Total Particulate Nitrogen and Carbon

1.1 Scope and Application

1.1.1 ~~This method covers the determination of~~Elemental analysis is used to determine particulate nitrogen and carbon (PNC) in estuarine ~~or sea water,~~ and surface

~~Summary of Method~~

~~waters. The sample~~method measures the PNC irrespective of source (inorganic or organic).

1.1.2 This procedure is ~~filtered through a glass fiber filter~~primarily for use in the Chesapeake Bay Program's (CBP) data gathering and monitoring programs but can be used for other programs that require the analysis of suspended material for carbon and nitrogen.

1.1.3 This procedure conforms to EPA Method 440.0.

1.1.4 The Storet code for particulate carbon is 49569 (Carbon Particulate, Field Filtered, Suspended, WTR, mg/L) and for particulate nitrogen is 49570 (Nitrogen Particulate, Field Filtered, Suspended, WTR, mg/L).

1.2 Summary of Method

1.2.1 The sample is filtered through a glass fiber filter ~~the~~to collect suspended matter. The filter is then placed in a combustion chamber for analysis.

1.2.2 ~~The nitrogen determination is made by burning the glass fiber filter in a closed system in the presence of oxygen. The nitrogen in the sample is converted to molecular nitrogen (N_2) and oxides of nitrogen (NO_x) during the combustion. A carrier gas first transports the combustion products to a reduction tube where the NO_x is converted to N_2, and then to a thermal conductivity detector for measurement~~In a combustion analyzer, the carbon, hydrogen, and nitrogen content in organic and inorganic compounds can be determined. Combustion of the sample occurs in pure oxygen under static conditions. Helium is used to carry the combustion products through the analytical system to atmosphere, as well as for purging the instrument. Helium was selected for this purpose because it is chemically inert relative to tube packing chemicals, and it has a very high co-efficient of thermal conductivity. The products of combustion are passed over suitable reagents in the combustion tube to assure complete oxidation and removal of undesirable by-products such as sulfur, phosphorous, and halogen gases. In the reduction tube, oxides of nitrogen are converted to molecular nitrogen and residual oxygen is removed. In the mixing volume the sample gasses are thoroughly homogenized at precise volume, temperature, and pressure. This mixture is released through the sample volume into the thermal conductivity detector. Between the first of three pairs of thermal conductivity cells an

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absorption trap removes water from the sample gas. The differential signal read before and after the trap reflects the water concentration and, therefore, the amount of hydrogen in the original sample. A similar measurement is made of the signal output of a second pair of thermal conductivity cells, between which a trap removes carbon dioxide, thus determining the carbon content. The remaining gas now consists only of helium and nitrogen. This gas passes through a thermal conductivity cell and the output signal is compared to a reference cell through which pure helium flows. This gives the nitrogen concentration.

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1.3 Interferences

1.3.1 There are no known interferences for the determination of particulate carbon and nitrogen in estuarine/costal water. The presence of carbon and nitrogen compounds associated with laboratory surfaces, finger-prints, detergents and dust necessitates the utilization of careful techniques (i.e., the use of forceps and gloves) to avoid contamination in all parts of this procedure.

1.4 Apparatus and Materials

1.4.1 ~~Carlo Erba NA 1500 Nitrogen/Carbon Analyzer, or other instrumentation.~~Elemental combustion analyzer for determination of carbon and nitrogen

1.4.2 Clean metal forceps.

1.4.3 Plastic syringe, 60 mL.

1.4.4 ~~Filter holders with O-rings, 13 mm.~~Glass fiber filter manifold

1.4.5 ~~Dichromic acid washed DOC bottle.~~

~~Dichromic acid washed 50 mL graduated~~Graduated cylinder (various volumes)

1.4.6 Glass transfer pipettes.

1.4.7 Desiccator.

1.4.8 Glass fiber filters, mm diameter, with a nominal pore size of 0.7 µm.

1.4.9 Muffle ~~oven~~furnace capable of 550°C.

1.4.10 Analytical ~~balance~~ microbalance.

1.4.11 Freezer capable of < -20°C

1.4.12 Pre-muffled nickel sleeves and tin capsules

1.5 Reagents

1.5.1 ~~Cleaning Solvents~~

~~Reagent Grade Water: see section 1.9.~~

Acetanilide, 99.9% + purity, C_8H_9NO (CASRN 103-84-4) or chloramine-T (N-chloro-p-toluenesulfonamide sodium salt) - Primary standards and used for CCV analysis.

