

Standard Operating Procedure for:

Total Dissolved Nitrogen

University of Missouri Limnology Laboratory

Prepared by: Dan Obrecht

Date: April 30, 2021

Approved by: Christopher Brunet

Date: May 25, 2021



Table of Contents

1	Identification of the method	3
2	Applicable matrix or matrices	3
3	Detection limit	3
4	Scope of the method	3
5	Summary of the method	3
6	Interferences	3
7	Health and Safety	4
8	Personnel qualifications	4
9	Equipment and supplies	4
10	Reagents and standards	5
11	Quality Control	5
12	Analysis	6
13	Data acquisition, calculations, and reporting	14
14	Computer hardware and software	14
15	Method performance	15
16	Pollution prevention	15
17	Data assessment, acceptable criteria for quality control measures and actions for out-of-control or unacceptable data	15
18	Waste management	16
19	References	17

1 Identification of the method

- 1.1 Total dissolved nitrogen analysis via second derivative spectroscopy following Persulfate Digestion (Standard Methods 4500-N C).

2 Applicable matrix or matrices

- 2.1 This method is suitable for the analysis of [environmental samples](#). The method can be used for wastewater samples, but will require dilution of samples to fit within the [calibration curve](#).

3 Detection limit

- 3.1 [Method Detection Limit](#): 0.035 mg N/L
- 3.2 This [Method Detection Limit](#) was determined by taking the calculated concentrations of the lowest calibration standard from multiple [analytical runs](#) (using seven or more individual values taken from at least three [analytical runs](#)) and calculating a standard deviation from those concentrations. This standard deviation is then multiplied by the one-sided t-statistic at the 99% confidence level for the appropriate degrees of freedom ($n - 1$).

4 Scope of the method

- 4.1 This standard operating procedure is intended to provide MU Limnology [operators](#), [technicians](#), and [analysts](#) with guidance on the analysis of total dissolved phosphorus. This document is not intended to replace individual training in this method by an experienced MU Limnology [technician](#).

5 Summary of the method

- 5.1 10 mL aliquots of well mixed filtrate are placed into acid washed culture tubes. A digestion solution is then added to each tube and the tubes are autoclaved to digest/transform all forms of nitrogen into nitrate (NO₃). Tubes are acidified and centrifuged prior to analysis. Samples are placed in a spectrophotometer and absorbency readings are taken at three wavelengths (230, 225 and 220 nm). The second derivative of the three absorbency values is related to the nitrate concentration in the sample.
- 5.2 Desired performance criteria for this method are:
- Operating Range: 0.035 — 1.000 mg N/L
 - Sample Volume: 10 mL
 - Run Time: 3 to 4 hours
 - Digestion Time: 1.5 hours
 - Samples per run: 26 — 39

6 Interferences

- 6.1 Bromide can interfere at seawater concentrations (68 mg Br⁻/L) so the method is not suitable for seawater.
- 6.2 Iron (Fe) and copper (Cu) do not interfere at concentrations of 2 mg/L, but seriously interfere at concentrations of 20 mg/L.
- 6.3 When nitrate concentrations exceed 3.0 mg N/L the UV absorbance spectrum shifts upward, which may result in poor accuracy.
- 6.4 Inorganic turbidity can interfere with the method via light absorption. Highly turbid samples tend to have poor replication. Removal of turbidity by centrifugation reduces this interference and results in better replication.

7 Health and Safety

- 7.1 Environmental water samples may contain microorganisms that can result in infection/illness. Laboratory personnel working with environmental waters should take precautions such as wearing gloves and washing hands frequently to minimize risks.
- 7.2 The [calibration standards](#), [QC](#) samples and [environmental samples](#) pose no unusual hazards to laboratory personnel that are wearing standard [PPE](#) such as lab coats, gloves, and safety glasses/goggles.
- 7.3 The method does call for samples to be acidified with 0.1 mL of sulfuric acid prior to analysis. This results in the tubes having low pH (<2). Laboratory personnel should review the MSDSs for all chemicals used in the method.
- 7.4 The digestion process of the method uses an autoclave that heats samples to >250 °F (121 °C). Care should be taken when operating the autoclave to avoid burns. Autoclaves should be allowed to cool completely before removing the samples within. Opening the autoclave while it is still hot could result in steam burns.

