Wetland Biogeochemistry Laboratory
Standard Operation Procedure

SOP #: WBL-AN-023

Determination of Ammonia and Nitrate+Nitrite in Extracts using Continuous Segmented-Flow Analysis (NEON)

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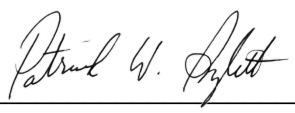

Patrick Inglett, Laboratory Director

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1. APPLICABLE MATRIX

This procedure is for analysis of ammonia and nitrate + nitrite in KCl extracts of soil, but can also be utilized for surface waters, ground waters, saline waters, and porewater.

2. SCOPE AND APPLICATION

2.1 The purpose of this SOP is to document and standardize the procedure used by laboratory personnel in the Wetland Biogeochemistry Laboratory (WBL) for the analysis of ammonia (NH_3/NH_4^+) and nitrate + nitrite ($NO_3^- + NO_2^-$) in soil extracts. This method is specific to the needs of the National Ecological Observatory Network (NEON) project.

2.2 The WBL uses a SEAL AutoAnalyzer 500 (AA500), which allows simultaneous determination of multiple analytes via separate flow channels. For ammonia determination, this protocol is based on Standard Method (SM) 4500-NH3 G (Automated Method) and SEAL Analytical Method No. A-005-19 Rev. 3 which uses salicylate instead of phenol. For nitrate + nitrite analysis, a modified SM4500-NO3 F *Automated Cadmium Reduction Method* (Seal Analytical AutoAnalyzer Applications Method No. A-044-19 Rev. 6) is used. The applicable range for ammonia and nitrate + nitrite analysis for the AA500 is from 0.01 to 1.0 mg/L N. Higher concentrations may be measured by using sample dilution.

2.3 Detection limit of ammonia-N and nitrite + nitrate-N is **0.01** mg N/L.

3. SUMMARY OF METHOD

3.1 Ammonia Analysis: During analysis, sodium salicylate and dichloroisocyanuric acid sodium salt dehydrate react with ammonia to form a blue-green color. The absorbance of blue-green color is measured at 660 nm and is directly proportional to the concentration of ammonia in the sample.

3.2 Nitrate + Nitrite Analysis: The WBL uses an AutoAnalyzer 500, and a modified version of SM4500-NO3 F *Automated Cadmium Reduction Method* for nitrite + nitrate analysis, which uses imidazole instead of ammonium chloride (modified SEAL Analytical method no. A-044-19 Rev.6).

During analysis, nitrate is reduced to nitrite by copper/cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrite formed by cadmium reduction, plus any nitrite originally present in the sample, is determined as an azo dye at 540 nm following its diazotization with sulfanilamide and subsequent coupling with N-(1-Naphthyl)ethylenediamine dihydrochloride. These reactions occur in an acidic (pH<2) matrix.

Imidazole buffer reduces interferences from iron, copper, or other metals. Samples with turbidity or color that absorbs in the analytical wavelength may interfere. Turbidity or suspended matter should be removed by filtration prior to analysis to avoid blockage of the OTCR. Since nitrate is soluble filtration does not affect the analysis.

4. DEFINITIONS

See WBL-OM-003 *Acronyms and Definitions* for common laboratory terminologies.

5. INTERFERENCES

5.1 Ammonia: Tri-sodium citrate dihydrate is used as a complexing agent to eliminate interferences caused by precipitation of calcium and magnesium compounds. Sodium nitroprusside is used to enhance the sensitivity. Turbidity and sample color that absorbs at the analytical wavelength will also interfere.

5.2 Nitrate+nitrite: Imidazole buffer reduces interferences from iron, copper, and other metals. Samples with turbidity or color that absorbs in the analytical wavelength may also interfere.

6. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

6.1 Read and understand the appropriate MSDS sheets and WBL SOP Chemical Hygiene Plan on all reagents used in a particular analysis.

6.2 Always use caution when working with acids and/or bases.

6.3 The instrument waste from the colorimeter must be collected in a satellite waste container (properly labeled as hazardous waste). When the satellite container is full, call for collection by Environmental Health & Safety.