1.5.2 Quality Control Sample (QCS) - For this procedure, the QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. If a certified sediment or particulate sample is not available then use the standard material (Acetanilide). The materials below are listed as suggestions.

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 is used for the carbon QCS.

1.5.2.2 SRM 2781 from NIST is a certified particulate nitrogen sludge sample. It is 4.78% nitrogen and is used for the QCS.

1.5.3 Deionized water consistent with ASTM Type II or better.

1.6 Sample Handling

1.6.1 Filter preparation

1.6.1.1 Whatman glass fiber filters, or equivalent, are placed in a ~~metal~~ceramic crucible, loosely arranged.

1.6.1.2 Place filters in muffle oven for 1.5 hours at 550°C.

1.6.1.3 Remove from muffle ~~oven~~furnace, and cover crucible with aluminum foil until cool.

1.6.1.4 ~~Place~~Store muffled filters in a clean ~~labeled petri dishes~~dessicator until ready for use.

1.6.2 Sample preparation

1.6.2.1 Remove ~~foil pouches~~sample containers containing ~~sample~~ filters from freezer and open them.

1.6.2.2 ~~Put foil pouches~~Place the samples and containers in a drying oven at ~~50 ±~~104 +/- 1°C ~~overnight~~for 24 hours.

1.6.2.3 ~~Next~~The next day, remove ~~foil pouches~~the samples from the oven, ~~fold closed~~ and desiccate until ready for analysis.

1.6.2.4 Clean the metal forceps, and ~~ANA metal sealing tray~~preparation area using reagent water and a kimwipe. Never use acid on metal.

1.6.2.5 Using metal forceps, place a clean ~~tin cup~~nickel sleeve in filter loading devise.

1.6.2.6 With metal forceps remove glass fiber filter (sample) from the ~~foil pouch.~~sample container, and place in ~~tin cup~~nickel sleeve

1.6.2.7 Place sample ~~in~~and nickel sleeve into auto sampler wheel ~~an desicate~~and dessicate until analysis.

1.7 Procedure

1.7.1 Standards and Standard Curve Equipment Preparation

1.7.1.1 Tin cups must always be handled with clean metal forceps. ~~The forceps, metal ANA sealing plate and metal spatula must be rinsed with fresh reagent water and completely dried with Kimwipes. Always work on a clean aluminum foil covered~~ Always work on a clean surface whenever handling standards or samples.

1.7.1.2 Calibrate the electronic microbalance at the ~~200 mg~~proper range each day prior to weighing any standards.

1.7.2 Standardization

1.7.2.1 For PN analysis of glass fiber filters, ~~Chloramine~~ acetanilide, chloramine-T (N-chloro-p-toluenesulfonamide sodium salt) or other suitable standards are used as the standard. Standards should weighed into tin capsules and weigh approximately 0.05 to 2.0 mg of standard..

1.7.2.2 For PC analysis of glass fiber filters, acetanilide or another suitable primary standard should be used to standardize the analyzer. Standards should be weighed into the tin capsule. Final weight of standard should be between 0.05 and 2.0 mg.

1.7.2.3 Using metal forceps to handle tin cups, weigh a clean tin cup (see cleaning procedure, 1.7.1) on calibrated microbalance, and tare the balance to eliminate the weight of the cup from the weight measurement.

1.7.2.4 ~~Apply balance brake and remove cup. Place cup on clean ANA metal sealing plate and unfold cup somewhat.~~

~~Using~~Use a clean metal spatula, place approximately 0.05 to 2.0 mg ~~Chloramine T~~of standard into cup~~ and, using~~. Use forceps (2 pairs~~),~~) to seal the tin cup~~.~~

~~Place sealed standard on microbalance, weigh, and record weight on data sheet.~~

~~For the standard curve, 3 blanks (empty clean tin cups), then 7 Chloramine T standards of known weight are analyzed on~~ by pinching the ~~Carlo Erba C/N analyzer in conjunction with the Carlo Erba E.A.G.E.R. computer software~~top closed.

1.7.2.5 ~~Chloramine T standards in the standard curve are analyzed as **STD** sample types and **CHLOR-T** standard types. Blanks are analyzed as **BLANK** sample types.~~

Record the weight of the standard on a raw data schedule. Place the tin cup inside the nickel sleeve and put in proper location of the sample carousel.