8 Personnel qualifications

- 8.1 Samples will be analyzed by MU Limnology staff who have been trained to the [operators](#) or [technician](#) level in this method and who are familiar with all of the MU Limnology sampling handling and labeling procedures and appropriate [SOPs](#).
- 8.2 The [calibration standards](#), secondary [QC](#) standards, environmental [reference](#), spiked [reference](#), and color reagent will be prepared by MU Limnology [technicians](#).

9 Equipment and supplies

- 9.1 Glass culture tubes: Tubes are 16mL in volume, 15 mm x 125 mm in size.
- 9.2 10 mL micropipette
- 9.3 Repipettes 1) Digestion Solution and 2) H₂SO₄
- 9.4 Analytical Balance
- 9.5 Autoclave

- 9.6 Centrifuge
- 9.7 Spectrophotometer
- 9.8 Whatman GF/F Glass Microfiber Filters, 0.7 µm pore size, 47 mm, CAT No. 1825-047
- 9.9 47 mm Magnetic Filter Flask, PALL
- 9.10 Internal Vacuum System or Portable Vacuum Pump
- 9.11 Sodium Hydroxide, Fisher Chemical, S318-500
- 9.12 Potassium Peroxydisulfate, Alfa Aesar, 13145
- 9.13 Sulfuric Acid, Fisher Chemical, A300-500
- 9.14 Nitrate Standard Solution, RICCA, R5307900-120A
- 9.15 Ammonium Standard Solution, RICCA, 691-4

10 Reagents and standards

- 10.1 [Calibration standards](#) should be prepared as shown below in Table 1, in a clean volumetric flask. All stock solutions should be added quantitatively using a calibrated micropipette. Rinse all glassware three times with [UPDI](#) and then fill ~75% of the way to the line. Add the appropriate stock solution, fill to the line with [UPDI](#), cover with parafilm and invert three times to mix. Standards may be stored in glass bottles at 4°C.

Table 1. Nominal Concentration of Nitrogen [Calibration Standards](#)

Standard Concentration (mg N/L)	2.5	1.0	0.5	0.25	0.1
Total Standard Volume (mL)	1000	1000	1000	1000	1000
Volume 1000 mg N/L Stock (ml)	2.5	1	0.5	0.25	0.10

- 10.2 [Reference](#) and spiked [reference](#) are made using environmental water that has been filtered through a 0.7 µm glass fiber filter. Both the [reference](#) and spiked [reference](#) should be made using the same water, at the same time, with a 1 L bottle of each being prepared. The spiked [reference](#) will be spiked to increase nitrogen concentration by 0.4 mg N/L. The stock solution used to make the [calibration standards](#) is also used to make the spiked [reference](#).

11 Quality Control

- 11.1 Reagent Coloring
Prior to beginning analysis, all reagents should be visually expected for discoloring.
- 11.2 [Check Standards](#)

[Check standards](#) must be analyzed with every run. [Check standards](#) will be identical to the [calibration standards](#) used in the run and will be included at the end of the run.

11.3 Secondary [QC](#) Standards

Secondary [QC](#) standards must be analyzed with every run. These standards should be identical in concentration to the highest calibration standard but prepared from a different stock solution.

11.4 Environmental [Reference](#) and Spiked [Reference](#)

Three tubes of the environmental [reference](#) and three tubes of the spiked [reference](#) will be included in every run and analyzed identically to the samples.

12 Analysis

12.1 Calibration and standardization

- Each [analytical run](#) will contain [calibration standards](#) of known nitrogen concentrations. The calculated second derivative using the three absorbency values from the [calibration standards](#) are used, along with their nitrogen concentrations, to generate a linear equation in an Excel Spreadsheet. This linear equation is then used to determine the nitrogen concentrations of the [environmental samples](#) based on their second derivative values.

12.2 Set-up of Nitrogen [Analytical Run](#)

- Ten mL aliquots of [environmental samples](#) should be pipetted into clean 16 mL (125 mm x 16 mm) glass culture tubes in either duplicate or triplicate. [Environmental samples](#) that are known to have nitrogen concentrations that exceed 1.0 mg N/L may be pre-diluted with [UPDI](#) water in order to bring the concentration down into the method's range (<1.0 mg N/L).
- Each nitrogen [analytical run](#) will include 30 [QA/QC](#) tubes out of the rack of 108. This includes five different [calibration standards](#), [UPDI](#) blanks, secondary standards, [references](#), and spiked [references](#). There are four of each [calibrations standards](#) <1.0 mg N/L and [UPDI](#) blanks (three on the front of the run, with one at the back). Two secondary standards are at the front of the run, while two [calibration standards](#) of 2.5 mg N/L are located at the back of the run, as are the [references](#) and [spiked references](#). This leaves 78 slots for [environmental samples](#), which allows for analysis of 39 [environmental samples](#) if run in duplicate or 26 [environmental samples](#) if run in triplicate.
- A nitrogen data sheet will be filled out with each [analytical run](#). The sheet will identify the [environmental samples](#) within the [analytical run](#), including site name, sample depth/type, date of collection, etc. Other information recorded on the analytical sheet includes information about pre-dilution of samples suspected of exceeding the analytical range (>1.0 mg N/L).

