Standard Operating Procedure for:

Total Nitrogen
(3020R02 Total N.doc)

Missouri State University

and

Ozarks Environmental and Water
Resources Institute (OEWRI)

Prepared by: _____ Date: _____
OEWRI Quality Assurance Coordinator

Approved by: _____ Date: _____
OEWRI Director

Approved by: _____ Date: _____
MSU Chemistry Faculty

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        Total nitrogen analysis by spectrophotometry.

2   Applicable matrix or matrices
        This method is suitable for the analysis of water samples collected from rivers, streams, lakes, and wastewater.

3   Detection Limit
        As described here, the detection limit is ≤ 0.1 mg TN/L and the upper range 5 mg TN/L.

4   Scope of the test method
        This Standard Operating Procedure provides Missouri State University (MSU) laboratory personnel with guidance on the procedure for determining total nitrogen (TN) in surface water samples. TN is a measure of all forms of nitrogen present in a sample. This method may give poor recoveries for organic compounds which contain nitrogen-nitrogen double bonds or terminal nitrogen groups (e.g., H-N=C). As described here, the detection limit is 0.1 mg TN/L and the upper range 5 mg TN/L. The upper range may be extended by sample dilution.

5   Summary of test method
        The procedure outlined below follows that described by Crumpton, Isenhart, and Mitchel (1992). A water sample is combined with an alkaline persulfate oxidizing solution and heated to approximately 120ºC in an autoclave or pressure cooker. This quantitatively converts most nitrogen compounds to nitrate. The digested sample is acidified with hydrochloric acid, and then its absorbance is measured at three wavelengths (230, 225, and 220 nm). The absorbance data are used to compute the second derivative at 225 nm. Comparison of the second derivative with that of similarly-treated standards allows estimation of total nitrogen.
        The desired performance criteria for this measurement are:
        1.   Detection limit: ≤ 0.1 mg TN/L
        2.   Precision: ± 20% RPD
        3.   Accuracy: ± 20%
        4.   Minimum Quantification Interval: 0.1 mg TN/L

        The applicable range for the method is ≤ 0.1 mg TN/L to 5 mg TN/L and may be extended by dilution.

6   Definitions
6.1   The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

6.2   Analytical batch: The set of samples processed at the same time.

6.4   Calibration standard: A solution prepared from the primary dilution standard solution or stock standard solutions. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

6.5   De-ionized water blank (DIB): This blank should not contain any concentration of analyte and is not processed or digested like the standards, checks, or samples. The de-ionized water blank should be less than the laboratory reagent blank (LRB).

6.6   Digestion Efficiency Check (DEC): This is a urea intermediate standard solution that is used to confirm effective digestion of nitrogen forms.

6.5   Field blank (FB) : An aliquot of deionized water treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

6.6   Field duplicate (FD): Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

6.7   Laboratory control check sample (LCC): A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

6.8   Laboratory matrix spike (LS): An aliquot of a sample to which a known amount of analyte is added before sample preparation. The LFM is used to evaluate analyte recovery in a sample matrix.

6.9   Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

6.10   Laboratory reagent blank (LRB): An aliquot of deionized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

6.11   Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.
        a.   To calculate the MDL:
        b.   Prepare a solution with the concentration of TN near the estimated MDL
        c.   Analyze seven portions of this solution over a period of at least three days
        d.   Include all sample processing steps in the determination
        e.   Calculate the standard deviation (s).

      f.   From a table of the one-sided *t* distribution select the value of *t* for 7 – 1 = 6 degrees of freedom at the 99% level. This value is 3.14

      g.   The product 3.14 times *s* is the desired MDL.

6.12   Relative Percent Difference (RPD): calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

6.12   Quality control check sample (QCC): A sample containing analytes of interest at known concentrations (true values). The quality control check sample is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

7   Interferences

7.1   This method may give poor recoveries for organic compounds which contain nitrogen-nitrogen double bonds or terminal nitrogen groups (e.g., H-N=C).

