

Standard Operating Procedure for the
Digestion and Analysis of Fresh Water Samples
For Total Nitrogen and Total
Dissolved Nitrogen
CCAL 33A.0

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Table of Contents

1.0	Scope and Application	3
2.0	Summary of Method	3
3.0	Definitions	3
4.0	Interferences	4
5.0	Safety	4
6.0	Equipment and Supplies	5
7.0	Reagents and Standards	6
7.1	Preparation of Reagents	6
7.2	Preparation of Cadmium Column	7
7.3	Preparation of Standards	7
8.0	Sample Handling and Storage	7
9.0	Quality Control	8
10.0	Calibration and Standardization	8
11.0	Procedures	9
11.1	Digest Procedure	9
11.2	Calibration and Analysis Procedure	9
11.3	System Notes	10
12.0	Data Analysis and Calculations	10
13.0	Method Performance	11
14.0	Pollution Prevention	11
15.0	Waste Management	11
16.0	References	11
17.0	Tables, Diagrams, Flowcharts, and Validation Data	13
17.1	Nitrate/Nitrite Nitrogen Reaction Manifold	13
17.2	Data System Parameters	14

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1.0 Scope and Application

- 1.1 This method details the persulfate digestion and analysis of total nitrogen (TN) in fresh waters by automated colorimetric analysis. The practical range of determination for this method is 0.01 to 2 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 90°C
- 6.10 50 mL centrifuge tubes with screw on caps

7.0 Reagents and Standards

7.1 Preparation of Reagents

Solution 7.1.4 needs to be filtered through a prewashed GF/F filter after preparation

7.1.1 *Persulfate Reagent:*

Add 24 g of sodium hydroxide (NaOH) to approximately 1 L of DI water in a clean 2 L HDPE bottle 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 0.3 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.)

7.1.2 *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.

7.1.3 Color Reagent:

a) Reagent 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.

b) Reagent 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 dehydrate (EDTA), dilute to 1 L and invert to mix. This solution is stable for about one month.

7.1.4 Ammonium Chloride Buffer (used to prepare cadmium column):

Dissolve 10 g of reagent grade ammonium chloride in 900 mL of alkaline water (7.1.2). 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% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 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 (7.1.4) 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 0.4 mg N/L standards. Results should agree to within 1 %.

7.3 Preparation of Standards

- 7.3.1 *Calibration and Check Standards:*
A 1000 mg/L potassium nitrate stock standard is prepared from 7.222 g potassium nitrate (oven dried at 80°C) diluted to 1 L with DI water. A 10 mg/L intermediate standard is prepared by dilution with DI water. Working standards in concentrations of 0.40, 0.20, 0.05 and 0.01 mg/L nitrate nitrogen are prepared from the intermediate stock standard.
- 7.3.2 *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 dessicator) 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. Final concentration of digest check standard, 0.25 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 consistency check sample are digested each digest batch to verify TN recovery. Two DI water blanks are digested to determine the nitrogen blank 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 17.2 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, 50 mL centrifuge tube. (Note: Bring samples to room temperature and shake well just prior to subsampling to minimize pipetting and sampling errors.)

11.1.1.2 Add 1 mL TN digest standard and 19 mL DI water to a clean, labeled, 50 mL centrifuge tube.

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, 50 mL centrifuge tube.

11.1.1.4 Add 20 mL of DI water to each of two clean, labeled, 50 mL centrifuge tubes.

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

Note: For low volume samples, 8 mL of sample with 2 mL of persulfate digest solution may be used. Digest time is constant.

11.1.3 Weigh centrifuge tubes and record weight.

11.1.4 Place centrifuge tubes in polypropylene centrifuge tube racks and place the racks in a forced air oven set at 90°C. Digest for 12 – 16 hours. Remove racks from the oven and cool to room temperature.

11.1.5 Reweigh the centrifuge tubes 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 17.2.

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 participates in the USGS Standard Reference Water QA 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

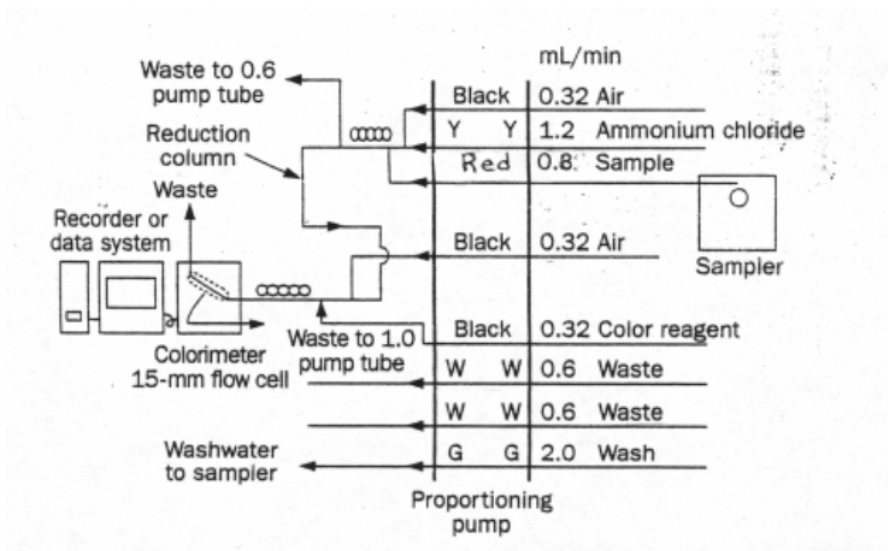
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17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Nitrate/Nitrite Nitrogen Reaction Manifold



Nitrate-Nitrite Manifold Specifications

Carrier is ammonium chloride reagent

Interference Filter is 520 nm

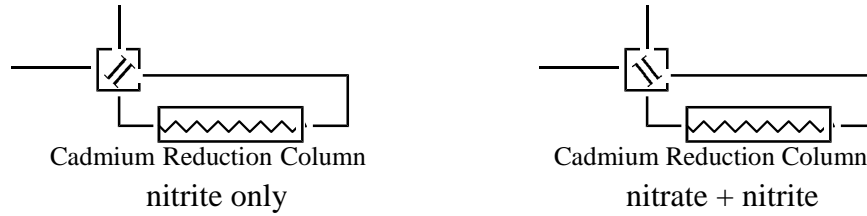
Pump tubing is Tygon

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.



17.2 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	0.40	0.20	0.05	0.025

Calibration Fit Type: Linear Regression

Sampler Timing:
 Minimum Probe in Wash Period: 30 s
 Probe in Sample Period: 90 s