12.3 Digestion of Nitrogen

- To each tube ([calibration standards](#) and [check standards](#) as well as [environmental samples](#)) add 1.5 mL of digestion solution. The solution should be made up immediately prior to digestion, with dosed tubes being placed in the autoclave right after being dosed with digestion solution (the solution has a short life span).
- To make digestion solution, first rinse the [repipette](#) bottle located in Room 326 with [UPDI](#) water to ensure cleanliness (make sure to pump [UPDI](#) water through the [repipette](#) mechanism to rinse plumping). Measure out 200 mL [UPDI](#) in the graduated cylinder that is reserved for this use (also located in Room 326) and pour into the [repipette](#).
- Measure out 12 grams of Sodium Hydroxide using the analytical balance and add to the [UPDI](#) in the [repipette](#) bottle, cover with parafilm, and swirl until all of the Sodium Hydroxide has dissolved.
- Measure out 12 grams of Potassium Peroxydisulfate using the analytical balance and add to the [repipette](#). Swirl to dissolve the Potassium Peroxydisulfate. Note that this chemical is added in surplus, and not all of it will dissolve readily. Swirl until the rate of dissolution decreases to a point where it is not noticeable. NOTE: the digestion solution is sensitive to the order in which the chemicals are added, make sure to dissolve Sodium Hydroxide completely before adding Potassium Peroxydisulfate.
- Prior to use, the water level in the autoclave needs to be checked. It should be near the top of the heating element where it connects to the autoclave wall. If the autoclave needs more water, add [DI](#).
- A small strip of autoclave tape will be placed on the rack before it is put into the autoclave. The autoclave is closed, and the lid tightened. The six wingnuts need to be tightened evenly so the gap between the lid and the autoclave is even. If the lid is tightened unevenly there will be steam leaks. Make sure the emergency release valve is in the down position.
- Turn the autoclave on, once it reaches 250 °F set a timer for 1 hour. You will want to check the autoclave throughout the digestion period to make sure the temperature remains between 250 – 260 °F.
- Once the digestion is completed, the autoclave is turned off and left to cool on its own. Opening the autoclave while it is still hot, poses a hazard for steam burns. Also, release of the steam from the autoclave while it is still hot could result in samples being boiled out of the tubes. After the autoclave has cooled, the tubes can be removed and placed into the refrigerator if not being analyzed right away.

12.4 Acidification and Centrifuging of tubes

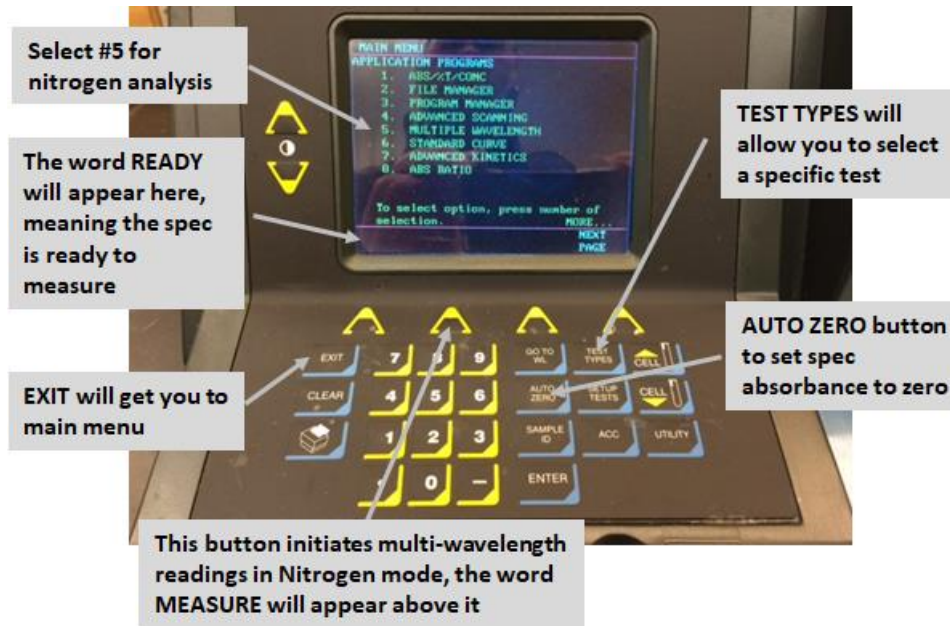
- Remove rack from refrigerator to warm. Tubes should be at room temperature before analyzing – at least 30 minutes. Verify the rack has been autoclaved by putting autoclave tape on the datasheet.