7.2   Sample turbidity may interfere. Turbidity can be removed by filtration of the digested solution through a 0.45 µm pore diameter membrane filter prior to analysis, or by centrifugation.

7.3   Sample color that absorbs strongly around 225 nm (after digestion) interferes.

8   Health and safety

8.1   This analysis involves handling freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.2   The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

8.3   Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) is available to all personnel involved in the chemical analysis.

8.4   The following chemicals have the potential to be highly toxic or hazardous; for detailed explanations consult the MSDS.

      a.   Sodium hydroxide

      b.   Hydrochloric acid

8.5 This procedure requires use of an autoclave or pressure cooker capable of heating samples to 120ºC. All safety directions for using these devices should be followed carefully.

9   Personnel qualifications

Laboratory and field personnel shall have a working knowledge of this analytical procedure and will have received training from an MSU employee knowledgeable of the proper sample analysis procedures. Prior to the first batch of sample analyses, the analyst will complete a demonstration of capability exercise as described below in the Quality control section.

10 Equipment and supplies

10.1 Balance, analytical: capable of accurately weighing to the nearest 0.0001 g

10.2 Glassware: Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

10.3 Glass culture tubes: 20 mm OD × 150 mm long, with linerless polypropylene caps. Clean tubes before use by heating to 120ºC with digestion reagent, or by soaking in 5% HCl. New tubes may be used without prior cleaning.

10.4 Spectrophotometer: Hitachi UV-2001 or Shimadzu UV-1600, or equivalent.

10.5 Spectrophotometer cells: 1 cm or longer path length (flow cells may be used)

10.6 Heating unit: Use one of the following:
   a. Autoclave
   b. Pressure cooker

11 Reagents and standards

11.1 Deionized water: Use deionized water that has been purified with a Barnstead/Thermolyne purification system (or equivalent) that includes ion exchange and organic purification cartridges. Use this water for all procedures as well as the de-ionized water blank (DIB). Use 100 mL of de-ionized water for the de-ionized water blank (DIB).

11.2 6 M HCl (6N HCl): Add concentrated HCl to an equal volume of water with mixing.

11.3 6 M NaOH: Prepare by one of the following methods:
   a. Dissolve 240 g ACS reagent grade NaOH per liter of water.
   b. Dilute 320 mL 19 M NaOH (commercially available) per liter.

11.4 Nitrate solutions: Prepare two sets of the following, using different sources of potassium nitrate (e.g., different lot numbers from the same supplier or different suppliers). Use one to prepare calibration standards and the other to produce quality control standards.
   a. Stock nitrate solution (1.00 mg N/mL):

1. Dry KNO_3 in an oven (105ºC) for 24 hours.
2. Dissolve 7.218 g in water with 2 mL $CHCl_3$ (preservative) and dilute to 1 L.
3. This solution is stable for at least 6 months.
4. 1.00 mL = 1.00 mg NO_3-N.
5. Commercially prepared nitrate solutions may be purchased instead.

    b. Intermediate nitrate solution (0.10 mg N/mL):
        11.5.1.1 Dilute 25.0 mL nitrate stock solution to 250 mL.
        11.5.1.2 This solution is stable for six months.
        11.5.1.3 1.00 mL = 0.100 mg NO_3-N.

11.5 Urea $(CO(NH_2)_2)$ stock solution (1.00 mg N_{org}/mL):
    a. Dissolve 536 mg urea and dilute to 250.0 mL.
    b. Store in refrigerator.
    c. 1.00 mL = 0.10 mg N_{org}.

11.6 Urea intermediate standard solution (0.100 mg N_{org}/mL):
    a. Dilute 10.0 mL urea stock solution to 100 mL with deionized water.
    b. Store in refrigerator
    c. Prepare monthly.
    d. 1.00 mL = 0.100 mg N_{org}.

11.7 Other compounds can be used for the digestion test solution, for example, glycine and glutamic acid.
    a. *Glycine digest-check stock solution (1 mL = 1.0 mg-N)*: Dissolve 3.98 g glycine (C2H5NO2•HCl, FW=111.5) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

11.8 Preparation of nitrate calibration standards and checks: Prepare standards according to Table 1 below. Prepare fresh daily.