7. APPARATUS AND EQUIPMENT

7.1 SEAL AutoAnalyzer 500 segmented flow analysis system:
- 7.1.1 AutoSampler AS2-2
- 7.1.2 AA500 Pump Module
- 7.1.3 AA500 Chemistry Module: Heating bath temperature: 37°C
- 7.1.4 AA500 Photometer Module:
  Channel 1: Nitrate + Nitrite- 10 mm flowcell/ 540 nm filter
  Channel 2. Ammonia- 10 mm flowcell / 660 nm filter

- 7.1.5 Pump Tubing / Manifold Configuration:

Tubings: 6 + 2 air + sample wash

a) Ammonia Channel (Channel 002)

Air	Blk/Blk
Debubbler	Wht/Wht
Sample	Yel/Yel
Complexing reagent	Orn/Orn
Salicylate	Orn/Wht
DCI	Orn/Wht
Sample Matrix	Orn/Grn

b) Nitrate + Nitrite Channel (Channel 001)

Air	Blk/Blk
Debubbler	Wht/Wht
Sample	Yel/Yel
Imidazole Buffer	Orn/Orn
Color reagent	Orn/Wht
Sample Matrix	Orn/Wht
Sample Matrix	Orn/Grn

7.2 Personal Computer (PC)
7.2.1 AACE Software Version 8.03 SP1

7.3 Lab Supplies:
Note: All flasks listed in this SOP are volumetric.

7.3.1 Working standard supplies:
12 – 100 mL volumetric flask

7.3.2 Reagent supplies:
Ammonia:
3 – 2000 mL amber bottles
1- 100 mL glass bottle
1- 250 mL amber bottle
1 – 250 mL volumetric flask
1 – 100 mL volumetric flask
3 – 2000 mL volumetric flasks
Nitrate + Nitrite:
2 – 250 mL amber plastic bottles
3 – 500 mL amber plastic bottles
1 – 1000 mL amber plastic bottle
1 – 1000 mL amber glass bottle
2 – 500 mL volumetric flasks
4 – 1000 mL volumetric flasks

1 – 2000 mL volumetric flask

7.3.3 Miscellaneous:
Disposable 4mL Auto Analyzer sample cups
Plastic weigh boats
Plastic disposable beakers
Spatula
Parafilm
Balance
Pipettes
Nalgene dropper bottle
Magnetic stir bars
Stir plate
pH meter and probe
100 mL graduated cylinder
250 mL graduated cylinder

8. REAGENTS

8.1 Reagents for Ammonia Determination

8.1.1 Chemicals-
Brij-35
Dichloroisocyanuric acid sodium salt dihydrate (DCI)
Ethylenediamine tetra-acetic acid disodium (EDTA)
Sodium hydroxide (NaOH)
Sodium nitroferricyanide
Sodium salicylate
Tri-sodium citrate dihydrate
Potassium chloride (KCl, high purity, see Section 9)
1000 mg N/L Ammonia Standard Stock solution, (purchased/traceable)
Certified QC Ammonia Standard (purchased/traceable)
Distilled de-ionized, or double de-ionized (DDI) water at 18.2 MΩ purity

8.1.2 Complexing reagent (**2 weeks**):

EDTA	60 g
Tri-sodium citrate dihydrate	240 g
Sodium nitroferricyanide	1 g
Brij-35	6 mL
DDI	to 2000 mL

Into a 2000 mL volumetric flask containing ~1200 mL DDI, add 60 g of EDTA, 240 g of tri-sodium citrate dehydrate, and 1 g of sodium nitroferricyanide. Mix to dissolve and bring to volume. Add 6 mL of Brij-35. Store in an amber bottle for up to 2 weeks.

8.1.3 Dichloroisocyanuric acid (DCI) reagent (**daily**):

Sodium hydroxide	10 g
Dichloroisocyanuric acid sodium salt dihydrate	0.25 g
DDI	to 100 mL

Into a 100 mL volumetric flask containing ~80 mL DDI, completely dissolve 10 g of sodium hydroxide. Add 0.25 g of dichloroisocyanuric acid sodium salt dihydrate. Mix to dissolve and bring to volume. Store in a glass bottle and prepare fresh daily.

8.1.4 Salicylate reagent (**2 weeks**):

Sodium salicylate	75 g
DDI	to 250 mL

Into a 250 mL volumetric flask containing ~150 mL of DDI, add 75 g of sodium salicylate. Mix to dissolve and bring to volume. Store in an amber bottle for up to 2 weeks.

8.1.5 System wash reagent (**2 weeks**):

Brij-35	4 mL
DDI	to 2000 mL

Into a 2000 mL volumetric flask containing ~1800 mL of DDI, add 4 mL of Brij-35. Mix well and bring to volume. Prepare fresh every 2 weeks.