- Prior to analysis, all tubes in the nitrogen run need to be acidified. A [repipette](#) bottle containing the appropriate sulfuric acid is in Room 326. Each tube receives 0.1 mL of the acid. Once all tubes have been dosed, they should be inverted to mix.
- After acidification, you should centrifuge the tubes. Centrifuging will help remove any inorganic sediment from suspension, which is desirable as these materials will absorb light. Samples that contain suspended sediment will not replicate as well as those in which the sediment has been spun out of suspension.

12.5 Analyzing Total Dissolved Nitrogen using the Genysis 2 Spectrophotometer

- Turn on the Genysis 2 Spectrophotometer at least 30 minutes prior to analysis.
- After the spectrophotometer has warmed up and gone through its diagnostic checks, you will need to select the Nitrogen method. From the initial screen select Multiple Wavelength Absorbency (#5). After the screen switches to the Multiple Wavelength screen, press the Test Types key and select Nitrogen. The spectrophotometer will adjust the location of the cell holder and set up with the appropriate wavelengths (230, 225, & 220 nm).
- Auto Zero the spectrophotometer with nothing in it (no cuvette). After rinsing out a 5 cm quartz cuvette (regular glass cuvette will not work at low wavelengths) well (and wiping down the two clear sides) place the cuvette with [UPDI](#) water (2/3 full) into the spectrophotometer and close the lid. Press the Measure button (second triangular button from the left side). The spectrophotometer will take a few seconds to collect the three readings. Once the readings are finalized, calculate the second derivative for the [UPDI](#) water. The second derivative should be <0.08.
- Turn vacuum on at wall and evacuate the [UPDI](#) water from the cuvette.

Location of select keys on the spectrophotometer.



- Start with the standards at the beginning of the rack. Carefully pour sample into the cuvette, making sure not to disturb the bottom sediment in the tube. In order to do this, you will want to make sure not to pour too much sample into the cuvette (2/3 full is fine). Make sure you do not touch the clear sides of cuvette as fingerprints/smudges could affect absorbency readings. If you need to wipe down cuvette, use Kimwipes located near the spectrophotometer. Place cuvette into the spec, close the lid, and press measure. Once the spec is done taking the readings, record the three absorbency values on the data sheet. Use the calculator located near the spectrophotometer to calculate the second derivative value. You can compare your results with those from previous runs by looking at the Nitrogen Logbook, located on the shelf above the spectrophotometer. If your results differ substantially from previous results, inform the lab manager.
- After you have completed all the [calibration standards](#) at the front of the run, enter appropriate information into the nitrogen logbook. You will need to calculate the cut-point for dilutions using information from the 1.0 mg N/L Calibration Standard. This value is calculated by subtracting the 230 nm absorbency value from the 225 nm absorbency value, and multiplying by 1.1 to get cut-point value.
- Example: $1121 (\text{Abs}_{225}) - 752 (\text{Abs}_{230}) = 369 * 1.1 = 406$
- In this hypothetical [analytical run](#), any [environmental samples](#) in which the difference between the 230 nm absorbency and 225 nm absorbency exceeds 406 will require a “dilution” to get the concentration within the analytical range.

- Dilutions are done by using a smaller cuvette (shorter light path, thus less light is absorbed). All samples that require dilution are poured back into the test tube and saved for the end of the [analytical run](#). After all samples that can be analyzed using the 5 cm cuvette have been dealt with, the 2 cm quartz cuvette is rinsed thoroughly, and the spec zeroed with this cuvette filled with [UPDI](#) water. Samples needing dilution, as well as the 2.5 mg N/L [calibration standards](#) located at the back of each rack, are analyzed using the smaller cuvette. All procedures are the same for this cuvette; the only difference is the calculated 2nd derivative will be multiplied by 2.5 to get the final nitrogen concentration. Make sure to note any samples that are run using the smaller cuvette as being a 2.5 times dilution.

