

**Standard Operating Procedure for the
Analysis of Particulate-Phase Organic Carbon
in Great Lakes Waters**

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August 2, 1994

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1.0 Scope and Application

This Standard Operating Procedure describes the analysis of glass fiber filters from particulate-phase Great Lakes waters samples and associated quality control blanks and duplicate samples for particulate organic carbon (POC). The analysis is by catalytic combustion followed by packed column gas chromatographic separation with thermal conductivity detection. This SOP covers standard and instrument preparation, instrument calibration and maintenance, elemental analysis of carbon, and calculation of results.

2.0 Safety and Waste Handling

All applicable safety and waste handling rules are to be followed. These include the proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden. Refer to the GLNPO Safety, Health, and Environmental Compliance Manual for specific rules.

3.0 Summary of Procedure

Filtered Great Lakes water samples are analyzed for POC in a ship-board or land-based laboratory. Sub-samples of the exposed glass fiber filters are loaded into small tin capsules and placed in the autosampler of a Carlo Erba Elemental Analyzer 1108. At preset intervals, the tin capsules are dropped into a vertical quartz reactor tube inside a 1000°C furnace. After a capsule drops into the reactor tube, the carrier gas is temporarily enriched with oxygen causing instantaneous oxidation of the sample. Quantitative oxidation is achieved by passing the resulting mixture of gases over a tungstic anhydride catalyst. The gas mixture then passes over elemental copper to remove excess oxygen and to reduce nitrogen oxides to elemental nitrogen. The sample gases pass through a packed chromatographic column, are separated, eluted, and detected by a thermal conductivity detector (TCD). Organic carbon is quantified by the external standard method.

4.0 Description of Instrumentation

The Carlo Erba EA 1108 Elemental Analyzer is a commercially-available instrument comprised of a combustion furnace, gas chromatographic oven, and thermal conductivity detector. It can be configured to detect carbon, hydrogen, nitrogen, and sulfur simultaneously (an oxygen determination mode is also possible). The instrument is equipped with a pneumatic autosampler and a PC-based computer data system (Carlo Erba Eager 200). The analytical method uses one of two available furnaces to house a catalytic reactor tube. The reactor tube is packed with an upper part which functions as an oxidation catalyst (tungstic anhydride), and a lower portion which functions as the reduction reactor (elemental copper). After exiting the reactor tube, the gas-phase sample travels through a water trap (anhydron), and then, into a packed chromatographic column. The sample components are separated by the column as CO₂, H₂, N₂, and H₂S. These species are detected by a thermal conductivity detector (TCD).

5.0 Preparation

5.1 Preparation of Calibration Standards

- 5.1.1 The calibration standard stock solution of potassium hydrogen phthalate (KHP, AR grade or better) is prepared by dissolving 4.2509 grams of crushed, dried (110 °C for two hours) $\text{KHC}_8\text{H}_4\text{O}_4$ in organic-free, distilled, deionized water (from now on referred to as organic-free water). Add 0.2 mL of concentrated sulfuric acid (AR grade or better) and dilute to 1 liter in a volumetric flask. The concentration of the solution is 2000 mg of carbon/L.
- 5.1.2 The stock solution is stored at 4 °C in a clean glass bottle. Label the bottle with reagent name, concentration, date prepared, expiration date, and analyst's initials.
- 5.1.3 Working calibration standards are prepared from the stock KHP solution. Use Class A volumetric pipets and volumetric flasks. Store the working calibration standards in clean glass bottles with Teflon-lined caps at 4 °C. Label the bottles with reagent name, concentration, date prepared, expiration date, and analyst's initials. Prepare the working standards following these directions:

mL Stock KHP, diluted to 100 mL	µg carbon/50 µL spike
5.0	5.0
10.0	10.0
25.0	25.0
50.0	50.0
75.0	75.0
100.0	100.0

- 5.1.4 Stock and working calibration standards are prepared on a monthly basis.

