

D.9 Particulate Nitrogen and Particulate Carbon

**CEDR Method Codes: PN L01
 PC L01**

1.1 Scope and Application

- 1.1.1 Elemental analysis is used to determine total particulate carbon (PC) and total particulate nitrogen (PN) in estuarine and surface waters. The method measures all carbon and nitrogen compounds irrespective of source (inorganic or organic).
- 1.1.2 This procedure is used for the analysis of Chesapeake Bay Program (CBP) tidal and nontidal water quality samples. Measurements of PC and PN by this direct method were shown to be more accurate than low-level results calculated from the difference between whole-water and filtered-water concentrations. (D'Elia, 1997)
- 1.1.3 This procedure conforms to EPA Method 440.0 (Zimmerman, et. al 1997). The MDL is dependent on the volume of sample filtered and the weight of particulate matter. In a 250 mL sample, CBP laboratories routinely calculate MDLs of 0.1 mg C/L and 0.02 mg N/L respectively.

1.2 Summary of Method

- 1.2.1 A known volume of sample is filtered through a glass fiber filter to collect suspended particulate matter. The filter is dried and then placed in a combustion chamber where the carbon and nitrogen compounds are oxidized. Helium gas carries the oxidation products through a reduction tube where nitrogen oxides are converted to N₂ and carbon oxides are converted to CO₂. These compounds are separated through a series of traps or columns and then are detected by thermal conductivity.

1.3 Interferences

- 1.3.1 There are no known interferences for the determination of particulate carbon and nitrogen in estuarine/costal water.
- 1.3.2 The presence of volatile organic compounds, as well as contaminated laboratory surfaces, fingerprints, detergents and dust necessitates the utilization of clean techniques. Use forceps and gloves to avoid contamination in all parts of this procedure.

1.4 Apparatus and Materials

- 1.4.1 Elemental combustion analyzer for the determination of carbon and nitrogen
- 1.4.2 Clean metal forceps.
- 1.4.3 Plastic syringe, 60 mL.
- 1.4.4 Glass fiber filter manifold
- 1.4.5 Graduated cylinder (various volumes)

- 1.4.6 Glass transfer pipettes.
- 1.4.7 Desiccator.
- 1.4.8 Glass fiber filters: 25 mm diameter, with a nominal pore size of 0.7 μm .
- 1.4.9 Muffle furnace capable of 550°C.
- 1.4.10 Analytical microbalance.
- 1.4.11 Freezer capable of $\leq -20^{\circ}\text{C}$
- 1.4.12 Pre-muffled instrument sample containers, e.g., nickel sleeves and tin capsules.
- 1.5 Reagents and Standards
 - 1.5.1 Calibration Standards - Acetanilide ($\text{C}_8\text{H}_9\text{NO}$), 99.9% + purity, or chloramine-T (N-chloro-p-toluene sulfonamide sodium salt).
 - 1.5.2 External Reference Materials - The QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. If certified sediment materials are not available for nitrogen and carbon, use the standard material (Acetanilide) in place of the external standard. The materials below are currently used by CBP laboratories.
 - 1.5.2.1 Pacs-2 from the National Research Council of Canada is a certified particulate carbon sample. It is 3.3 % carbon and should be used for the carbon QCS.
 - 1.5.2.2 SRM 2781 from NIST is a certified material for nitrogen in a sludge matrix. It is 4.78% nitrogen and should be used for the nitrogen QCS.
 - 1.5.3 Nitrogen-free, reagent-grade DI water.
- 1.6 Filter Preparation and Sample Collection
 - 1.6.1 Filter Preparation (prior to field collection)
 - 1.6.1.1 Place new glass fiber filters in a ceramic dish or crucible.
 - 1.6.1.2 Place filters in muffle furnace for 1.5 hours at 550°C.
 - 1.6.1.3 Remove from muffle furnace and cover with aluminum foil until cool.
 - 1.6.1.4 Store muffled filters in a clean desiccator or sealed container until ready for use.
 - 1.6.2 **Sample Collection**
 - 1. Samples must be filtered as soon as possible after collection, preferably in the field. If filtering is delayed, keep water samples refrigerated or in a cooler on ice.

2. Use a forceps to transfer a pre-combusted 0.7 μm glass fiber filter onto the base of a vacuum filtration apparatus. Concentrate particulates on the filter pad by pouring a known volume of water through the filter under vacuum pressure of ≤ 10 in. Hg. or < 5 psi.).
3. To prevent lysis of phytoplankton cells, do not apply vacuum suction to dryness; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.
4. Remove filter with forceps. Carefully fold in half and place into a labeled filter container.
5. Freeze filters at $\leq -20^{\circ}\text{C}$ and analyze for PN and PC within 28 days.

1.6.2 Sample preparation

- 1.6.2.1 Remove filters from freezer and place in labeled containers suitable for drying.
- 1.6.2.2 Transfer to a drying oven at $\leq 105^{\circ}\text{C}$ until dry. Remove, allow to cool and then desiccate until ready for analysis.
- 1.6.2.3 Clean the metal forceps, and preparation area using reagent water and a Kimwipe™. Never use acid on metal.
- 1.6.2.4 Using metal forceps, place a clean nickel sleeve in filter loading devise.
- 1.6.2.5 With metal forceps remove the glass fiber filter (sample) from the sample container, and place in an instrument capsule
- 1.6.2.6 Place prepared capsules into a clean desiccator until analysis.

