

D.1. Alkaline Persulfate Digestion for Nitrogen and Phosphorus, Total and Total Dissolved

Method Codes: TN L01 (Total Nitrogen) TDN L01 (Total Dissolved Nitrogen)
TP L04 (Total Phosphorus) TDP L01 (Total Dissolved Phosphorus)

a) Scope and Application

- i) This method describes the digestion procedure for the determination of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) in fresh and estuarine surface waters by the alkaline persulfate oxidation technique. The method is suitable for the determination of total nitrogen (TN) and total phosphorus (TP) with necessary precautions to ensure that particulates are fully digested.
- ii) Typical analytical ranges are 0.01 to 3.0 mg-N/L for total nitrogen and 0.01 to 0.5 mg-P/L for total phosphorus. Analytical ranges may be extended by digesting and analyzing a diluted sample. A higher calibration range for nitrogen is permitted for filtered samples only (i.e., TDN).

b) Summary of Method

- i) The persulfate oxidation technique for nitrogen in water is performed under heated alkaline conditions, where all organic and inorganic forms of nitrogen are oxidized to nitrate. As the reaction proceeds, NaOH is consumed and the pH drops to < 2.2, which allows the oxidation of all phosphorus compounds to orthophosphate.
- ii) An aliquot of digested sample is analyzed for nitrate and orthophosphate using an automated colorimetric method (Sections VI.D.3 & D.5) to produce total nitrogen and total phosphorus concentrations.

c) Interferences

- i) Some particulate nitrogen compounds in unfiltered samples may be resistant to alkaline persulfate digestion (i.e., refractory) and yield low total nitrogen results.
- ii) Samples preserved with acid will result in low recoveries of nitrogen unless the pH is neutralized to the same pH as the reagents. (USGS 2003.)
- iii) Organic carbon reacts with the persulfate oxidation reagent to form carbon dioxide. Concentrations over 150 mg-C/L may deplete the persulfate before all nitrogen compounds are oxidized and cause a low bias for total nitrogen values.

d) Apparatus and Materials

- i) Autoclave or pressure cooker capable of maintaining 100-120 °C for at least 30 minutes.
- ii) Glass Digestion Tubes: 30 mL culture tubes with polypropylene liner-less screw-caps or 40 mL vials with Teflon-lined screw-caps. Acid-rinse digestion tubes with HCl to clean. New tubes should be conditioned prior to their first use by filling with persulfate oxidizing reagent and autoclaving at 100-120 °C for 30 minutes.

e) Reagents and Standards

i) Reagent Water: Nitrogen-free reagent water.

ii) Borate Buffer Solution: Add approximately 800 mL of reagent water to a two-liter volumetric flask. Quantitatively transfer ~~123.6 g of~~ boric acid (H_3BO_3) and ~~46.16 g of~~ low-nitrogen (< 0.001% N) sodium hydroxide (NaOH) to the flask. Allow the solution to cool and dilute to 2.0 liters with reagent water. This solution is stable for two months at room temperature.

Comment [m1]: We decided in other methods to omit weights & volumes for the reagents and standards. Deleted here to be consistent.

iii) Persulfate Oxidizing Reagent: Add 400 mL of reagent water to a one-liter volumetric flask. Quantitatively transfer and dissolve ~~3.0 g of~~ low-nitrogen NaOH and ~~20.1 g of~~ low-nitrogen potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$ with < 0.001% N) in the flask. Dilute to one-liter with reagent water and store in a glass reagent bottle. Prepare this solution ~~is stable for two hours at room temperature.~~ just before use.

iv) Instrument Wash Water: Prepare auto-analyzer wash water by mixing oxidizing reagent and reagent water in an Erlenmeyer flask, in the same proportion as added to the digestion tubes, e.g., 2:1 (v/v) ratio. Cover with foil and autoclave the solution for at least 30 min. at 100-120°C; cool and add Borate Buffer (or 3N NaOH if following SM 4500-P J).

v) Calibration Standards: A laboratory may purchase or prepare stock and working standards. If standards are prepared in the laboratory, a purchased standard should be used as the calibration check standard.

Comment [m2]: New section (v.)

1. Potassium nitrate (KNO_3) for nitrogen (Section VLD.5). Use primary standard-grade KNO_3 , dried at 45°C. The primary stock standard is stable for ~ 3 months if stored at 4°C.
2. Potassium dihydrogen phosphate (KH_2PO_4) for phosphorus (Section VLD.8). Use anhydrous, primary standard grade KH_2PO_4 , that has been oven-dried and desiccated. The primary stock solution is stable for 6 months if ~~preserved with chloroform and~~ stored at 4°C.
3. Prepare a series of working standards just prior to digesting each analytical batch by diluting suitable volumes with reagent or ASW water.
4. Standards should bracket the expected concentrations of samples. Analytical ranges may be extended by digesting and analyzing a diluted sample. However, for total nitrogen determinations in unfiltered samples, a calibration range for higher than 3 mg N/L is not recommended.
5. When analyzing samples of known salinity it is recommended that the working standards and instrument wash water be diluted with artificial sea water to yield similar salinity.
6. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and Refractive Index corrections be made to the sample concentrations.