Table 1. Preparation of nitrate calibration standards.

Standard solution	Prepare using	Volume of standard solution (mL)	Final Volume (mL)	Concentration (mg NO_3-N/L)	Use
LRB	Deionized water	0.00	250.0	0.00	Calibration blank
TN-1	Intermediate nitrate standard solution (0.10 mg N/L)	0.10	100.0	0.10	Calibration standard
TN-2		0.20	100.0	0.20	Calibration standard
TN-3		0.50	100.0	0.50	Calibration standard

TN-4		1.00	100.0	1.00	Calibration standard
TN-5		2.00	100.0	2.0	Calibration standard
TN-6		5.00	100.0	5.0	Calibration standard
LCC		1.00	100.0	1.0	Lab control check
QCC	Alternate intermediate nitrate standard*	1.00	100.0	1.0	Quality control check
DEC	Urea intermediate standard	2.0	100.0	2.0 mg N_{org}/L	Digestion efficiency check

LRB = Laboratory Reagent Blank; TN = Calibration standards; LCC = Laboratory Control Check; QCC = Quality Control Check; DEC = Digestion Efficiency Check
* Prepared using alternate nitrate standard solution, i.e., not the same standard used to prepare the calibration standards.

11.9 Digestion reagent: Prepare 1.5 M NaOH by diluting 250 mL of 6M NaOH solution (made in section 11.3) to one liter with deionized water. Dissolve 60.0 g potassium persulfate ($K_2S_2O_8$, N < 0.001%) per liter of 1.5 M NaOH to prepare the digestion reagent.

Note: If the total nitrogen in a reagent blank is greater than 0.01 mg/L, recrystallize the potassium persulfate as follows:
- Dissolve 75 g $K_2S_2O_4$ (reagent grade, < 0.001% N) in 500 mL 60ºC water.
- Filter the solution rapidly through loosely packed Pyrex wool and cool in ice water to about 4ºC while stirring continuously.
- Collect the crystals by vacuum filtration on a sintered-glass filter and wash with small amounts of ice water.
- Dry as rapidly as possible in a vacuum over anhydrous calcium chloride.
- Store in a vacuum desiccator over anhydrous calcium chloride.

12. Sample collection, preservation, shipment and storage
12.1 Samples are collected in 500 mL plastic bottles and preserved with concentrated sulfuric acid (H_2SO_4) to pH <2. CAUTION: highly corrosive; will cause chemical burns to exposed skin, wear protective gloves.

12.2 Sample bottles are stored on ice for transport to the laboratory.

12.3 Preserved and refrigerated samples can be stored for up to 28 days.

13. Quality control
13.1 Quality control program: The minimum requirements of the quality control program for this analysis consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory must maintain performance records that define the quality of the data that are generated.

a.   Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). Analyze one matrix spike and one set of matrix spike duplicates for every 20 samples analyzed.

b.   Analyses of laboratory blanks are required to demonstrate freedom from contamination. Analyze one LRB for every 20 samples analyzed.

c.   The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

d.   The laboratory should maintain records to define the quality of data that is generated.

13.2   Initial demonstration of performance. The following must be satisfied before the analytical procedure may be used for samples and before a new analyst may analyze samples.

a.   Method Detection Limit (MDL): To establish the ability to detect the analyte, the analyst shall determine the MDL by carrying through 7 or more separately prepared reagent blank solutions through the analytical procedure in Section 15. The average value, X, and the standard deviation of the values, s, shall be calculated. The MDL is equal to 3s (3 × standard deviation). The MDL and average value, X, must both be less than 0.10 mg N/L.

b.   Initial Precision and Recovery: To establish the ability to generate acceptably precise and accurate results, the operator shall perform 10 replicates of a mid-range standard (0.50 mg NO_3-N/L for low range, 1.0 for high range), according to the procedure in Section 15. Using the results of the replicates, compute the average value, X, and the standard deviation, s, for the analyte. The value of X should be within ± 20% of the true value. The standard deviation should be less than or equal to 20% of the average value.

