

Standard Operating Procedure for the
Digestion and Analysis of Fresh Water Samples
For Total Nitrogen and Total
Dissolved Nitrogen
CCAL 33A.4

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1.0 Scope and Application

- 1.1 This method details the persulfate digestion and subsequent automated colorimetric analysis of total nitrogen (TN) in an unfiltered sample, or total dissolved nitrogen (TDN) in a filtered sample, of fresh water samples. The practical range of determination for this method is 0.01 to 2.00 mg/L as N. Method detection limit for this analysis is 0.01 mg/L N.
- 1.2 The persulfate digestion for TN is particularly useful where total nitrogen in the system is dominated by organic nitrogen, and the TN level is near or below the 0.1 mg N/L detection limit of the alternative total Kjeldahl nitrogen (TKN) method. The persulfate digestion method produces low toxicity waste and is less cumbersome than classical TKN methods (Ameel et al., 1993). If needed, TKN can be calculated from TN if nitrate/nitrite concentration data are available.
- 1.3 This method has been proven to be sensitive and reliable for extremely unproductive lake and stream samples (Ameel et al., 1993).

2.0 Summary of Method

- 2.1 Digestion with persulfate oxidizes all forms of nitrogen to nitrate. An automated analysis method is used for the colorimetric determination of nitrate and nitrite. Nitrate is reduced to nitrite when passed through a copperized cadmium reduction column. The nitrite is diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, the absorbance of which is measured colorimetrically. Concentration of nitrate/nitrite is determined by comparison of absorbance signal with calibration results obtained from prepared standards of varying concentrations.

3.0 Definitions

- 3.1 DI water: Water that has been through a deionization system to produce water similar to ASTM Type I reagent with 16.7 Mohms resistivity (ASTM) (Reference 16.3).

- 3.2 Method Detection Limit (MDL): The minimum concentration of an analyte that can be measured and reported with 99% confidence, based on a one-sided 99% confidence interval (t -value at a significance level of 0.01 and $n-1$ degrees of freedom) from at least seven repeated measurements of a low concentration standard measured within an analysis run.

$$\text{MDL} = ts$$

Where,

t = Student's t value at a significance level of 0.01 and $n-1$ degrees of freedom

s = standard deviation of at least seven repeated measurements of a low level standard

4.0 Interferences

- 4.1 The persulfate digestion method is effective in recovering TN from almost all forms of organically bound nitrogen including nicotinic acid. However, N-O and N-N bonds are not quantitatively converted to nitrate by either persulfate or TKN digestions. The ability of the persulfate digestion to convert organic nitrogen bound to three carbon atoms requires further study.
- 4.2 In high nitrogenous compounds, the persulfate digestion may not yield quantitative TN recovery. If in doubt, redigest a diluted aliquot and reanalyze to confirm that the oxidative capacity of the mixed persulfate reagent is not exceeded.
- 4.3 Incomplete digestion of nitrogenous compounds to nitrate occurs when the persulfate digestion does not remain alkaline for a long enough period of time.
- 4.4 In rare cases where the digestate is not acidic following digestion, samples should be redigested after confirmation of quality of the persulfate digest solution. The persulfate reagent should be made fresh if there is obvious recrystallization within the solution.

5.0 Safety

- 5.1 The toxicity or carcinogenicity of each reagent has not been precisely determined; however, each chemical should be regarded as a potential health hazard. Exposure to these chemicals should be reduced to the

lowest possible level. Cautions are included for known extremely hazardous materials.

5.2 The following chemicals have the potential to be highly toxic or hazardous. For detailed explanations, consult the MSDS.

- 5.2.1 Sulfuric acid
- 5.2.2 Hydrochloric acid
- 5.2.3 Phosphoric acid
- 5.2.4 Sodium hydroxide
- 5.2.5 Ammonium hydroxide
- 5.2.6 N-(1-naphthyl)-ethylenediamine dihydrochloride
- 5.2.7 Cadmium
- 5.2.8 Potassium persulfate

6.0 Equipment and Supplies

Note: *Brand names, suppliers and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

- 6.1 Analytical balance with resolution to 0.1 mg
- 6.2 Glassware: including volumetric flasks and pipettes as required
- 6.3 Technicon Auto-Analyzer II
 - 6.3.1 Multichannel proportioning pump
 - 6.3.2 Colorimetric detector
 - 6.3.3 Data system
 - 6.3.4 Alpkem manifold and method 100-70W
- 6.4 Safety glasses
- 6.5 Nitrile gloves
- 6.6 Lab coat or apron
- 6.7 Laboratory exhaust fume hood
- 6.8 High density polyethylene (HDPE) bottles
- 6.9 Forced air oven, with over-temperature control; set at 100°C
- 6.10 40 mL borosilicate vials with screw on caps