8.1.6 Matrix solution to match samples (2 M KCl) (see purity testing, Section 9)

Potassium chloride	149.1 g
DDI water	to 1000 mL

Into a 1000 mL volumetric flask containing ~600 mL of DDI water, add 149.1 g of KCl. Mix to dissolve and bring to volume.

8.2 Reagents for Nitrate + Nitrite Determination

8.2.1 Chemicals-
Imidazole
Hydrochloric acid (HCl), concentrated
Sulfanilamide (SAN)
Sulfuric acid (H_2SO_4), concentrated
Phosphoric acid, concentrated
Cupric sulfate

Triton X-100
Iso-propanol
N-(1-Naphthyl) ethylenediamine dihydrochloride (NED)
1000 mg/L Nitrate Stock Solution (purchased/traceable)
1000 mg/L Nitrite Stock Solution (reduction efficiency, purchased/traceable)
Certified Nitrate Standard (QC, purchased/traceable)
Potassium chloride (KCl, high purity, see Section 9)
Distilled de-ionized, or double de-ionized (DDI) water at 18.2 MΩ purity

8.2.2 Cupric sulfate reagent, 0.01 M (**6 months**):

Cupric sulfate	2.5 g
DDI	to 1000 mL

Into a 1000 mL volumetric flask containing ~800 mL of DDI water, add 2.5 g of cupric sulfate. Mix well with magnetic stir bar until the cupric sulfate is dissolved and bring to volume. Store in a plastic bottle for up to 6 months.

8.2.3 Stock imidazole buffer (0.1 M) (**1 month**):

Imidazole	36.2 g
HCl	to pH 7.5
DDI water	to 2000 mL

Into a 2000 mL volumetric flask containing ~1600 mL of DDI water, add 32.6 g of imidazole. Mix well with magnetic stir bar until imidazole is dissolved. Suspend a pH probe into the flask and carefully add concentrated HCl dropwise until the pH has been adjusted to pH 7.5, about 2 mL of HCl (Note: Allow time between additions for the acid to be mixed into solution to obtain an accurate measurement). After using the magnetic wand to remove the stir bar and bring to volume with DDI water. Mix well and transfer to a plastic (HDPE) bottle. Store at room temperature.

8.2.4 Working imidazole buffer (**daily**):

Triton-X 50% solution	1 mL
Stock imidazole buffer	750 mL
0.01 M cupric sulfate	1.8 mL
DDI water	to 1000 mL

To make Triton X-100, 50% solution, thoroughly mix 50 mL of Triton X-100 and 50 mL of iso-propanol. Into a 1000 mL volumetric flask, add 750 mL of the stock imidazole buffer. Add 1.8 mL of the 0.01 M cupric sulfate

and 1 mL of Triton-X 50% solution. Bring to volume with DDI water. Mix well by swirling. Prepare fresh daily.

8.2.5 Color reagent (**1 month**):

Conc. Phosphoric acid	100 mL
SAN	10 g
NED	0.5 g
DDI water	to 1000 mL

Into a 1000 mL volumetric flask containing ~ 500 mL of DDI water, add 100 mL of the concentrated phosphoric acid, 10 g of SAN, and 0.5 g of NED. Swirl to mix well. Add DDI water and bring to volume.

8.2.6 Cupric sulfate, 2% w/v (**6 months**):

Cupric sulfate	10 g
DDI water	to 500 mL

Into a 500 mL volumetric flask containing ~400 mL of DDI water, add 10 g of cupric sulfate. Mix well with a magnetic stir bar until the cupric sulfate is dissolved. Bring to volume. Store in a plastic bottle for up to 6 months. This solution is used for column (OTCR) activation only.

8.2.7 System wash:

Triton X-100, 50% solution	1 mL
DDI water	to 1000 mL

To make Triton X-100, 50% solution, thoroughly mix 50 mL of Triton X-100 and 50 mL of iso-propanol. Into a 1000 mL volumetric flask containing ~750 mL of DDI water, add 1 mL of Triton X-100 50% solution. Mix well and bring to volume.

8.2.8 Matrix solution to match samples (2 M KCl) (see purity testing, Section 9):

Potassium chloride	149.1 g
DDI water	to 1000 mL

Into a 1000 mL volumetric flask containing ~600 mL of DDI water, add 149.1 g of KCl. Mix to dissolve and bring to volume.