1.7 Procedure

1.7.1 Standards and Calibration

- 1.7.1.1 Metal cups must always be handled with clean metal forceps. Always work on a clean surface whenever handling standards or samples.
- 1.7.1.2 Calibrate the electronic microbalance at the proper range each day prior to weighing any standards.

1.7.2 Calibration

- 1.7.2.1 For PN, acetanilide, chloramine-T (N-chloro-p-toluenesulfonamide sodium salt) or other suitable standards should be used to calibrate the analyzer. Standards should be weighed in the cup of the instrument capsule. The final weight of standard should be between 0.05 and 2.0 mg.
- 1.7.2.2 For PC, acetanilide or another suitable primary standard should be used to calibrate the analyzer. Standards should be weighed in the cup of the instrument capsule. The final weight of standard should be between 0.05 and 2.0 mg.

- 1.7.2.3 Using metal forceps to handle the cups, weigh a clean cup (see cleaning procedure, 1.7.1) on a calibrated microbalance, and tare the balance to eliminate the weight of the cup from the weight measurement.
- 1.7.2.4 Use a clean metal spatula, place approximately 0.05 to 2.0 mg of standard into the cup. Use forceps (2 pairs) to seal the cup by pinching the top closed.
- 1.7.2.5 Record the weight of the standard. Place the cup inside the holder (nickel sleeve) and put in proper location of the sample carousel.
- 1.7.2.6 Purge the instrument prior to calibrating by running three empty holders (nickel sleeves) through the system. The system is now ready for a blank followed by the number of standards necessary for the instrument used.

1.7.3 Sample Analysis

1.7.3.1 Analytical Batch

- a. Calibration Standards: One PN calibration standard and one PC. (Single-point calibration for each.)
- b. Initial Calibration Verification (ICV) – External QCS for nitrogen and external QCS for carbon
- c. Method Blank – Pre-combusted blank filter
- d. Twenty samples
- e. Laboratory Duplicate Sample
- f. Method Blank – Pre-combusted blank filter
- g. Continuing calibration verification (CCV) – Mid-range calibration standard for PN and PC?
- h. Repeat steps d. through g. until all samples are prepared.

- 1.7.3.2 Place auto sampler tray into the instrument, with the purges blanks and standards in the front of tray. Run a QCS for each nitrogen and carbon prior to the running of samples.
- 1.7.3.3 If the calibration and QCS samples are acceptable then allow the instrument to continue to run samples.
- 1.7.3.4 Run an acetanilide sample after every ten samples to confirm that the instrument is still within calibration.

- 1.7.3.6 Divide the results for particulate carbon or nitrogen in micrograms, by the volume of sample in milliliters that was filtered through the glass fiber filter. The result will then be in $\mu\text{g/mL}$ which is equivalent to mg/L .

1.8 Quality Control

- 1.8.1 This method should be performed by analysts experienced in the theory and application of elemental analysis. A minimum of 6 months experience with an elemental analyzer is recommended. (Zimmerman, 1997). Analyst training and a demonstration of capability should be documented.
- 1.8.2 Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter 6, Section C.8. Since PN and PC cannot be spiked, utilize seven aliquots of a low concentration sample. If there are no low concentration samples available, dilute an aliquot or composite of samples that have been run previously. Dilute into a range that is somewhere between the detection limit and reporting limit.
- 1.8.2 Calibration:
- 1.8.2.1 If the instrument uses a multipoint calibration curve then make the curve high enough that no samples will exceed it. If a single point calibration is used then run a QC sample that exceeds the range of samples being tested to prove performance.
- 1.8.2.2 For multipoint curves the correlation coefficient must be 0.995 or better.
- 1.8.3 Method Blank: A pre-combusted (muffled) filter, from the same lot as that used to filter the particulates is recommended. See Chapter 6, Section C.6.1.
- 1.8.4 Laboratory duplicate: see Chapter 6, Section C.6.3.
- 1.8.5 Reference materials: See section 1.5.2 above. The recovery of the QCS material should be tracked and monitored for performance. Rules for acceptance are summarized in the table below.
- 1.8.6 The frequency, acceptance criteria and corrective actions for PN and PC are summarized in Table D.9-1.

Table D.9-1. Frequency of Calibration, Blank and QC Samples for Particulate Nitrogen and Carbon

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day	90-110% recovery of CRM	Repeat calibration.
Initial Calibration Verification - <i>External QCS or 2nd source of primary standard</i>	After calibration standards, prior to sample analysis	90-110% recovery of known concentration	Recalibrate and verify prior to analysis.
Method Blank	Beginning and end of preparation batch (20 samples)	\leq PQL or reporting limit	Reanalyze another aliquot of filter/blank solution. Investigate possible sources of contamination.

Continuing Calibration Verification (CCV)	Beginning and end of preparation batch.	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or QCS.
Matrix Spike Sample	Not Applicable	Not Applicable	Not Applicable
Laboratory or Field Duplicate Sample	At least 1 per 20 samples	≤ 30% RPD	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

1.9 References

- 1.9.1 [D'Elia, C.F., Magnien, R.E., Zimmermann, C.F., Vass, P.A., Kaumeyer, N.L., Keefe, C.W., Shaw, D.V., Wood, K.V. 1987. Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring. University of Maryland Center for Environmental and Estuarine Studies, publication number UMCEES 87-19 CBL, p 26.](#)
- 1.9.2 [Zimmerman, C. F., C. W. Keefe, and J. Bashe. Method 440.0 Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/009, 1997.](#)