12.6 Analyzing Total Dissolved Nitrogen Analysis using the Cary 60 Spectrophotometer

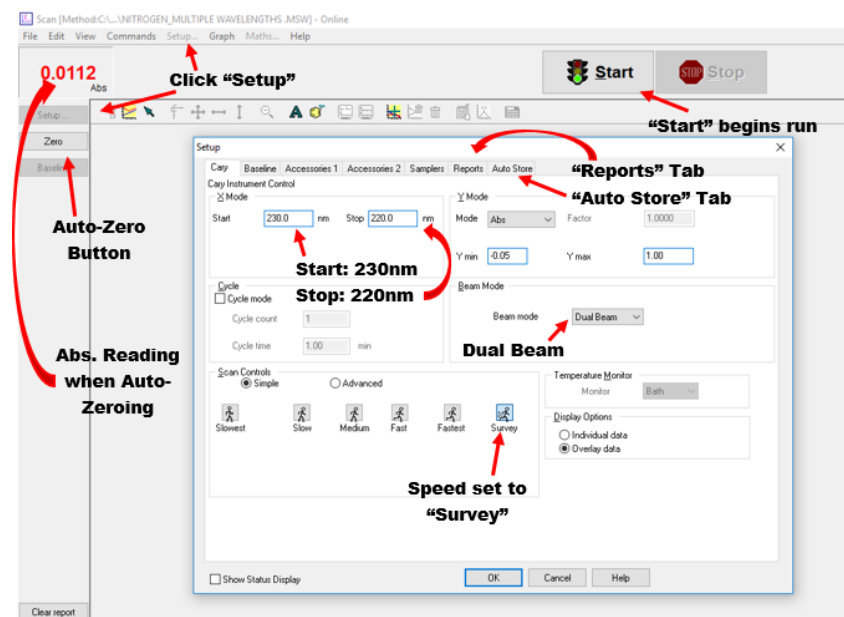
- The Cary 60 is run in tandem with the HPZ240 computer located above and to the right of the spec. Turn on the Cary60. The “ON” button will turn green once the lamp has heated up and the machine is ready to use.
- Open the nitrogen methods, titled “NITROGEN_Multiple Wavelengths.MSW” on the desktop. Make sure that all settings are correct by opening the Setup tab located at the left of the screen.

Input Start: 230 nm

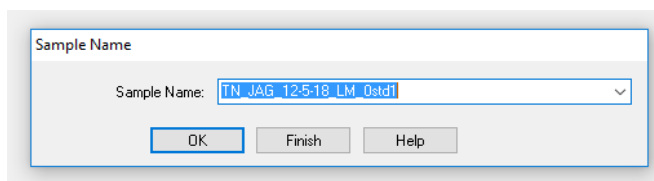
Input Stop: 220 nm

Speed: Survey

Beam Mode: Dual Beam



- Click the “Autostore” tab and make sure that “Storage on (prompt at end)” is selected.
- Click the “Reports” tab and make sure “ASCII (csv)” is selected (no log).
- Close the Setup menu. Once you have verified that all the settings are correct, you are ready to begin the run. Rinse the 5cm quartz cuvette 3x with [UPDI](#). The cuvettes are located on the shelf above the Cary60 in small, Tupperware containers labeled according to cuvette size and type.
- Fill the cuvette 2/3 of the way with [UPDI](#) and ensure that the sides of the cuvette are free of fingerprints and drops of water. Place the cuvette in the spec, close the lid, and click “Auto-Zero.
- Empty the cuvette into the Acid Solution waste container, rinse 3 times with [UPDI](#), and fill it again with [UPDI](#) water. Place the filled cuvette in the spec.
- Click “Start.” A Sample Name pop-up window will appear. Name the sample (such as [UPDI_test_1](#)) and click OK. The absorbance, found in the top left of the screen, should read zero. Steps 11-13 must be repeated until you get two consecutive 0.0000 readings, +/- 0.0003. If you would like to re-zero the spec, click “Finish” in the pop-up window. Don’t save when prompted. The nitrogen data template only has room for two [UPDI](#) readings. If it takes you more than two readings to get consecutive zeros, take care when copy and pasting into the nitrogen template.
- At this point, you are ready to begin running your standards. Be sure to [UPDI](#) rinse 3 times between each tube and provide a unique name for each standard in the pop-up window. Include Total/Dissolved Nitrogen, initials, date, project, standard (e.g., TN_JAG_2018_12_06_LM_0std1, this is the first replicate of the 0 standard). Note that date is listed as YYYY_MM_DD. The program will automatically count-up from the last number you type in. If your tubes are in order, you should not have to change the sample name unless you have a dilution and switch tubes.