1.7.2.6 ~~If the correlation coefficient for the standard curve is below the minimum acceptable value, up to 2 standards and/or 1 blank may be omitted from the standard curve (minimum of 2 blanks and 5 standards are required for a standard curve). A standard can be omitted by bypassing the standard in the sample table and recalculating the curve.~~

Prior to calibrating the instrument purge by running three nickel sleeves through the system. The system is now ready for a blank followed by the number of standards necessary for the instrument of use.

1.7.3 Analysis of Glass Fiber Filter Samples

1.7.3.1 ~~Set flow rates as outlined~~ Write a sample schedule. Press the glass microfiber filters into the nickel sleeves and place in the ~~Carlo Erba NA 1500 Nitrogen/Carbon Analyzer Instruction Manual. Set control panel parameters as follows:~~

~~Filament temperature 190°C~~
~~Oxygen injection stop 67 seconds~~
~~Peak enable 10 seconds~~
~~Cycle stop 410 seconds~~
~~(this may need to be increased if carbon peak on Chromatogram is not completed during cycle time).~~

~~Set~~ autosampler ~~tray with space #1 aligned with window on the front of the~~

autosamplerin the order denoted on the sample schedule.

1.7.3.2 Place auto sampler tray into the instrument, with the purges blanks and standards in spaces 1-10the front of tray. Run a QCS for each nitrogen and carbon prior to the running of autosampler tray and, using the Carlo Erba E.A.G.E.R. computer software, generate the standard curvesamples.

1.7.3.3 After an acceptable standard curve has been generated, place one wrapped sample or standard in each space of the autosampler tray and, using the E.A.G.E.R. software, and analyze the samples.

Run a QCS for each nitrogen and carbon prior to the running of samples.

1.7.3.4 In order to demonstrate that the instrument was still in calibration at the end of the analytical run, the last sample analyzed in any series of analyses must be a standard.

If the calibration and QCS samples are acceptable then allow the instrument to continue to run samples.

1.7.3.5 Samples will have a known volume ofRun an acetanilide sample water filtered onto them. Divideafter every ten samples to confirm that volume (the instrument is still within calibration.

1.7.3.6 For aqueous samples divide the result, for particulate carbon or nitrogen, in mL)micrograms by 10, and place this value in the amount of sample weight column of the sample table of the E.A.G.E.R. file.

The cycle time used in the E.A.G.E.R. program parameters should be 10 seconds longer than the cycle stop time set on the C/N analyzer instrument panel.

Quality Control

The correlation coefficient of the calibration curve must ≥ 0.990.

Run a duplicate of each sample, and a Chloramine T standard after every 5 samples (sample and it's duplicate count as one sample). If the samples are not all analyzed in duplicate, a minimum of one duplicate must be analyzed after every 10 samples, and at least 2 duplicates must be analyzed per analytical run The calibration check standards are analyzed as unknown sample types. Recovery must be within 90-110% of actual value. If recovery of a standard falls outside these limits, it is considered to be "out-of-control," and all samples analyzed after the last "in-control" standard must be reanalyzed.

~~Prepare and analyze at least two standards (2 "in-control" standards must occur consecutively before proceeding with analyses).~~
~~Re-prepare and reanalyze each sample which was analyzed after the last "in-control" standard.~~

~~Particulate Carbon~~

~~Scope and Application~~

~~This method covers the determination of particulate carbon in estuarine or sea water and the filter is then placed in a combustion chamber for analysis~~ through~~.~~

1.7.4~~The carbon determination is made by burning~~ the glass fiber filter ~~in a closed system in the presence of oxygen. The carbon in the sample is converted to carbon dioxide during the combustion. A carrier gas then transports the CO_2 to a thermal conductivity detector for measurement.~~ The result will then be in

~~Apparatus and Materials~~

~~Carlo Erba NA 1500 Nitrogen/Carbon Analyzer, or other instrumentation.~~

~~Clean metal forceps.~~

~~Plastic syringe, 60 mL.~~

~~Filter holders with O-rings, 13 mm.~~

~~Hydrochloric acid washed DOC bottle.~~

~~Hydrochloric acid washed 50 mL graduated cylinder.~~

~~Glass transfer pipettes.~~

~~Desiccator.~~

~~Muffle oven.~~

~~Analytical balance.~~

~~Reagents~~

~~Reagent Grade Water: see section 1.9.~~

~~Sample Handling~~

~~Filter preparation~~

~~NOTE:~~

~~Whatman glass fiber filters are placed on aluminum foil in a muffle pan. Filters should~~

not be touching each other.