5.2 Preparation of Calibration Check Standards

- 5.2.1 The calibration check standard stock solution of ethylenediamine tetra-acetic acid (EDTA, di-sodium salt, AR grade or better) is prepared by dissolving 6.1983 grams of EDTA in organic-free water. Add 0.2 mL of concentrated sulfuric acid (AR grade or better), taking precaution to prevent precipitation, and dilute to 1 liter in a volumetric flask. The concentration of the solution is 2000 mg of carbon/L.
- 5.2.2 The stock solution is stored at 4 °C in a clean glass bottle. Label the bottle with reagent name, concentration, date prepared, expiration date, and analyst's initials.

- 5.2.3 Working calibration check standards are prepared from the stock EDTA solution. Use Class A volumetric pipets and volumetric flasks. Store the working calibration check standards in clean glass bottles with Teflon-lined caps at 4 °C. Label the bottles with reagent name, concentration, date prepared, expiration date and analyst's initials. Prepare the working standards following these directions:

mL stock EDTA	µg carbon/50 µL spike
15.0 diluted to 100 mL	15.0
20.0 diluted to 25 mL	80.0

- 5.2.4 Stock and working calibration check standards are prepared on a monthly basis.

5.3 Preparation of Elemental Analyzer

- 5.3.1 Read the operating manuals for the elemental analyzer and data system. The instrument is initially installed following instructions in the manuals. Field assistance from the manufacturer may be necessary. The data system requires the installation of a circuit board into the PC (see the data system manual). If the instrument is to be used in a ship-based laboratory, proper installation requires both vibration isolation and secure mounting.

- 5.3.2 Verify that the instrument has been properly and securely installed. Locate the main power switch on the left side of the back panel. The instrument may be turned off at this switch when not in use for extended time periods, or it can be left in stand-by for shorter periods. The instrument power should be off during preparation.

- 5.3.3 The instrument operates using three pneumatic systems. Helium and oxygen must be UHP grade (99.999%) or better. The gases required are:

Helium: Helium is used as the carrier gas. A combination oxygen/hydrocarbon trap (e.g., Supelco OMI-1) is installed in the helium line, as close to the instrument as possible. Set the helium supply pressure to 200-300 kPa at the tank.

Oxygen: Oxygen is used during the combustion step in the reactor tube. Set the oxygen supply pressure to 100 kPa at the tank.

Air: Air is used to operate pneumatic valves in the instrument and to operate the autosampler. Set the air supply pressure to 350-400 kPa at the tank.

- 5.3.4 The instrument's analytical configuration is determined by the materials used to pack the reactor tube. A CHNS configuration will be described here. Refer to the operating manual for other possible configurations. Figure 1 shows the CHNS packing.

- 5.3.5 Pack the reactor tube by marking the sizes of the packing layers (see Figure 1) on the outside of the quartz tube with a permanent marker and filling to the marks with the appropriate material. The packing materials are held in place with quartz wool.

- 5.3.6 Place the Viton O-ring on the empty portion of the packed reactor tube, with the flat part facing down.
- 5.3.7 Lower the reactor tube into the left furnace of the instrument with the copper layer going in first (towards the bottom). Adjust the O-ring so that it is approximately 1 inch from the top of the reactor tube.
- 5.3.8 Attach the autosampler to the top of the reactor tube.
- 5.3.9 Install the nut and washer followed by a Viton O-ring with the flat end facing up. Attach the coupling to the bottom of the reactor tube.
- 5.3.10 A water trap is used to keep water out of the detector. An unstable, high baseline will result if a water trap is not used. Pack the water trap by filling the glass tube with anhydron (magnesium perchlorate). Use 5 mm of quartz wool to hold the material in place.
- 5.3.11 Connect the fittings to the water trap and clip it into the mounting bracket in front of the chromatographic oven. Check the water trap daily, and repack when needed.
- 5.3.12 A leak check of the pneumatic systems is necessary at the start of each day of analysis. Turn on the main power switch. Depress the filament standby push-button, which is located in the lower left corner of the instrument control panel.
- 5.3.13 Follow these steps to do a leak check:
 - A. Set the air pressure to 350 kPa using the pressure regulator on the front panel of the instrument.
 - B. Wait 2 minutes.
 - C. Turn the tank pressure regulator off. If the system maintains the 350 kPa pressure for 2 minutes, the air system is leak-tight.
 - D. Turn the helium and oxygen pressure regulators on the front panel to zero and cap off the carrier gas vents (measuring and reference) and oxygen vent located on the lower front panel with the caps provided with the instrument.
 - E. Adjust the pressure regulators on the front panel to 100 kPa for helium and 150 kPa for oxygen.
 - F. Wait 2 minutes.
 - G. Turn the front panel valves for helium and oxygen off. If the system maintains the 100 kPa, for helium, and 150 kPa, for oxygen, pressures for 3 minutes, the Systems are leak-tight. Replace the caps with the fittings.
- 5.3.14 If there are leaks, refer to the operating manual (section 3.2 in the Carlo Erba EA 1108 Manual). Leaks can be traced using a liquid leak detector (e.g., Snoop) to check for loose connections.