Comment [m3]: CBL's 45C, DHMH - 110C, ODU 104C, DCLS 104C,

vii) Digestion Check Standards: A laboratory must analyze one nitrogen and one phosphorus digestion check standard to demonstrate that all compounds containing N and P were completely digested. Standards may be purchased or prepared in the laboratory. Some laboratories use certified reference materials to check the calibration as well as the completeness of digestion. Listed below are various digestion check standards cited in the reference methods.

1. Nitrogen Digestion Check Standards

- a. Glutamic Acid, Stock and Working Standards – Stock solutions are stable for ten months when preserved with chloroform and stored at 4°C. Prepare the working digestion check standard the day of digestion, diluting to volume with wash water solution.
- b. Nicotinic Acid p-toluenesulfonate – See Standard Methods 4500-P J (APHA 2005a).
- c. Glycine – Stable for 6 months at 4°C. (USGS 2005)

2. Phosphorus Digestion Check Standards

- a. Glycerophosphate (*B*-glycerophosphoric acid-disodium salt-5-hydrate) stock solutions are stable for 10 months when preserved with chloroform and stored at 4°C. Prepare the working digestion check standard the day of digestion.
- b. Adenosine triphosphate – See Standard Methods 4500-P J (APHA 2005a).

f) Procedure

i) Preparation of Analytical Batch

- (1) Prepare a series of standard solutions covering the analytical range by diluting either the stock or standard solutions.
- (2) Add 10.0 mL of sample (or a smaller aliquot diluted to 10.0 mL) to a clean glass test tube or vial.

Use a wide-bore pipette for taking aliquots of whole-water samples. A magnetic stirrer may be necessary to obtain a representative subsample from samples with high suspended solids.
- (3) Pipette 10.0 mL of each calibration standard, method blank, LCS, CCV, digestion check standard, etc., into a test tube.
- (4) Add reagent water to 3 test tubes as method blanks. **Include two oxidizing reagent blanks which contain only the oxidizing reagent.**
- (5) Pipette 5.0 mL of the persulfate oxidizing reagent to each tube. Immediately cap tightly to prevent volatilization, and then invert twice to mix.
- (6) Prepare a flask of instrument wash water (mix reagent water and persulfate oxidizing agent in a 2:1 (v/v) ratio) and cover with aluminum foil.

Comment [m4]: This is from old procedure.
Purpose? Baseline Absorbance?

ii) Digestion

- (1) Autoclave the analytical batch at 100 - 120°C for at least 30 minutes on the liquid automatic cycle. No volatilization occurs after this point.
- (2) Remove tubes from autoclave and cool to room temperature. **Digested samples can be held at room temperature at this point for up to one month before analysis.**
- (3) Add 1.0 mL of buffer solution to each tube and mix. The pH of the sample should be around 2.5 after the addition of buffer solution.

Comment [m5]: New text

- (4) Add 3N NaOH or buffer to the digested wash water.
- (5) Remove any suspended particles remaining in digests by decantation or filtration prior to colorimetric analyses.

iii) Analysis – See procedures for Nitrate + Nitrite and Phosphate

iv) Calibration and Data Reduction

Prepare a standard curve by plotting the absorbances or peak heights of the digested calibration standards against their known concentrations. Designate a digested method blank as having a concentration of 0.0 mg N/L and 0.0 mg P/L.

Do not select a curve-fitting function that forces a zero y-intercept. The calibration curve will have a positive y-intercept that approximates the baseline-corrected absorbance of the digestion blank.

g) Quality Control

- i) Samples with results greater than the highest calibration standard must be diluted and re-digested.
- ii) Digestion check standards: **Must achieve a 90% recovery of the known concentration.**
- iii) Method blanks: see Chapter VI, Section C.
- iv) Matrix spike samples: see Chapter VI, Section C.
- v) Laboratory duplicates: see Chapter VI, Section C.
- vi) Laboratory control samples: see Chapter VI, Section C.
- vii) Method detection limits (MDL): Method detection limits should be established as specified in Chapter VI, Section C.

Comment [m6]: New

h) References

APHA 2005a. Standard Methods for the Examination of Water and Wastewater, 21st Edition, p. 4-160, Method 4500-P J. (2005).

APHA 2005b. Standard Methods for the Examination of Water and Wastewater, 21st Edition, Method 4500-C. (2005)

USGS 2003. [USGS WRIR 03-4174: Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. Methods I-2650-03 \(total dissolved nitrogen and total dissolved phosphorus\) and I-4650-03 \(total nitrogen and total phosphorus\).](#)

Valderrama, J.C., 1981. The Simultaneous Analysis of Total Nitrogen and Total Phosphorus in Natural Waters: Marine Chemistry, v. 21, p. 109-122.