13.3   The LRB, LCC, QCC, CEC, and DEC should be measured along with the standards at the start of the analytical cycle. The criteria are as listed in Table 2:

Table 2. Quality Control solutions and acceptable ranges.		
Solution	Acceptable range	Comments
DIB	≤ 0.01mg TN/L	Less than LRB
LRB	≤ 0.10 mg TN/L	less than or equal to the required detection limit
LCC	0.80 - 1.20 mg TN/L	within ± 20% of the true value
QCC	0.80 - 1.20 mg TN/L	within ± 20% of the true value

DEC	2.0 – 3.0 mg TN/L	corresponds to 80 – 120% efficiency for oxidation of urea to nitrate

13.4   See Table 3 for QC samples that must be analyzed with each sample batch of ten samples.

Table 3. Quality control samples and acceptance criteria.	
Solution	Acceptance Criteria
DIB	≤ 0.01mg TN/L
LRB	< 0.10 mg TN/L
LCC	0.8 – 1.20 TN/L
Lab Duplicate (LD)	greater of ±20% or ± 0.20 mg TN/L
Field Duplicate (FD)	greater of ±20% or ± 0.20 mg TN/L
2 matrix spike solutions (LS1 and LS2)	both 80% - 120% recovery

14.   Calibration and standardization
14.1   Calibration: Calibration employs a quadratic equation to represent the relationship between the second derivative of absorbance observed for each standard and its concentration. Based on this equation, the absorbance of each standard should predict the concentration of the standard to within ± 20% accuracy.

15.   Procedure
15.1   Preparation of matrix spike samples:
  a.   Prepare two matrix spike samples using 10-mL aliquots of water sample (or a smaller aliquot diluted to 10 mL) from the same sample.
  b.   Spike with 0.10 mL intermediate urea standard (0.10 mg N_{org}/L) solutions. This should increase observed concentration by 1.0 mg/L. Carry each through the sample preparation and analysis procedure (15.2 and following).

15.2   Preparation of samples and digestion: All standards, checks, and samples should be processed in the same manner. The only exception is the de-ionized water blank (DIB) which is only de-ionized water.
  a.   Transfer a portion of each well-mixed sample to a beaker or flask, and then add NaOH until the solution is just neutral (measure using litmus, pH paper, or a pH meter).
  b.   Combine 10 mL sample (or an aliquot of sample and enough water to equal 10 mL) and 1.5 mL digestion reagent in a culture tube and seal securely with polypropylene cap. The analyst may determine an

        appropriate volume based on previous measurements at a site or other information, and then adjust the volume of sample used to minimize the need for later dilutions or reruns.

c.   Place tubes in rack in either autoclave or pressure cooker.

d.   For autoclave, follow manufacturer's directions and heat at 120ºC for 30 minutes.

e.   For pressure cooker: Add sufficient deionized water to pressure cooker to bring water to a depth of at least 5 cm. Heat the pressure cooker on a hotplate set to high until the water in the cooker is boiling, as evidenced by a steady stream of steam emerging from the pressure cooker's vent. Maintain constant boiling (adjusting heat as needed) for 60 minutes. Allow pressure cooker to cool in air for at least 30 minutes. After this initial cooling, a stream of cold water from a faucet may be used to speed up the cooling process. Do not open the cooker until it has cooled to near room temperature.

f.   CAUTION: The tubes may be under pressure. Wearing of eye protection is essential. Open each tube carefully to vent any pressure buildup. (Note: After the digestion procedure, many samples will contain some precipitate and/or will appear cloudy. This normally clears up when acid is added in the next step.)

15.3   Sample treatment:

a.   Add 0.4 mL 6*M* HCl to each sample and stir. (The light colored precipitate that forms during the digestion process will usually dissolve completely upon acidification.)

b.   Filter turbid samples through a 0.45 μm membrane filter, or centrifuge.