9. KCl PURITY TESTING

IMPORTANT: Each batch of KCl powder purchased from vendors must be analyzed for ammonium and nitrate + nitrite concentration to reduce inorganic N contamination during sample analyses. This purity assessment will be conducted for KCl used by both the WBL and NEON field personnel. Only KCl powder batches meeting NEON specifications for ammonium and nitrate + nitrite concentration (preferred: <=0.02 mg N/L for each analyte) should be used for matrix and standard solution preparation. If the concentration of ammonium and nitrite + nitrite is >0.02 mg N/L for any analyte for a particular KCl powder batch, NEON should be notified prior to using that batch for matrix or standard solution.

9.1. In a 200 mL volumetric flask containing ~100 mL of DDI water add 29.82 g of KCl powder. Mix well to dissolve and bring to volume with DDI water. This results in a 2M KCl solution. Analyze the solution for ammonia (using salicylate-hypochlorite method, see Section 14), and for nitrite and nitrate (using sulfanilamide method with and without open tubular cadmium reactor (OTCR), Section 14). For KCl purity testing, make standards curves in DDI water for inorganic N estimation (see Section 10).

10. STANDARDS

NOTE: Standard preparation outlined below is for analysis of samples and standards. If analyzing KCl solution for purity testing, the sample matrix is DDI in place of KCl.

NOTE: Each analysis run should contain separate calibration curves based on standards made using DDI and KCl. The KCl-based calibration curve will be used initially, however, if estimated nitrate + nitrite and/or ammonia concentration of the analyzed samples appear to be negative (e.g., less than zero, due to samples and standards having different KCl matrix purities), the analysis for these samples will be based on the standards prepared in DDI. This will be noted in the testMethod field as ‘WBL-AN-023-waterCurve’ whereas a standard entry of ‘WBL-AN-023’ indicates use of KCl standards.

10.1 Ammonia 10 mg N/L stock standards (**monthly**):

1000 mg N/L ammonia stock standard	1 mL
Sample matrix (2M KCl or DDI)	to 100 mL

Into a 100 mL volumetric flask containing approximately 50 mL of sample matrix (8.6), pipette 1 mL of the 1000 mg N/L Ammonia stock standard. Bring to volume and mix well. Store at 4°C. Record the preparation of the stock standard in the standard prep logbook. The solution is good for 1 month.

10.2 Nitrate 10.0 mg N/L stock standard (**monthly**):

1000 mg N/L nitrate stock standard	1 mL
Sample matrix (2M KCl or DDI)	to 100 mL

Into a 100 mL volumetric flask containing ~80 mL of sample matrix, add 1 mL of 1000 mg/L nitrate standard. Bring to volume and mix well. Store at 4°C. Record the preparation of the stock standard in the standard prep logbook. The solution is good for 1 month.

10.3 Calibration standards (concentrations in mg N/L) for a simultaneous run in AA500 (prepared as needed, or monthly). Prepare standards using both DDI and KCl matrices:

Blank, 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 (or higher).

Using the matrix solution (2 M KCl (8.1.6, 8.2.8) or DDI) and the 10 mg/L NH3 and the 10 mg N/L NO3 stock standards, prepare the calibration standards according to the following table:

Standard (mg/L)	Vol. of 10 mg/L NH3 Stock	Vol. of 10 mg/L NO3 Stock	Final Volume
Blank	-	-	100 mL
0.01	0.1 mL	0.1 mL	100 mL
0.02	0.2 mL	0.2 mL	100 mL
0.05	0.5 mL	0.5 mL	100 mL
0.1	1.0 mL	1.0 mL	100 mL
0.2	2.0 mL	2.0 mL	100 mL
0.4	4.0 mL	4.0 mL	100 mL
0.6	6.0 mL	6.0 mL	100 mL
0.8	8.0 mL	8.0 mL	100 mL
1.0	10.0 mL	10.0 mL	100 mL

Store at 4°C. Record the preparation of the working calibration standard in the standard prep logbook. The solution is good for 1 month.

10.4 Reduction Efficiency Check solutions (1.0 mg N/L as nitrite)
Prepare a working reduction check solution by adding 0.1 mL of the 1000 mg/L nitrite stock solution (8.2.1) into a 100 mL flask containing ~80 mL of sample matrix. Bring to volume and mix well. This solution should be run after a 1.0 mg/L nitrate standard to compare responses and calculate the efficiency of the OTCR to reduce nitrate to nitrite. This solution can be stored at 4°C for up to a month.

