DEPRECATED

Wetland Biogeochemistry Laboratory
Standard Operation Procedure

SOP #: WBL-AN-020

Determination of Ammonia in Water or Extracts via the Salicylate Method using the Bran+Luebbe AutoAnalyzer 3

Effective Date: November 1st, 2018

Prepared and approved by:

Sophia Barbour

Sophia Barbour, Laboratory Manager and QA Officer

Determination of Ammonia in Water or Extracts via the Salicylate Method using the Bran+Luebbe AutoAnalyzer 3

1. APPLICABLE MATRIX

This procedure is for analysis of ammonia in surface waters, ground waters, saline waters, porewater, and extracts.

2. SCOPE AND APPLICATION

2.1 The purpose of this SOP is to document and standardize the procedure used by laboratory personnel and students in the Wetland Biogeochemistry Laboratory (WBL) for the analysis of ammonia in water and extracts.

2.2 The WBL uses a Bran+Luebbe AutoAnalyzer 3 (AA3), and a protocol based on Standard Method (SM) 4500-NH3 G (Automated Method) and SEAL Analytical Method No. G-171-96 which uses salicylate instead of phenol for ammonia analysis. The applicable range for ammonia analysis for the AA3 is from 0.01 to 1.0 mg/L NH3-N. Higher concentrations may be measured by using sample dilution.

2.3 Detection limit of water NH3-N is **0.01** mg N/L.

3. SUMMARY OF METHOD

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.

The WBL uses an AA3 and a variation of SM4500-NH3 G and SEAL Analytical Method No. G-171-96 for ammonia analysis. The calibration range for ammonia analysis for the AA3 is from 0.01 to 1.0 mg/L NH3-N. Higher concentrations may be measured by using sample dilution.

4. DEFINITIONS

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

5. INTERFERENCES

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.

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 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 Bran+Luebbe AutoAnalyzer 3 segmented flow analysis system:
- 7.1.1 Compact Sampler
- 7.1.2 AA3 Pump
- 7.1.3 AA3 Chemistry Manifold: Heating bath temperature: 50°C
- 7.1.4 AA3 Digital Colorimeter: 10 mm flow cell / 660 nm filter
- 7.1.5 Pump Tubing / Manifold Configuration:

Air	Blk/Blk
Debubbler	Wht/Wht
Sample	Yel/Yel
Complexing reagent	Orn/Orn
Salicylate	Orn/Wht
DCI	Orn/Wht
Sample Wash	Pur/Wht

7.2 Personal Computer (PC)
- 7.2.1 AACE Software Version 5.48.3

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

7.3.1 Working standard supplies:
1 – 100 mL volumetric flask
10 – 50 mL volumetric flasks

7.3.2 Reagent supplies:
2 – 1000 mL amber bottles
1 – 250 mL glass bottle
1 – 100 mL volumetric flask
3 – 1000 mL volumetric flasks
1 – 2000 mL volumetric flask

7.3.3 Miscellaneous:
Plastic weigh boats
Plastic disposable beakers
Spatula

Parafilm
Balance
Pipettes

8. REAGENTS

8.1 Brij-35
8.2 Dichloroisocyanuric acid sodium salt dihydrate
8.3 Ethylenediamine tetra-acetic acid disodium (EDTA)
8.4 Sodium hydroxide (NaOH)
8.5 Sodium nitroferricyanide
8.6 Sodium salicylate
8.7 Tri-sodium citrate dehydrate
8.8 Potassium chloride (KCl)
8.9 1000 mg N/L Ammonia Standard Stock solution, (purchased/traceable)

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

EDTA	30 g
Tri-sodium citrate dihydrate	120 g
Sodium nitroferricyanide	0.5 g
DDI	to 1000 mL
Brij-35	3 mL

Into a 1000 mL volumetric flask containing ~600 mL DDI, add 30 g of EDTA, 120 g of tri-sodium citrate dehydrate, and 0.5 g of sodium nitroferricyanide. Mix to dissolve and bring to volume. Add 3 mL of Brij-35. Store in an amber bottle for up to 2 weeks.

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

Sodium hydroxide	3.5 g
Dichloroisocyanuric acid sodium salt dihydrate	0.2 g
DDI	to 100 mL

Into a 100 mL volumetric flask containing ~80 mL DDI, completely dissolve 3.5 g of sodium hydroxide. Add 0.2 g of dichloroisocyanuric acid sodium salt dihydrate. Mix to dissolve and bring to volume. Store in a glass bottle and prepare fresh daily.

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

Sodium salicylate	300 g
DDI	to 1000 mL

Into a 1000 mL volumetric flask containing ~600 mL of DDI, add 300 g of sodium salicylate. Mix to dissolve and bring to volume. Store in an amber bottle for up to 2 weeks.

8.13 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.

8.14 Matrix solution to match samples (2 M KCl):

Potassium chloride	149.1 g
DDI	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. STANDARDS

9.1 Ammonia 10 mg N/L stock standard (**monthly**):

Into a 100 mL volumetric flask containing approximately 50 mL of 2 M KCl (8.14), 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.

9.2 Calibration standards (concentrations in mg N/L):

Blank, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, and 1.0 (or higher).

