/or troubleshooting.

9.5.5. Failing blanks can indicate contamination. There are multiple approaches to fixing this, such as:

- Check that standard and sample tubes are not filled above the autosampler wash receptable line. Ensure that the autosampler wash receptable is filling and emptying appropriately.
- Acid soak with diluted HCl all glassware used to make reagents and standards overnight and replace all bottles. Remake reagents and standards. Acid soak the standards rack glass tubes overnight.
- Clean the system, replace the pump tubing, and change the membrane (see 9.4). Replace the probe sample line.
- For deeper contamination issues, it may be necessary to acid soak the reagent tubing, replace the tubing connecting glass joints, change the sample probe, replace the Kel-F line from 10-turn coil to detector, and/or replace the reagent and autosampler wash tubing lines (connected to the manifold, autosampler, and reagent tubing).
- If the problem persists, the Buffer Reagent may have become contaminated and should be re-made with a new bottle of potassium tartrate (4.1.3.). If the stock bottle becomes contaminated, see "SEAL AQ2 Technical Tips Removal of Ammonia Contamination from Sodium Potassium Tartrate" for guidance.

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9.5.6. It has been established that excessive amount of acid in the samples can result in negative or irregularly shaped peaks. The samples are acidified in the field to pH < 2 according to the preservation requirements. Typically, it is done by adding the contents of a 2 mL 1:1 H_2SO_4 ampule to a 500 mL of sample. Occasionally smaller size bottles are used. If in such cases the sampler does not scale down the amount of H_2SO_4 added, the samples may produce negative or irregularly shapes peaks during analysis. Such samples should be re-analyzed diluted. If there are more than one small size sample bottle submitted by the same customer, dilute all of these samples in the same way. Please notify your supervisor that there is a potential problem with the over-acidification of samples in the field.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

10.1. Review the Laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

10.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

10.3. Read the MSDS information for safe reagent and sample handling.

10.4. The instrument waste from the dialyzer and colorimeter produced during analysis is corrosive due to the high pH. All corrosive waste should be collected in bottles clearly labeled as hazardous waste. The waste should be disposed of as Basic Corrosive.

10.5. Noncorrosive, nonhazardous waste is disposed of down the sink with tap water.

10.6. The sodium nitroferricyanide (4.1.6.) used to prepare the salicylate reagent (4.2.7.) produces cyanide gas if it encounters acid. Wear all personal PPE when preparing and thoroughly rinse flasks and containers after acid washing. Dispose excess sodium nitroferricyanide crystals by lab packing. Excess salicylate reagent can be disposed of down the sink when flushed thoroughly with water.

10.7. Excess DIC (4.1.2.) and DIC Reagent (4.2.8.) should be disposed of in the same hazardous waste container as the instrument waste (see section 10.4.).

11. REFERENCES/DEFINITIONS

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

11.1. EPA method 350.1, Rev. 2.0 (1993).

11.2. SEAL Auto Analyzer Method G-427-14 Rev. 3

11.3. SEAL Method Comparison Table for Ammonia G-427-14 (2019)

11.4. SEAL Analyzer AA3 Operation Manual System

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11.5. SEAL AQ2 Technical Tips "Removal of Ammonia Contamination from Sodium Potassium Tartrate."

11.6. Quality Manual, Bureau of Laboratories, FDEP.

11.7. Laboratory Health and Safety Plan.

11.8. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

11.9. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.10. DEP SOP NU-041, Reagent Preparation Procedure.

11.11. DEP SOP NU-042, Standard Preparation Procedure.

11.12. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

11.13. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

Appendix of Significant Changes

12/08/2020 In Section 3, the instrumentation, equipment, and supplies were updated and inputted into table format. In Section 4, chemicals were updated and inputted into table format. Preparation procedures for reagents and standards were reformatted. Updated amount Triton X-100, 30% added to the System Wash Reagent, EDTA/NaOH Reagent, Sulfuric Acid Reagent, and Buffer Reagent. Updated amount of NaOH in the Buffer reagent. The ALTQC preparation was clarified. Updates to Section 5 were made to the sample analysis procedure. The calibration curve blank level standard limits were added to Section 8. Section 9 Maintenance and Troubleshooting was updated.

07/25/2022 In Section 3, the use of reagent straws was removed as the reagent lines are submerged directly into the reagents. Straw is replaced with tubing or lines in the remainder of the SOP. In Section 9, cleaning specifications were amended to represent the current cleaning procedure to avoid damage to the instrument and the NH_3 membrane. A note about the air tubing affecting the baseline was added to 9.5.

07/01/2024 In Section 4, the holding time for NH_3 intermediate and spiking solution, NH_3 working calibration standards, and ALTQC working standard were changed to 30 days. The storage temperature was changed from room temperature to refrigerator temperature. For the preparation of working standards, the volume of 10 mg/L intermediate standard added, the volume of sulfuric acid added, and the final volume of working standards were all increased to ensure sufficient volume throughout 30 days of usage.

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