10.5 Quality Control Check Standard:
A certified QC standard, purchased from a secondary source (ERA), is used as a stock solution to prepare a working QC solution. Prepare and analyze this solution to validate the calibration curve. This solution must be prepared according to the manufacturer's instructions using appropriate clean flasks and the sample matrix solution (2 M KCl (8.1.6, 8.2.8)). This solution can be stored at 4°C for up to a month.

11. SAMPLE HANDLING AND PRESERVATION

11.1 Samples for ammonia and nitrate + nitrite determination must be stored at -20°C. Samples should be analyzed within 48 hours of thawing and can be stored at 4°C for up to 21 days thawed after the initial analysis if reruns are required. Samples should be refrozen and stored at -20°C until data reporting and contract invoicing is complete.

11.2 Samples that are stored at -20°C must be allowed to come to room temperature before processing.

12. QUALITY CONTROL

The following quality controls checks are used with each analytical set.

Type	**Frequency**
Instrument Calibration	7-10 Standards, daily or failure of CCCS.
Method Reagent Blank	1st analysis of blank KCl immediately after standard curve.
Continuing Calibration Blank (CCB)	Continued analysis of blank: 1 per 10 samples if analytical set <100, or 1 per 20 samples if analytical set >100.
Practical Quantification Limit (PQL)	At least 1 per analytical set <100, or at least 2 per analytical set >100, at a concentration of 3-5 times the MDL.
Continuing Calibration Check Standard (CCCS)	Continued analysis of 0.8 ppm std. 1 per 10 samples if analytical set <100, or 1 per 20 samples if analytical set >100.
Quality Control Check Standards (QC)	Analyzed at the beginning of each analytical set to verify the standard curve. One QC is also analyzed 1 per 20 samples.
Reduction Efficiency Check	Analyzed once after the highest nitrate standard during the gain check to determine if cadmium reduction (OTCR) is effectively reducing nitrate to nitrite.
Repeat (Rep)	At least 1 per analytical set and 1 per 20 samples analyzed.
Spike (spike added prior to sample analysis) (Sp)	At least 1 per analytical set and 1 per 20 samples analyzed.

13. CALIBRATION AND STANDARDIZATION

13.1 At the beginning of each analytical run, a blank and the following working standards are analyzed to calibrate the instrument: 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg N/L for both channels (NH3 and NO3+NO2). Quality control and calibration checks are also used to determine the accuracy of calibration (Section 10.4, 10.5, 12).

13.2 Only Class A volumetric flasks and properly calibrated pipettes are used for preparing calibration and check standards. Intermediate stocks and standards are prepared using a purchased, traceable and certified standard solution. Quality control (secondary source) samples are prepared using certified solutions from commercial vendors.

14. SAMPLE ANALYSIS

AA500 startup procedure (Refer to SEAL AA500 Operations Manual for a detailed discussion of AACE procedures).

14.1 Turn on surge protectors to provide power to the PC, pump, autosampler, chemistry module, and the photometer. Check to make sure the blue LED lights on the photometer modules are on.

14.2 After starting the PC go to **Programs/B+L Applications/AACE**. After AACE is opened, open the System window and note the **.anl** file that appears in the bottom left field. The **.anl** file that will be there will be the last **.anl** file used. Make sure that the **.anl** file is specific to ammonium (channel 2) and nitrate + nitrite (channel 1). If this is the desired **.anl** file then click Charting. If you need to load a different analysis file you can double click on the **.anl** to change to another file. **Note:** The default Base and Gain values are specific to each analysis file and channels. If you start charting with a different analysis file than the one you intend to use, you need to make sure to switch to the proper analysis file before setting your Gain. By starting **Charting**, AACE will automatically start a download to establish communication between the AA500 and the PC and between the digital photometer and the pump and autosampler. When the download is complete and the dark current has been automatically measured, two charts are opened, and it is now possible to monitor the baseline specific to channel 1 (NOx) and channel 2 (NH3).

14.3 Ammonia analysis in channel 2:

14.3.1 With the wash and ammonia reagent lines in DDI, engage the platen and begin pumping fluids through the system. After ~10 minutes place line in system wash reagent, which contains the Brij-35 necessary to establish a good bubble pattern. Place the wash line in the sample wash solution. Keep pumping for ~10 minutes and then place the complexing reagent, salicylate, and DCI reagent lines in their respective reagent receptacles for ammonia analysis. Monitor the baseline and adjust the baseline to 5% of the chart if necessary. To adjust the Base, place the cursor over the chart and right click the mouse. Choose **Set Base**.