7.0 Reagents and Standards

7.1 Preparation of Reagents

Solutions 7.1.1 & 7.1.3.2 need to be filtered through a prewashed GF/F filter after preparation

7.1.1 Persulfate Digest Reagent

Add 19.2 g of sodium hydroxide (NaOH) to approximately 1 L of DI water in a clean 2 L volumetric flask and mix until the NaOH is dissolved. Add 80.4 g of low-nitrogen potassium persulfate ($K_2S_2O_8$) to the NaOH Solution and adjust volume to 2 L. The mixed reagent contains 0.296 eq/L of $K_2S_2O_8$ and 0.24 eq/L of NaOH. (Note: Reagent grade potassium persulfate generally contains a significant nitrogen contamination level. Use a source of potassium persulfate with the lowest nitrogen blank available or recrystallize to remove most of the nitrogen present.) This solution is stable. Refilter if crystallization occurs.

7.1.2 Analysis Reagents

7.1.2.1 Sulfanilamide Color Reagent

Slowly add 100 mL 85% phosphoric acid to approximately 600 mL DI water. Add 40.0 g sulfanilamide and dissolve completely with stirring. Add 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Continue to stir until dissolution complete. Dilute to 1 L and invert to mix. This solution is stable for about one month.

7.1.2.2 Ammonium Chloride Buffer, pH 8.5

**Caution: Toxic fumes – prepare in a hood*

Add 105 mL concentrated hydrochloric acid to approximately 500 mL DI water. Cool solution in an ice bath. Add 95 mL concentrated ammonium hydroxide. Mix well and allow solution to cool. Add 1.0 g disodium ethylenediamine tetraacetic acid dihydrate (EDTA), dilute to 1 L and invert to mix. This solution is stable for about one month.

7.1.3 Cadmium Column Reagents

7.1.3.1 Alkaline Water

Add 0.1 mL concentrated ammonium hydroxide to 100 mL DI water. Add 3 mL of this dilute solution to 3.5 L of DI water. Adjust pH to 8.5 with dilute NH_4OH solution, adding dropwise. Store refrigerated to inhibit microbial growth.

7.1.3.2 Ammonium Chloride Buffer

Dissolve 10 g of reagent grade ammonium chloride in 900 mL of alkaline water (7.1.3.1). Dilute to 1 L. Store in the refrigerator; allow reagent to come to room temperature before use. This solution is stable.

7.2 Preparation of Cadmium Column

- 7.2.1 Check the size of the coarse cadmium granules before washing and discard exceptionally large granules.
- 7.2.2 Wash granules first with acetone, then with 1N HCl (color should be silvery). At this point the cadmium may be dried and stored in an air-tight container.
- 7.2.3 Wash the clean cadmium with 50 to 100 mL of 2% CuSO₄ • 5H₂O, until no blue color remains in the solution and the semi-colloidal copper particles begin to enter the supernatant liquid. Rinse with DI water.
- 7.2.4 Continue to wash with water until cadmium appears black; the number of water rinses is not critical.
- 7.2.5 Fill the column with ammonium chloride buffer (7.1.3.2) and transfer the granules to the column.
- 7.2.6 Condition the column in-line by running 186 mg N/L standard through the column for 12.5 minutes. Check the column conditioning by analyzing a minimum of six 0.100 mg N/L standards. Results should agree to within 1 %.

7.3 Preparation of Standards

7.3.1 *Calibration Standard:*

Standards are prepared by dilution of a standard purchased from a vendor that provides traceability to NIST standards. A mixed stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. The stock standard with various concentrations of nitrate and phosphorus is used to prepare working standards in the table below.

	mg NO ₃ -N/L	mg PO ₄ P/L
1	0.01	0.010
2	0.05	0.050
3	0.20	0.100
4	0.40	0.200

7.3.2 *Second Source Check Standard:*

Standards are prepared by dilution of a standard purchased from a vendor that provides traceability to NIST standards; this reagent is from a source other than that of the calibration standards. A mixed stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. Check standard concentrations are the same as calibration standard 3.