Using the 2 M KCl matrix solution (8.14) and the 10 mg/L stock standard, prepare the calibration standards according to the following table:

Standard (mg/L)	Vol. of 10 mg/L Stock	Final Volume
0.01	0.05 mL	50 mL
0.025	0.125 mL	50 mL
0.05	0.25 mL	50 mL
0.075	0.375 mL	50 mL
0.1	0.5 mL	50 mL
0.25	1.25 mL	50 mL
0.5	2.5 mL	50 mL
0.75	3.75 mL	50 mL
1.0	5.0 mL	50 mL

9.3 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. Concentrations of these solutions vary and must be prepared according to the manufacturer's instructions. All preparation and dilutions are carried out with appropriate clean flasks and the 2 M KCl matrix solution (8.14).

10. SAMPLE HANDLING AND PRESERVATION

10.1 Samples for ammonia 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.

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

11. 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	1 per 20 samples in an analytical set.
Continuing Calibration Blank (CCB)	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)	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 standard curve. One QC is also analyzed 1 per 20 samples.
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.

12. CALIBRATION AND STANDARDIZATION

12.1 At the beginning of each analytical run, a blank and the following working standards are analyzed to calibrate the instrument: 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, and 1.0 mg N/L. Quality control and calibration checks are also used to determine the accuracy of calibration.

12.2 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.

13. SAMPLE ANALYSIS

AA3 startup procedure (Refer to Bran+Luebbe's Operations Manual for a detailed discussion of AACE procedures).

13.1 Turn on surge protectors to provide power to the PC, pump, autosampler, chemistry manifold, and the colorimeter. Check to make sure the separate switch on the colorimeter is turned on.

13.2 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. 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. 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 digital colorimeter and the PC and between the digital colorimeter and the pump and autosampler. When the download is complete and the dark current has been automatically measured, a chart is opened and it is now possible to monitor the baseline.

13.3 With the wash and 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 DCl reagent lines in their respective reagent receptacles. 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**.

13.4 Monitor the system 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.

13.5 While waiting for system equilibration the analysis file for the day's analytical run can be set up. If the proper **.anl** file was loaded before starting **Charting** then that will be the default folder that will open when opening **Set Up/Analysis**. Highlight the **.anl** file and press **New Run**. This will create a file for that day's run. For example, the file name 020822A means year 2002, month of August, the 22nd day,

with A being the first run of that day. Multiple runs can be generated on the same day with a B, C, etc., suffix attached to the year/month/day format.

13.6 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. At this point the analyst can transfer the reagent and wash lines into vessels containing DDI 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. Turn off the digital colorimeter and 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.

14. CALCULATIONS AND DATA REPORTING

14.1 After completion of the analysis, select **Retrieve/View Chart** to check that AACE has marked all the peaks correctly. 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 would have to be made again. This extra step may be avoided by making any corrections to the file that is intended for use.

14.2 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.

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

14.4 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.

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

15. 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.

16. METHOD PERFORMANCE

This method was validated through a single laboratory studied and an inter-laboratory method validation study (SM4500-NH3 G). Historical performance based on recoveries from 2011 is as follows:

Ammonia in the water:

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

17. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QC MEASURES

Type	Purpose	Criteria
Instrument Calibration	Standardize the instrument.	**Linear regression $R^2 > 0.995$** All standards must be within 10% of their true value
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.	**Mean of observed values for an analytical set are <MDL or <0.02 mg/L, whichever is higher**
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 <0.02 mg/L, whichever is higher**
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%**
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%**
Quality Control Check Standards (QC)	Determine if calibration is accurate.	**Accuracy 95-105%**
Repeat (Rep)	Assess analytical precision.	**Precision (RSD) < 20%**
Spike (spike added prior to sample analysis) (Sp)	Determine if there is matrix interference.	**Recovery 85-115%**

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. Standard DEP qualifiers (Chapter 62-160, F.A.C.) may be used when requested by clients or project manager, or if project is known to be used for DEP reporting.

18. CORRECTIVE ACTIONS

QC Activity	Acceptance Criteria	Recommended Corrective Action
Initial Calibration Standards	Correlation coefficient R > 0.995	Reanalyze standards. If same response is obtained, re-optimize instrument and restart analysis. If same response is obtained, prepare new standards & restart analysis.
Initial Instrument Blank Method Reagent Blank	<MDL response & value	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)	Mean of observed values for an analytical set are <MDL or <0.02 mg/L, whichever is higher	Recalibrate and reanalyze the affected portion of the run.
Practical Quantification Limit (PQL)	Mean of observed values from an analytical set within 10% of true value	Reevaluate system, recalibrate & reanalyze the affected portion of the run.
Continuing Calibration Standard (CCCS)	Mean of observed values from an analytical set within 5% of true value	Recalibrate and reanalyze the affected portion of the run.
Quality Control or Check Standards (QC)	Accuracy within established limits (95-105%)	Reanalyze or re-prepare QC check standard. If same response is obtained, prepare new primary & 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.
Repeat (Rep)	Precision within established limits (<20%)	Determine and eliminate cause of problem (baseline drift, carryover, etc). Reanalyze all affected samples.
Spike (Sp)	Recovery within established limits (85-115%)	Check pipette, remake spike, and reanalyze. In the meantime, spike a DDI water or Blank Matrix. If acceptable, reanalyze affected samples. If not acceptable, but the recovery of DDI water or blank matrix is acceptable, then it is likely sample matrix interference. Make proper notation on the analytical report.

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 Analytic. 1999. Method No. G-171-96 REV. 14. AutoAnalyzer Applications.

19.4 Bran+Luebbe AutoAnalyzer 3 Operations Manual.

20. REVISIONS