14.3.2 Monitor the channel 2 window and re-adjust the baseline using **Set Base** after the baseline has shifted from the addition of the reagents. When the baseline appears stable the system is ready for analysis.

14.3.3 Once the base is set, load 1.0 mg/L NH3-N high standard onto the autosampler so the Gain can be set. With a high standard passing through the photometer, use the Gain in the AACE to set the peak height at ~90% (or within 75-95%) of full scale.

14.4 Nitrate + Nitrite analysis in channel 1:

14.4.1 Place the reagent lines into the dedicated plastic bottle for DDI water with Triton X-100 (8.2.7.), engage the platen and begin pumping. During this time you can check for leaks, flow problems, or problems with the bubble pattern that are independent of the OTCR. The electronic valve for the OTCR should be turned off.

14.4.2 Switch the reagent line from the wash solution to their corresponding reagent. When the imidazole buffer has reached the 4-way-valve, open the valve to insert the OTCR into the liquid stream. Wait for the baseline and bubble pattern to stabilize again.

14.4.3 Now it is necessary to load several aliquots of the 1.0 mg/L NO3-N high standard onto the autosampler so the Gain can be set. With a 1.0 mg/L high standard passing through the photometer, use the Gain in AACE to set the peak height at ~90% of full scale. Ideally, you will be able to set the standard calibration by the first cup or two of high standard so the other cups will give you an opportunity to check the stability of the high standard response.

14.4.4 A 1.0 mg/L NO2-N standard should be the last cup run when setting the standard calibration. This standard should be prepared in sample matrix without acidification, as the addition of acid can oxidize NO2 to NO3. This standard is run as a reduction check. The response of this standard can be compared to the 1.0 mg/L NO_3-N to check the efficiency of the OTCR reducing NO3 to NO2. For reduction efficiency below 90% the column needs to be reactivated (see 14.4.5).

14.4.5 Proper performance of the OTCR is a critical component of this analysis. When the coil has poor reduction efficiency there will be a low recovery of nitrate as nitrite. If the coil is overactive, it is possible that nitrite will be further reduced also resulting low recovery.

14.4.5.1 In the case of poor reduction efficiency, the OTCR should be reactivated. This is accomplished by diluting the Cupric sulfate (2% w/v solution) 1:1 with stock imidazole buffer in a plastic disposable beaker. Close the 4-way-valve and disconnect the OTCR. Place one end of the Tygon sleeve of the OTCR in the buffered copper solution. Using a syringe on the other end of the sleeve, draw this solution through the OTCR and let the solution stand in the coil for ~10 minutes. Draw stock imidazole buffer through the OTCR to

remove the buffered copper solution from the OTCR. Note: It is important that air is not introduced into the OTCR during handling. After an OTCR has been reactivated it should be conditioned similar to the process used for an overactive column. (See 14.4.5.2). The OTCR need not be reactivated every day before beginning analysis, but only when poor reduction efficiency is observed.

14.4.5.2 In the case of an overactive OTCR it is necessary to condition the coil by aspirating a 1.0 mg/L NO3-N standard continuously for ~10 minutes (or longer if it appears that the response shows that there is significant drift). Another way to condition the OTCR is to run multiple cups of the 10.0 mg/L NO_3-N with the autosampler.

14.4.5.3 After conditioning an overactive coil or a newly reactivated coil, run several sample cups of 1.0 mg/L NO_3-N, check the gain setting, run one 1.0 mg/L NO2 standard and recalculate the efficiency. Note: When running the NO2 standard check for tailing of the NO2 peak. If the NO2 peak shows tailing it is advisable to clean out the system. Disconnect the OTCR from the system by closing the 4-way-valve. Put the color reagent line in DDI water. Introduce 1N NaOH through the imidazole buffer line and the sample probe after the OTCR has been closed from the system. Flush with 1 N NaOH for ~15 minutes, followed by DDI water for five minutes, 5 N H2SO4 for ~15 minutes, and finally with DDI water for an additional 15 minutes. Rerun the 1.0 mg/L NO_3-N cups and 1.0 mg/L NO2 cup and recalculate the reduction efficiency.