7.3.3 *TN Digest Efficiency Check Standard:*

To prepare a 250 mg N/L stock solution, add 2.195 g nicotinic acid (oven dried at 80°C and cooled in a desiccator) to a 1 L volumetric flask and dilute to volume with DI water. Dilute 25 mL of the stock solution to 1000 mL with DI water to prepare intermediate standard, concentration 6.25 mg N/L.

8.0 Sample Handling and Storage

- 8.1 If required, unfiltered samples are filtered upon receipt through glass fiber filters into clean HDPE bottles and stored at 4°C in the dark. Samples are digested within 28 days to ensure sample integrity. If samples must be held prior to analysis, they are stored frozen at -18°C.

9.0 Quality Control

- 9.1 Preparation of stock standards is recorded on worksheets and documented by weight of standard added to a given flask before dilution to volume with DI water. All records of certification are kept on file at CCAL Laboratory.
- 9.2 Blank: DI water run after the calibration and before and after each check standard.
- 9.3 Quality Control Check Standard: Calibration standards run in rotation every ten samples to monitor stability and validate the calibration.
- 9.4 Method Detection Limit (MDL): Established for each analyte. Based on a one-sided 99% confidence interval (t-value) from at least seven repeated measurements of a low concentration standard. The t-distribution value is multiplied by the standard deviation of the population (n-1) to obtain the MDL.
- 9.5 Analytical Duplicate: Separate analysis from the same sample aliquot. Run a minimum of once every ten samples.
- 9.6 A nicotinic acid digest standard and a Quality Control Check Standard are digested each digest batch to verify TN recovery. Two DI water blanks are digested to determine the nitrogen present in the mixed persulfate digestion reagent.

10.0 Calibration and Standardization

- 10.1 Balances: calibrated yearly by external vendor.
- 10.2 Pipette delivery checked by weight to within 2% of theoretical weight of aliquot volume.
- 10.3 Calibration curve with $r^2 \geq 0.998$. (See 17.3 for calibration data set-up.)
- 10.4 Calibration verification with check standards, monitored throughout the run. If measurement exceeds +/- 10% of the theoretical value, the analysis should be terminated and the instrument recalibrated. The calibration must be verified before continuing analysis.

11.0 Procedures

11.1 Digest Procedure

11.1.1 Preparation of samples and reagents for digestion

11.1.1.1 Pipette 20 mL of sample into a clean, labeled, 40 mL vial. (Note: Bring samples to room temperature and shake well just prior to subsampling to minimize pipetting and sampling errors.)

11.1.1.2 Add 0.5 mL TN Digest Standard and 19.5 mL DI water to a clean, labeled, 40 mL vial. Final tube concentration after addition of Persulfate Reagent, 0.125 mg N/L. Alternatively, for combined TN/TP Digest Standard, add 0.5 mL TN Digest Standard, 0.5 mL TP Digest Standard (see CCAL SOP 35B.2) and 19.0 mL DI water to a clean, labeled, 40 mL vial. Final tube concentrations after addition of Persulfate Reagent 0.08 mg P/L and 0.185 mg N/L.

11.1.1.3 Add 20 mL of check sample (collected in bulk and an aliquot digested once each digest batch) to a clean, labeled, 40 mL vial.

11.1.1.4 Add 20 mL of DI water to each of two clean, labeled, 40 mL vial.

11.1.2 Pipette 5 mL of mixed persulfate digestion solution into each vial, cap and swirl immediately after addition.

Note: For low volume samples, dilute to 20 mL total volume

11.1.3 Weigh vials and record weight.

11.1.4 Place vials in plastic coated wire racks and place the racks in a forced air oven set at 100°C. Digest for 12 – 16 hours. Remove racks from the oven and cool to room temperature.

11.1.5 Reweigh the vials and record the weight. Analytical results will be corrected for weight loss; see section 12.0 Data Analysis and Calculations.

11.2 Calibration and Analysis Procedure

- 11.2.1 Prepare reagents and standards as outlined in Section 7.
- 11.2.2 Set up manifolds as shown in Section 17.1.
- 11.2.3 Samples are injected into the reaction path at a fixed time interval, determined by the cam timing set in the auto-sampler. Setup or confirm data system parameters as detailed in Section 17.3.
- 11.2.4 Pump DI water through all reagent and sample lines. Check for leaks and stable bubble pattern (smooth flow). Pump reagents through all lines until the system equilibrates (minimum of one-half hour). Put the cadmium column in-line, and allow system to stabilize for one hour. Do not allow air to pass through the column.
- 11.2.5 Record sample id's in the data template.
- 11.2.6 Calibrate the instrument with standards. Calibration regression equations must have $r^2 \geq 0.998$.