15.4   Spectroscopic measurements:

a.   Allow spectrophotometer to warm up at least 15 minutes before starting data collection.

b.   Use a 1-cm quartz (silica) cuvette for all measurements. Other cell path lengths may be used. A flow cell may be used to expedite measurements.

c.   Run a baseline adjustment scan over the range of 230 – 220 nm (or wider), with deionized water in the cuvette that will be used for measurements.

d.   Set the spectrophotometer to take readings at 230, 225, and 220 nm.

e.   Record absorption measurements of the following:
1.   Laboratory reagent blank
2.   Standards TN-1 and TN-2 (0.1 and 0.2 mg TN/L)
3.   Laboratory reagent blank
4.   Standards TN-3 and TN-4 (0.5 and 1.0 mg TN/L)
5.   Laboratory reagent blank
6.   Standards TN-5 and TN-6 (2.5 and 5.0 mg TN/L)
7.   Laboratory reagent blank

f.   Next, run the laboratory control standard check (LCC), quality control check (QCC), digestion efficiency check (DEC), laboratory reagent blank (LRB), and de-ionized water blank (DIB).

g. Run samples. With every batch of 10 samples maximum, also run:
1. Laboratory Control Check (LCC)
2. Quality Control Check (QCC)
3. Laboratory Reagent Blank (LRB)
4. Two matrix spike samples from the same sample
5. One laboratory duplicate (LD)
6. One field duplicate (FD)
7. One de-ionized water blank (DIB)

h. Any samples for which the absorbance at 220 nm is greater than 1.0 should be diluted with a reagent blank solution (not with deionized water) and rerun.

i. The procedure described above may be implemented with a programmed method that provides data output in spreadsheet format. A flow cell system may be used for spectrophotometric measurements.

16. Data acquisition, calculations, and reporting

16.1 Calculation of second derivatives: Carry out the computation, $4 \times (A_{230} + A_{220} - 2 \times A_{235})$ (this is actually $100 \times$ second derivative) for each data set. Note that the Hitachi UV-2001's computational method for three wavelength photometric measurements is based on a parameter that is directly proportional to the second derivative, so that its results are equivalent to those described above.

16.2 Calibration: For each range of standards, obtain a standard curve by plotting the second derivatives of standards (including the reagent blank) versus concentration. Fit the data to a 2nd-order equation using a spreadsheet program such as Excel. It is most convenient to fit the data with the second derivative as "x" and the concentration as "y" to facilitate calculation of concentrations of samples from absorbance data. Note that the Hitachi UV-2001's computational program provides concentration output based on this type of calculation.

16.3 Calculation of concentrations: The concentration of each solution will be calculated based on the 2nd-order equation for the regression data. The concentrations will represent the concentration of analyte in the 10-mL aliquot in (15.2.b).

16.4 Calculation of water sample concentrations, corrected for dilution: For samples for which dilution was required, the concentration in the original water sample is calculated as in equation 1.

a. Equation 1: $C_{sample} = C_{analysis} \times (10.0 \text{ mL}/V_{aliquot})$

b. Where: C_{sample} = the concentration in the original water sample,
$C_{analysis}$ = the concentration of the solution as determined in 16.3,
and $V_{aliquot}$ = the volume of the aliquot diluted to 10.0 mL in 15.2.b

c. If additional dilution was carried out (e.g., for samples with $A_{220} > 2.0$), include an additional correction factor.

16.5 Reporting results: Results should be reported to 0.1 mg TN/L precision.

16.6 Standard Deviation: The evaluation of MDL and precision require calculation of standard deviation. Standard deviations should be calculated as in equation 2.

    a.   Equation 2:

$$s = \left\{ \frac{\sum x^2 - [(\sum x)^2/n)]}{n - 1} \right\}^{1/2}$$

    b.   Where: n = number of samples,
    x = concentration in each sample.

    c.   Note: This is the sample standard deviation calculated by the STDEV function in Microsoft Excel.