14.5 With the gain set, a stable baseline and the demonstration of acceptable reduction efficiency, the system is ready for analysis.

14.6 During the analysis, log the order that standards and samples are loaded onto the tray using the tray map and update the tray protocol in AACE. Check the linear regression in AACE once the standards are run. Then calculate the QC standard recovery using the QC template. If the correlation coefficient and the recovery of the QC standard fall within acceptance criteria, then the run should be allowed to progress. Check the recoveries of the QC, Continuing Calibration Standard, the matrix spike and the precision of the analytical replicate every 20 samples to make sure the OTCR continues to perform during the analysis.

14.7 The run is completed when AACE marks the final baseline. The charting window closes automatically, and another window opens that tells the analyst that the analysis is complete. Make sure that the 4-way-valve for channel 1 is closed immediately after the analysis is complete. At this point the analyst can transfer the reagent and wash lines for both channels into vessels containing respective system wash to rinse out the system. The system should be left in the rinse mode for a

minimum of twenty minutes. When rinsing of the system has been completed, the platen should be disengaged. Switch off all modules by turning off the surge protector that serves as the master switch for the pump, autosampler and heating baths. Cover the chemistry module and the pump with their respective dust covers.

15. CALCULATIONS AND DATA REPORTING

15.1 After completion of the analysis, select **Retrieve/View Chart** to check that AACE has marked all the peaks correctly for both channels. Correct if necessary and save the corrections. **Note:** If the analyst decided to recalculate for baseline correction and then decides to use the corrected file, the chart that is selected to be checked for proper peak marking must be the corrected file. For example, if you use baseline correction to recalculate the chart labeled 030312A, AACE will automatically re-label the file as 030312AR1. Any corrections made in 030312A will not appear in 030312AR1 and will have to be made again. This extra step may be avoided by making any corrections to the file that is intended for use.

15.2 Select **Set Up/Analysis** and proofread your Tray Protocol to make sure all samples are correctly named and assigned to the proper cup positions on the sampler tray. Again, if the analyst decides to use a recalculated file, that file should be used to proofread the Tray Protocol. It is best to check the Tray Protocol for typographical errors before the run is completed so the corrections may be made in AACE. If an error is noticed in the Tray Protocol after the run is completed, the error is noted on the tray map and the correction is made in Excel after the run is downloaded from AACE.

15.3 To reformat the data from the AA500 report format to a form that can be downloaded directly into Excel, select **File/Export/ASCII file**. Press **Export**, **Ok**, and **Ok**.

15.4 Open Windows Explorer/AACE/Data and copy the .slk and .tmp files to a flash drive. In Excel this file can be opened off the flash drive to edit the file to put into the reporting format used by WBL. Copy and paste the edited file into an Excel report template and do the final data summary. Note: When closing the .slk file after editing, copying and pasting, say No to Save Changes. This preserves the original, unedited .slk file on flash drive.

15.5 Submit the final data and QC summary to the QA officer for approval.

16. DATA ARCHIVAL

Individual runs are archived on the PC. Archival of all files generated by AACE is done by saving on an external hard drive. Note: When performing file maintenance on the PC, delete analytical run data from AACE, not Windows Explorer.

17. METHOD PERFORMANCE

These methods have been validated through a single laboratory study and inter-laboratory method validation studies (SM4500-NH3 G and SM4500-NO3 F modified). Performance based on recoveries is as follows:

Ammonia in the sample:
Precision: 0.96% (UCL: 4.8%)
Accuracy: 106% (LCL-UCL: 97-115%)
Spike recovery: 101% (LCL-UCL: 96-106%)

Nitrate + Nitrite in the sample (Historical recoveries from 2011):
Precision – 2.3% (UCL: 14%)
Accuracy – 96% (LCL-UCL: 73-119%)
Spike recovery – 98% (LCL-UCL: 76-120%)