11.3 System Notes

- 11.3.1 If the baseline is excessively noisy, clean the manifold using the following procedure:
 - Place all reagent and carrier lines in rinse water and pump to clear reagents.
 - Place all lines in 1.2N HCl solution for several minutes.
 - Place lines in DI water and pump until thoroughly rinsed.
 - Be certain that no acid passes through the cadmium column.
- 11.3.2 If baseline has a large number of air spikes, check pump tubes for excessive wear and replace as necessary.
- 11.3.3 In line sample dilution for samples with TN concentrations below 1 mg N/L has been proven to be acceptable. Samples that exceed 1 mg N/L, or samples that contain precipitate following digestion, should be redigested using a smaller sample volume with dilution to 20 mL. For samples containing suspended organic matter, it is important to mix well prior to sampling to minimize subsampling errors. If samples contain suspended inorganic particles, a tan to red precipitate may result following the oxidative persulfate digestion. Redigest with dilution to confirm results.

12.0 Data Analysis and Calculations

- 12.1 The data system prepares a calibration curve by plotting response of injected standards versus known standard concentration. The resulting

regression equation is used to calculate the sample concentration.

- 12.2 All results and sample information are filed in the analysis data system by analysis run. Details specific to the instrumental analysis are noted in the Instrument Run Log created and maintained for the AAI. Analytical results are entered into electronic format and entries are verified by a second person.
- 12.3 Correct analytical results for weight loss due to evaporation; subtract the average persulfate reagent blank from the sample concentration and then correct the sample concentration for dilution by the persulfate digestion solution.

Note: "For low level samples the variability in the reagent blanks determines the limit of detection, not the error associated with the NO₃ and PO₄ analyses" Qualls (1989).

13.0 Method Performance

- 13.1 This method was validated through inter-laboratory studies. The CCAL Water Analysis Laboratory participates in the USGS Standard Reference Water QA program and the National Water Research Institute's (NWRI) Environment Canada Proficiency Testing (PT) Program.

14.0 Pollution Prevention

- 14.1 The chemicals used in this method pose little threat to the environment when properly managed.
- 14.2 All standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of disposable waste.
- 14.3 For further information on pollution prevention consult *Less is better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

- 15.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, and to protect the

environment by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is required.

- 15.2 For further information on waste management, consult “The Waste Management Manual for Laboratory Personnel”, and “Less is Better: Laboratory Chemical Management for Waste Reduction”, both available from the American Chemical Society’s Department of Government Relations and Science Policy, 1155 16th Street NW, Washington DC, 20036.