Place filters in muffle oven for 15 minutes at 550°C.

Remove from muffle oven, and cover pan with aluminum foil until cool.

Wrap muffled filters in aluminum foil and desiccate until use.

Sample preparation

Remove foil pouches containing sample filters from freezer. Remove caps from foil pouches and place caps and foil pouches in muffle pan.

Put vials in oven at 50 ± 1°C overnight.

Next day, remove foil pouches from oven, recap tightly, place foil pouches in divided cardboard box and desiccate until ready for analysis.

Clean the metal forceps, and ANA metal sealing tray using reagent water and a Kimwipe. Never use acid on metal.

Using metal forceps, place a clean tin cup in hole #2 of ANA metal sealing tray, and fan the top edges of the cup around edges of hole.

With metal forceps remove glass fiber filter (sample) from the glass vial, place in tin cup, seal cup using forceps (2 pair), and form into a ball which should be small enough to drop through the hole in the Carlo Erba C/N analyzer autosampler.

Replace foil wrapped sample into the glass vial, recap, and desiccate until analysis.

Procedure

Cleaning C/N analyzer tin cups

NOTE: Use the following procedure only if you have unusual contamination.

Pour approximately 50 mL of chloroform/methanol cleaning solution into a 100 mL glass beaker. (Level of solution in beaker should allow tin cups standing upright to be completely submerged.)

Using clean metal forceps, submerge each cup individually into the chloroform/methanol solution and stand cup upright. Make sure all cups are completely submerged.

~~Cover beaker with aluminum foil and let stand for at least 30 minutes.~~

~~Decant the cleaning solution from the beaker into a chloroform/methanol waste bottle without losing any cups. Remove any remaining solution from the beaker using a glass transfer pipette.~~

~~Using clean metal forceps, transfer cups individually to a 150 mL hydrochloric acid washed glass container of acetone. Hold cup momentarily in acetone then remove and drain acetone from cup. Do this twice for each cup.~~

~~Transfer cup to 150 mL hydrochloric acid washed glass container of fresh reagent water. Be sure cups are completely submerged and resting on the bottom of the container.~~

~~Cover container with aluminum foil and let stand at least 15 minutes.~~

~~Decant water from beaker and remove remaining water with a glass transfer pipette.~~

~~Place clean cups on a clean sheet of aluminum foil and dry in oven at 100 - 200°C for at least 2 hours. DO NOT exceed 200°C; tin cups will become brittle.~~

~~Wrap dry cups in aluminum foil or place into a glass, hydrochloric acid cleaned container and desiccate until use.~~

~~Standards and Standard Curve Equipment Preparation~~

~~Tin cups must always be handled with clean metal forceps. The forceps, metal ANA sealing plate and metal spatula must be rinsed with fresh reagent water and completely dried with Kimwipes. Always work on a clean aluminum foil-covered surface whenever handling standards or samples.~~

~~Calibrate the electronic microbalance at the 200 mg range each day prior to weighing any standards.~~

~~Standardization~~

~~For PC analysis of glass fiber filters, Chloramine-T (N-chloro-p-toluenesulfonamide sodium salt) or other suitable standards are used as the standard. Standards should weigh approximately 0.05 to 2.0 mg.~~

~~Using metal forceps to handle tin cups, weigh a clean tin cup on calibrated microbalance, and tare the balance to eliminate the weight of the cup from the weight measurement.~~

Apply balance brake and remove cup. Place cup on clean ANA metal sealing plate and unfold cup somewhat.

Using a clean metal spatula, place approximately 0.05 to 2.0 mg Chloramine T into cup and, using forceps (2 pairs), seal tin cup.

Place sealed standard on microbalance, weigh, and record weight on data sheet.

For the standard curve, 3 blanks (empty clean tin cups), then 7 Chloramine T standards of known weight are analyzed on the Carlo Erba C/N analyzer in conjunction with the Carlo Erba E.A.G.E.R. computer software.

Chloramine T standards in the standard curve are analyzed as **STD** sample types and **CHLOR-T** standard types. Blanks are analyzed as **BLANK** sample types.

If the correlation coefficient for the standard curve is below the minimum acceptable value, for a maximum 2 standards and/or 1 blank may be omitted from the standard curve (minimum of 2 blanks and 5 standards are required for a standard curve). A standard can be omitted by bypassing the standard in the sample table and recalculating the curve.