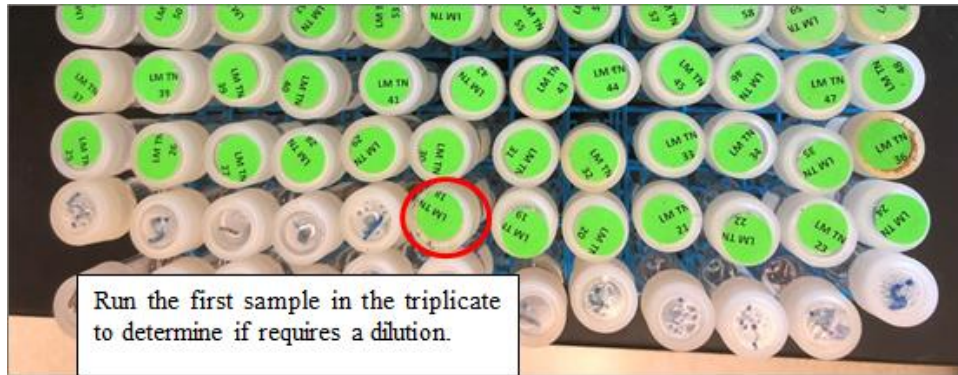


Sample Name

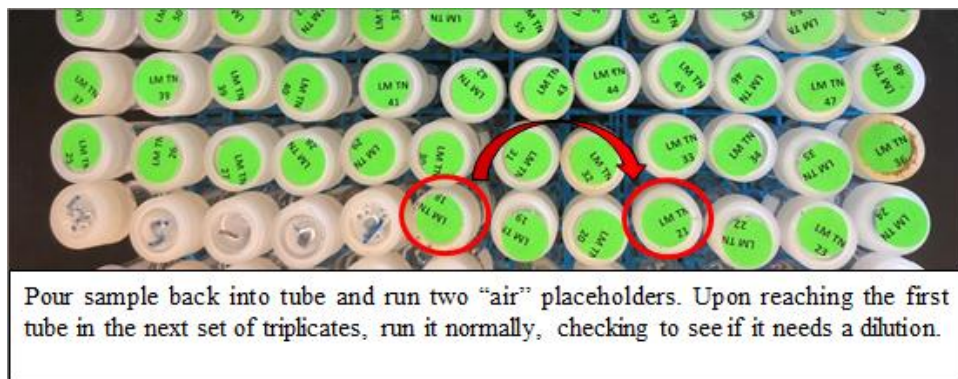
Sample Name:

- When you click Start, a Sample Name pop-up window will appear. Name the sample like: TN/TDN [Analyst](#) Initials_Date_Project_NAME. Clicking “OK” runs the sample. Click “Finish” to check absorbance and calculate 2nd derivative.

- After running your standards, click “Finish” in the pop-up window. Click Cancel when prompted to save. Instead, click on File à Save Data As and save the data file on the Desktop as a .csv file. You will be opening this file frequently throughout the run. After you have saved this file, minimize the Cary60 screen and open the data file you just saved.
- Next, open the Nitrogen Template excel file found on the Desktop. You will want this template, and the Cary 60 absorbance file you just saved, pulled up next to each other.
- Copy rows 1-5 in the absorbance file and paste them into the Screen tab of the Nitrogen Template. Note that you must Paste Special à Transpose when adding to the template. The template calculates the 2nd derivative from the absorbance readings automatically once you have pasted. For reference, the 2nd derivative is calculated as: $1000 * ((Abs230 - (2 * Abs225) + Abs220) / 25)$.
- Record standard 2nd derivatives in the logbook, located next to the computer.
- Run the first sample tube by clicking “Start.” Name the sample in the pop-up window, this time including Tube # instead of standard (EX: TN_JAG_2018_12_06_LM_Tube#18). The spec counts up from the first number you enter, so if your tubes are in order, you should only have to name the sample once.
- To determine whether you need to dilute your sample, you will have to check the absorbance after the first sample in every triplicate. To do this, click “Finish” in the Pop-Up window and do not save on prompt. Instead, click on File/Save Data As and overwrite the .csv file you saved in Step 15. Open this window, and copy/paste the sample you just ran into the Screen tab of the Nitrogen Template. Note that you must Paste Special à Transpose when adding to the template. The difference between Abs225 and Abs230 will automatically be calculated and multiplied by 1.1 once the cells have been populated. Select the median absorbency value from the 1 standards and enter it into the “Cut Point” cell. If the sample you just ran is greater than the “Cut Point” value, “Dilute” will appear next to the sample. Pour the cuvette contents back into the tube. Scan twice more on air. These will act as placeholders and will ensure your tube numbers do not get mixed up when you are copying and pasting into the template. When you get to the first replicate in the next set of tubes, be sure to check to see if it needs a dilution.
- If your sample does not require a dilution, “Okay” will appear next to the sample. You may now run the next three tubes.
- At the start of each set of triplicates:



- If a dilution is required:



- If a dilution is not required:



- Save tubes that require a dilution until the end of the rack as they will be analyzed with a 2cm quartz cuvette. After the run is complete through the 1.0 standard on the back row (save this standard if there are dilutions), re-zero the spec on the [UPDI](#) rinsed 2 cm quartz cuvette. Measure the 2.5 standards and the saved 1.0 standard. Make sure that you include “2.5Post” in the names of these standards when they appear in the pop-up window. This indicates that they have received a x2.5 post-dilution.

- Run all the remaining samples that require a dilution. Be sure to include “2.5Post” in the name of all of these samples to track that they were analyzed using the 2 cm quartz cuvette.
- When the run is complete, save your files in the Nitrogen Folder on the Desktop. Create a New Folder titled with the date of your run and put your files in there. In the file name, be sure to include TN/TDN [Analyst](#) Initials_Date_Project (EX: TN_JAG_2018_12_06_LM). You should save 2 files: the Nitrogen Template workbook, and the raw .csv file output from the Cary60 program.
- Clear off the computer Desktop and turn off the Cary60 spec.
- Backup your files to OneDrive. Select MULimnology, open the “Cary60” folder, open the “Nitrogen” subfolder, and click Upload in the top right of the screen. Upload the files you just saved.
- The Nitrogen template will create the standard curve and calculate nitrogen concentrations, but the user must make sure that samples are listed in the correct order. If there are no dilutions, copy and paste the entire Screen tab into the Manipulate tab. If there are dilutions, copy and paste from the Screen tab into the Manipulate tab so that tube numbers are in order. Also, manually enter “2.5” into the Post Dilution column.

12.7 Post Analysis Clean-Up

- Any remaining sample in the tubes should be dumped into the waste beaker located near the spectrophotometer. Triple rinse your test tubes with 3 tap, 3 [DI](#), then fill the tubes with [DI](#) to soak overnight. Remove the paper labels from the caps and place caps in a CAP RINSE bottle. Triple rinse with 3 tap, 3 [DI](#), and leave the caps to soak in [DI](#) overnight.
- Rinse the cuvette/s with [DI](#) water and place them back into the appropriate containers.
- Unwanted material located in the waste beaker and the catch bottle near the spectrophotometer needs to be dumped into the unwanted material container label “Acid Solution” located in room 326.
- Turn off the vacuum at the wall value, turn off spectrophotometer if it is not going to be used later in the day.
- Wipes up any spills with a sponge or paper-towel moistened with tap water.

13 Data acquisition, calculations, and reporting

- 13.1 Calculations of concentrations: Concentrations of unknown [environmental samples](#) are based on linear equation for the regression data from the [calibration standards](#).
- 13.2 Reporting Results: Results should be reported in milligrams nitrogen per liter (mg N/L) up to 3 decimal places (0.000). The detection limit of the method is 0.035 mg N/L.

14 Computer hardware and software

- 14.1 Windows 7 Enterprise

14.2 Cary WinUV Software

15 Method performance

- 15.1 [Method Detection Limit](#): 0.035 mg N/L.
- 15.2 Precision: Coefficient of Variation ([CV](#)) of duplicates/triplicates for unknown [environmental samples](#) should be <5 % and/or range of 0.100 mg N/L.
- 15.3 Calibration $r^2 > 0.99$

16 Pollution prevention

- 16.1 All reagents and standards will be prepared in appropriate volumes to reduce waste.
- 16.2 All samples and reagents will be handled according to [MU EHS](#) policies in order to ensure proper disposal.