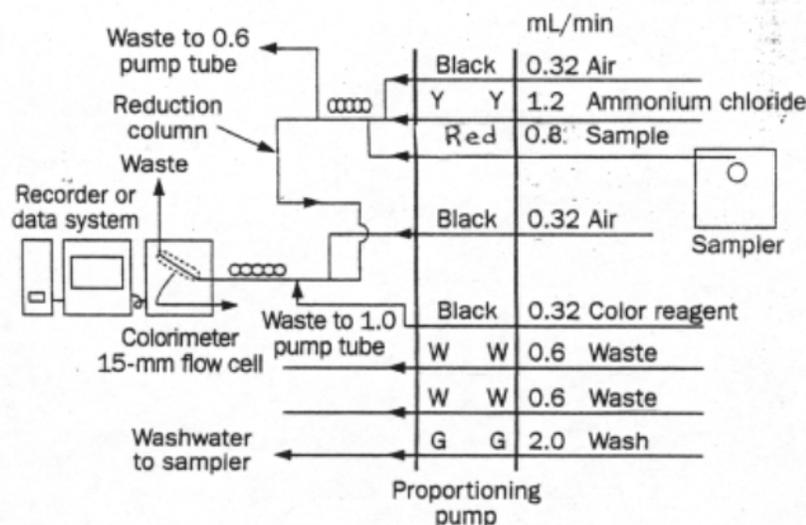
16.0 References

- 16.1 Standard Methods For The Examination of Water and Wastewater, Method 4500-NO₃, Nitrate Nitrogen Automated Cadmium Reduction Method, 4500-P Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus. American Public Health Association. 21st Edition, 2005.
- 16.2 Code of Federal Regulations. Protection of Environment. Section 40, Appendix B to Part 136. Definition and procedure for the determination of the method detection limit. Revision 1.11. Revised July 1, 1990. Office of the Federal Register, National Archives and Records.
- 16.3 ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, PA, 1991.
- 16.4 Code of Federal Regulations. Protection of Environment. Section 40, Appendix B to Part 136. Definition and procedure for the determination of the method detection limit. Revision 1.11. Revised July 1, 1990. Office of the Federal Register, National Archives and Records.
- 16.5 Ameel, J.J., Axler, R.P. and Owen, C.J. 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. American Environmental Laboratory. Volume 10/93.
- 16.6 D’Elia, C.F., Conner, E.E., Kaumeter, N.L., Keefe, C.V., Wood, K.V., and Zimmerman, C.F. 1997. Nutrient Analytical Services Laboratory Standard Operating Procedures. Chesapeake Biological Laboratory, P.O. Box 38, Solomons, Maryland. Technical Report Series No. 158-97, Center for Environmental and Estuarine Studies, The University of Maryland System.

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- 16.9 Koroleff, F. 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. P. 168-169. In K. Grasshoff, M. Eberhardt, and K. KremLing, eds., *Methods of Seawater Analysis*. 2nd ed., Verlag Chemie, Weinheimer, FRG.
- 16.10 Langer, C.L. and P.F. Hendrix. 1982. Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. *Water Res.* 16, 1451-1454.
- 16.11 Qualls, R.G. 1989. Determination of total nitrogen and phosphorus in water using persulfate oxidation: a modification for small sample volumes using the method of Koroleff (1983). Appendix A pp. 131-138. In the biogeochemical properties of dissolved organic matter in a hardwood forest ecosystem: their influence on the retention of nitrogen, phosphorus, and carbon. Ph.D dissertation, University of Georgia Institute of Ecology, Athens, Georgia, USA. University Microfilms, Inc., no. DEX9003448.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Total Nitrogen (Nitrate - Nitrite) Reaction Manifold



17.2 Total Nitrogen (Nitrate-Nitrite) Manifold Specifications

Carrier is ammonium chloride reagent

Interference Filter is 520 nm

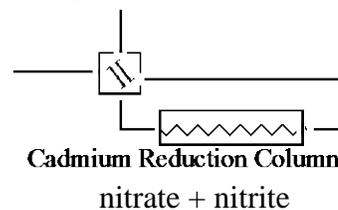
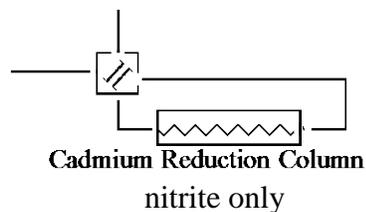
Pump tubing is Tygon

Manifold tubing is 0.030 mm i.d.

Mixing coils are 5 turn and a 20 turn with front injection fitting

15 mm x 2 mm flow cell

Note: Cadmium Column Switching Valve used to place the column in-line.



17.3 Data System Parameters

Cycle throughput: 30 samples/hr

Cycle Period: 120 s

Analyte Data:

Concentration Units mg N/L

Calibration Data:

Level	1	2	3	4
Concentration mg/L	0.01	0.05	0.20	0.40

Calibration Fit Type: linear regression

Sampler Timing:

Minimum Probe in Wash Period: 30 s

Probe in Sample Period: 90 s

18.0 Document Revision History

Original Document: March 2006

Version: 33A.0

Title: Standard Operating Procedure for the Digestion and Analysis of Fresh Water
Samples for Total Nitrogen and Total Dissolved Nitrogen

Edit Date: February 2010

New Version: 33A.1

Address update

Change from 50 mL centrifuge tubes to 40 mL vials

Section 6.9 and 11.1.4: change digest temperature to 100°C

Section 7.2: change preparation of standards from raw materials, to serial dilution of
purchased standards. Change standard concentrations. Add Second Source Check
Standard.

Section 13.1: add Environment Canada Proficiency Testing Program participation

Section 17.2: change standard concentrations and order of standards.

Edit Date: July 2011

New Version: 33A.2

Updates to reflect procedural, standard and reagent modifications for combined TN/TP
digestion and analysis.

Edit Date: June 2013

New Version: 33A.3

Revised 7.1 Preparation of Reagents for clarification.

Edit Date: April 2014

New Version: 33A.4

Section 1: change working range of method for appropriate significant figures.

Section 7.1: update filtering, stability and re-filtering for persulfate reagent. Update
reagent preparation storage for alkaline water.

Section 7.3: change preparation of standards to include a mixed intermediary standard
solution and provide a table of final standard concentrations.

Section 17.2: change calibration data values.

General editing.