18. DATA ASSESSMENT, ACCEPTANCE CRITERIA, and CORRECTIVE ACTION FOR QC MEASURES

Type	Purpose	Acceptance Criteria	Corrective Action
Instrument Calibration	Standardize the instrument.	**Linear regression $R^2 > 0.995$** All standards must be within 10% of their true value	Reanalyze standards. If same response is obtained, re-optimize instrument and restart analysis. If same response is obtained, prepare new standards and restart analysis.
Method Reagent Blank	Determine if there is any bias or contamination contributed by the method itself. When blanks are found to be positive, the impact on the samples is determined; sample results maybe qualified if concentrations are <5x the blank value.	**Value <MDL or <0.01 mg/L, whichever is higher**	Prepare new blank and restart analysis. If same response is obtained, determine cause of contamination (reagents, calibration standards, environment, equipment failure, etc.) and eliminate the source of contamination.
Continuing Calibration Blank (CCB)	Determine stability of calibration over the course of the run.	**Mean of observed values for an analytical set are <MDL or <0.01 mg/L, whichever is higher**	Recalibrate and reanalyze the affected portion of the run.
Practical Quantification Limit (PQL)	Determine stability of calibration on the low end of the curve; determine ability to consistently achieve the detection limit.	**Mean of observed values for an analytical set within Accuracy 90-110%**	Reevaluate system, recalibrate and reanalyze the affected portion of the run.
Continuing Calibration Check Standard (CCCS)	Determine stability of calibration over the course of the run.	**Mean of observed values for an analytical set within Accuracy 95-105%**	Recalibrate & reanalyze the affected portion of the run.

Quality Control Check Standards (QC)	Determine if calibration is accurate.	**Accuracy 95-105%**	Reanalyze or re-prepare QC check standard. If same response is obtained, prepare new primary and calibration standards. If that fails, check against an alternate QC source and stock solution. Obtain approval from QA officer or staff. Discard unacceptable QC once confirmed and document findings on QC result log.
Reduction Efficiency Check	Determine if cadmium reduction (OTCR) is effectively reducing nitrate to nitrite.	**Reduction Efficiency 90-110%**	Below 90% the column needs to be reactivated. For reduction efficiency above 110% the column needs to be conditioned.
Repeat (Rep)	Assess analytical precision.	**Precision (RSD) < 20%**	Determine and eliminate cause of problem (baseline drift, carryover, etc). Reanalyze all affected samples.
Spike (spike added prior to sample analysis) (Sp)	Determine if there is matrix interference.	**Recovery 85-115%**	Check pipette, remake spike and reanalyze. Meantime spike a DDI or Blank Matrix. If acceptable, reanalyze affected samples. If not acceptable, but the recovery of DDI or blank matrix is acceptable, then it is likely sample matrix interference. Make proper notation on the analytical report.

Data for actual samples that do not meet the above criteria are qualified as such, or the nature of non-conformance is reported to the customer, project manager, or principal investigator.

19. REFERENCES

19.1 Standard Methods for the Examination of Water and Wastewater 18th Edition. APHA 1992. 4500NH3 G. Automated Phenate Method. (p. 4-84).

19.2 U.S. EPA 1993. Methods for the Determination of Inorganic Substances in Environmental Samples. Method 350.1. Determination of Ammonia by Semi-Automated Colorimetry.

19.3 SEAL Analytical. 2024. Method No. A-043-19 Rev. 2., Ammonia in Water and Seawater (MT519), AutoAnalyzer 500, pp. 24.

19.4 SEAL Analytical, (2023). AA500 Auto Analyzer Operation Manual.

19.5 SEAL Analytical, (2023). AACE 8.03 SP1 Operation Manual.

19.6 Alpkem RFA Method #A303-S170.

19.7 Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992, 4500NO3 F Automated Cadmium Reduction Method. American Public Health Association, Washington, D.C. (pg 4-91).

19.8 F. Nydahl, Talanta 23, p. 349-357. (1976).

19.9 Methods for Chemical Analysis of Water and Wastes, August 1993, EPA-600/R-93/100, Methods 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry.

19.10 Patton, C.J., Doctoral Dissertation, Michigan State University, 1982, p. 87-121.

19.11 Fox, J.B., Anal. Chem., 51, p. 1493. (1979).

19.12 G. Norwitz and P.N. Keliher, Analyst, 109, p. 1281. (1984).

19.13 SEAL Analytical. 2024. Method No. A-044-19 Rev. 6., Nitrate and Nitrite in Water and Seawater (MT519A), AutoAnalyzer 500, pp. 29.

20. REVISIONS

ver. 1.1 This version clarifies the use of DDI standards in situations yielding negative estimates of nitrate+nitrite and/or ammonia N (e.g. the background N contamination in KCl concentration in samples are lower than the KCl matrix used in analysis).

ver. 1.0 Note: The analyses described here were previously conducted using Bran+Luebbe AutoAnalyzer 3 following WBL-AN-008 and WBL-AN-020.