17 Data assessment, acceptable criteria for quality control measures and actions for out-of-control or unacceptable data

- 17.1 Individual [analytical runs](#) will be entered into an Excel spreadsheet created specifically for nitrogen analysis. The second derivative values associated with the [Calibration Standards](#) will be entered in the appropriate cells and a regression analysis will be conducted.
- 17.2 The calculated concentrations of the [Calibration Standards](#) (automatically calculated by the spreadsheet) will be checked to determine if values are acceptable. Standards will be deemed acceptable if the average concentration of the three replicates are +0.025 mg N/L of the expected concentration or within 10% of expected concentration.
- 17.3 The r^2 of the regression will be reviewed and deemed acceptable if the value is >0.99.
- 17.4 Second derivative values for the [environmental samples](#) and [QC](#) samples will be entered into the spreadsheet. Nitrogen concentrations will automatically be calculated by the spreadsheet.
- 17.5 Replicates of [environmental samples](#) are deemed acceptable if the Coefficient of Variance ([CV](#)) of the calculated concentrations is <5 % or the range of calculated concentrations is <0.100 mg N/L. The spreadsheet is set up to determine if these two criteria are not met, and the data will automatically be flagged as needing to be re-analyzed.
- 17.6 [Check standards](#) placed at the end of the [analytical run](#) will be reviewed. Calculated nitrogen concentrations of these [check standards](#) should be +0.025 mg N/L or within 5 % of expected values. If the [check standards](#) fail to meet criteria the lab manager will be notified, and the [analytical run](#) will be evaluated to determine if there was “drift” during the course of the run. If there was machine drift (all [check standards](#) showing an upward or downward shift in results) the [analytical run](#) will be discarded.

- 17.7 Results from the [reference](#) samples and the spiked [reference](#) samples will be copied into an electronic Spike Log after each run. The Spike Log will automatically calculate averages for both the [reference](#) and spiked [reference](#) samples, determine recovery of the Spike, and flag any results if recovery is not +15 % of the spike concentrations of 400 mg N/L.

$$\frac{\%R = \text{matrix spike result} - \text{reference result}}{\text{matrix spike value}} \times 100$$

- 17.8 If the [calibration standards](#)/regression is deemed out-of-control (low r-square and/or concentrations of [calibration standards](#) are off) the lab manager will evaluate the run. The Lab Manager will make decisions concerning the removal of any [calibration standards](#) that may be outliers. After any outliers are removed, the regression will be re-calculated. If the [calibration standards](#)/regression is still out-of-control, the run will be discarded.
- 17.9 Environmental [sample replicates](#) that fail to meet criteria will be re-analyzed. Original and re-analyzed results will be evaluated to determine cause of initial poor replication (outlier or sample with high variability). Decisions concerning outliers are made following the protocol laid out in Standard Methods Test of Discordancy for a single outlier in a normal sample.

High outliers are dropped if: $(\text{Valuemax} - \text{Allavg}) / \text{AllStdDev} > 1.94$

Valuemax = Maximum value of replicates (suspected outlier)

Allavg = the average of all replicates

AllStdDev = the standard deviation of all replicates

1.94 = Critical Value for 6 values and a 1% level of significance

Low outliers are dropped in: $(\text{Allavg} - \text{Valuemin}) / \text{AllStdDev} > 1.94$

Valuemin = the minimum value of replicates (suspected outlier)

High variation is not considered problematic if the difference between two adjacent values, when values are ordered low to high, is multiplied by two and does not exceed the total range of values. Variable data that are considered problematic will be reviewed by the lab manager to determine appropriate steps, which may include re-analyzing the sample again if there is enough water left.

18 Waste management

- 18.1 All waste generated is considered hazardous.
- 18.2 All analyzed standards and reagents should be treated as waste upon completion of the run.
- 18.3 Waste should be kept in an approved container with proper labeling.
- 18.4 Waste will not be held for longer than 6 months and [MU Environmental Health and Safety \(EHS\)](#) will be notified an appropriate time before this point so that waste can be collected and disposed of.

19 References

- 19.1 Crumpton, W.G., T.M. Isenhart and P.D. Mitchell. 1992. Nitrate and organic N analysis with second derivative spectroscopy. *Limnology and Oceanography*. 37:907-913.
- 19.2 Standard Methods for the Examination of Water and Wastewater, 23rd Edition. 2017. Method 4500-N C. Nitrogen-Persulfate Method. American Public Health Association. Washington, DC.