Analysis of Glass Fiber Filter Samples

Set flow rates as outlined in the Carlo Erba NA 1500 Nitrogen/Carbon Analyzer Instruction Manual. Set control panel parameters as follows:

Filament temperature 190°C
Oxygen injection stop 67 seconds
Peak enable 10 seconds
Cycle stop 410 seconds
(this may need to be increased if carbon peak on Chromatogram is not completed during cycle time).

Set autosampler tray with space #1 aligned with window on the front of the autosampler.

Place blanks and standards in spaces 1-10 of autosampler tray and, using the Carlo Erba E.A.G.E.R. computer software, generate the standard curve.

After an acceptable standard curve has been generated, place one wrapped sample or standard in each space of the autosampler tray and, using the E.A.G.E.R. software, and analyze the samples.

~~In order to demonstrate that the instrument was still in calibration at the end of the analytical run, the last sample analyzed in any series of analyses must be a standard.~~

~~Samples will have a known volume of sample water filtered onto them. Divide that volume (in mL) by 10, and place this value in the sample weight column of the sample table of the E.A.G.E.R. file.~~

~~The cycle time used in the E.A.G.E.R. program parameters should be 10 seconds longer than the cycle stop time set on the C/N analyzer instrument panel.~~
ug/ml which is equivalent to mg/L.

1.8 Quality Control

1.8.1 ~~The correlation coefficient of the calibration curve must greater than or equal to 0.990.~~

Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter VI, Section C.8. Since PCN cannot be spiked, utilize seven aliquots of a low concentration sample. If there are no low concentration samples then 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 ~~Run a duplicate of each sample, and a Chloramine T standard after every 5 samples (sample and it's duplicate count as one sample). If the samples are not all analyzed in duplicate, a minimum of one duplicate must be analyzed after every 10 samples, and at least 2 duplicates must be analyzed per analytical run.~~

Calibration:

1.8.2.1 ~~The calibration check standards are analyzed as unknown sample types. Recovery must be within 90-110% of actual value. If recovery of a standard falls outside these limits, it is considered to be "out-of control," and all samples analyzed after the last "in-control" standard must be reanalyzed.~~

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 ~~Prepare and analyze at least two standards (2 "in-control" standards must occur consecutively before proceeding with analyses).~~

For multipoint curves the correlation coefficient must be 0.995 or better.

1.8.3 Method Blank: see Chapter VI, Section C.6.1.

1.8.4 Laboratory duplicate: see Chapter VI, Section C.6.3.

1.8.5 ~~Re-prepare and reanalyze each sample which was analyzed after the last "in-control" standard.~~

Reference materials: The laboratory must analyze a standard reference material or some other second source performance check with each run. The reference material should be charted for performance reasons. Rules for acceptance are summarized in the table below.

1.8.6 Summary table for QC parameters:

INDICATOR	ACCEPTANCE/ACTION LIMITS	ACTION	FREQUENCY (BATCH)
Correlation Coefficient	≥ 0.995	If < 0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
QCS (EPA)	± 10%	If QCS value is outside ± 10% of the target value, reject the run, correct the problem and rerun samples.	Beginning of run following the ICV.
QCS (NELAC)	± 3s	If the QCs exceeds 3SD then the run must be stopped and problem corrected before restarting the analysis. Acceptable performance must be shown before continuing.	Beginning of run following the ICV.
CCV	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 10 samples and at end of batch
Laboratory Reagent Blank / Calibration Blank[a]	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV, after every 10 samples and at the end of the run.
Method Quantitation Limit (MQL) The concentration of the lowest standard.	Determine the standard deviation at the concentration of the lowest standard. The MQL will be +3s.	When the value is outside the predetermined limit and the ICV is acceptable, reanalyze the sample. If the reanalysis is unacceptable, increase the concentration and reanalyze. If this higher concentration meets the acceptance criteria, raise the reporting limit for the batch.	Beginning of run following the LRB
Laboratory Fortified Sample Matrix	± 20%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	After every 10 samples
Laboratory Duplicate	± 20%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	After every 10 samples.

1.9 References

1.9.1 EPA 1997. "Methods for the determination of Chemical Substances in Marine and Estuarine Environmental Matrices" EPA/600/R-97/072, September 1997, Method 440.0 "Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis", Rev. 1.4.