16.7 Calculation of recoveries: Recovery of matrix spike solutions shall be calculated as in equation 3.

    a.   Equation 3:   % Recovery = [(S – U) / T] x 100%

    b.   Where:S = observed for spiked sample,
        U = observed for unspiked sample,
        T = spike value (T is normally 1.0 mg/L – see 15.1b).

    c.   Both S and U are concentrations based on 16.3, i.e., not adjusted for dilution.

17. Computer hardware and software

17.1 Word: This document and attached bench sheet are prepared using Microsoft Word. The Word document file name for this SOP is: 3020R01 Total N.doc

17.2 Excel: Quality control charts are created using Excel.

18. Method performance

18.1 The desired performance criteria for this measurement are:
    a.   Detection limit: ≤ 0.1 mg TN/L
    b.   Precision: ± 20%
    c.   Accuracy: ± 20%
    d.   Minimum Quantification Interval: 0.1 mg TN/L

18.2 The applicable range for the method is 0.1 mg TN/L to 5 mg TN/L and may be extended by dilution.

19. Pollution prevention

19.1 All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU Chemistry Department.

19.2 Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

20.   Data assessment and acceptable criteria for quality control measures

20.1   The analyst should review all data for correctness (e.g., calculations).

20.2   Precision values are calculated for pairs of duplicate analyses. Record the precision values as a percent on the bench sheet. The desired precision is ± 20%.

20.3   The desired detection limit is 0.1 mgN/L. The de-ionized water blank (DIB) should be less that the laboratory reagent blank (LRB), so ≤ 0.10 mg TN/L.

20.4   Percent recovery is calculated for LCC, QCC and spiked samples. Record these values on the bench sheet. The desired recoveries are; 100 ± 10% for LCC and QCC and 100 ± 20% for spiked samples.

20.5   The completed bench sheet is reviewed by the analyst's supervisor or the OEWRI QA coordinator.

21.   Corrective actions for out-of-control or unacceptable data

21.1   Quality control charts will be created for charting precision (LD), accuracy (LCC and LFM) and Laboratory Reagent Blanks (LRB) values.

21.2   The results for precision (± 20% RPD), accuracy (100 ± 10% for LCC and 100 ± 20% for LFM) and LRB data (0.1 mgN/L) are compared to the acceptable values for this analysis.

21.3   If a precision value exceeds 20% RPD then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control duplicate analysis. The RPD is > 20%."

21.4   If an accuracy value exceeds the UCLs for LCC or LFM, then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control LCC (or LFM) analysis. The limit is 100 ± 10% (or ± 20%)."

21.5   If a LRB value exceeds 0.1 mg/L then the analyst should write in the comments section of the bench sheet: "These data are associated with a blank value that exceeds the detection limit of 0.1 mg/L."

21.6   If there is sufficient volume of sample remaining for all samples, the batch may be re-analyzed. Quality control data from the first run must be recorded on the control charts.

21.7   If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

22.   Waste management

22.1   The wastes generated in this method are hazardous because of a low final pH. The samples may be neutralized by the addition of water in accumulation

container (e.g., a large beaker or flask). Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

22.2   Outdated chemicals are discarded following the procedures of the MSU Environmental Management Department

23.   References
23.1   "Nitrate and organic N analyses with second-derivative spectroscopy," Crumpton, Isenhart, and Mitchell, *Limnol. Oceanogr.*, 37(4), 1992, 907 – 913.

23.2   "18.0: Determination of Total Nitrogen," EPA Handbook of Methods for Acid Deposition Studies: Laboratory Analysis for Surface Water Chemistry," EPA publication 600/4-87/026, August 1987.

23.3   Standard Methods for the Examination of Water and Waste Water, Method 4500-N_{org} D, Persulfate Method (Proposed), APHA, 19th Edition, 1995.

23.4   "Simultaneous Determination of Total Nitrogen and Total Phosphorus in Water Using Peroxodisulfate Oxidation," Ebina, Tsutsui, and Shirai, *Water Res.* 17(12), pp. 1721 - 1726, 1983.

24.   Tables, diagrams, flowcharts and validation data
24.1   Tables can be found within this document. There are no diagrams, flowcharts or validation data.