5.3.15 When the leak check procedure is completed, release the filament standby button.

- 5.3.16 Set the helium and oxygen flow rates:
- A. Set the helium analytical flow rate to 100 mL/min. using the front panel pressure regulator. Measure the flow at VENT M on the lower front panel of the instrument using a soap-bubble flowmeter.
 - B. Set the helium reference flow rate to 40 mL/min. using the front panel pressure regulator. Measure the flow at the PURGE vent on the lower front panel of the instrument using a soap-bubble flow meter.
 - C. Set the oxygen flow rate to 12 mL/min. using the front panel pressure regulator. Measure the flow at the OXYGEN vent on the lower front panel of the instrument using a soap-bubble flowmeter.
- 5.3.17 Set the instrument control panel settings as follows:
- A. Left furnace -1000 °C
 - B. Right furnace -500 °C
 - C. Oven temperature - 70 °C
 - D. Filament temperature - 180 °C
 - E. Cycle - 180 seconds: the time needed for the complete analysis of one sample
 - F. Sample start - 15 seconds: the time into the run when the sample is dropped into the reactor tube
 - G. Sample stop - 40 seconds: the time into the run when the autosampler advances to the next sample
 - H. Oxygen inject - 35 seconds: the time into the run when the oxygen enrichment will stop
 - I. Peak Enable - 0 seconds: the time at which data acquisition will start
- 5.3.18 The settings for "F" and "G" may need to be adjusted in order to optimize the system for complete combustion of the sample. Combustion of the samples can be viewed through the "view finder" located on the face of the autosampler. It is recommended to occasionally observe the combustion of a sample to confirm that the control panel settings are optimum. If incomplete combustion is suspected, increase the oxygen inject time. Set the sample stop time to 5 seconds greater than the oxygen inject time.

6.0 Analytical Procedures

6.1 Instrument Start-up

- 6.1.1 Turn on the main power switch.
- 6.1.2 Turn on the gases at the tanks, and turn the instrument purge and carrier valves to the "on" position. These valves are located below the pressure gauges on the front panel of the instrument. Release the filament and furnace stand-by push-buttons on the instrument control panel. Allow the furnace to reach the set point temperature (1000°C).
- 6.1.3 Perform a leak check on the gas systems (section 5.3.13) at the start of each day of analysis. Check the gas flow rates (section 5.3.16).
- 6.1.4 Turn on the data system and launch the data acquisition software (Carlo Erba Eager 200).

6.2 Instrument Calibration

- 6.2.1 Cut out 6 filter discs from a muffled, 47 mm GF/F filter with a clean, 12 mm diameter cork boring tool (#6).
- 6.2.2 Spike 50 mL of each level of KHP calibration standard (section 5.1) on a separate disc using a micro-pipettor. Allow the spiked filter discs to dry. Rinse the micro-pipettor tip with organic-free water 5 times between spikes, and initialize the tip with calibration standard 5 times prior to spiking a filter disc.
- 6.2.3 While the spiked filter discs are drying, fill in the sample table in the data acquisition software. Start the analysis sequence with a by-pass, which consists of an empty tin sample container. Continue filling in the sample table by moving from the lowest to highest calibration standard concentration. Enter the amount of carbon spiked in milligrams in the "sample weight" column. Enter "standard" in the "sample type" column and "POC" in the "standard type" column.
- 6.2.4 Verify that the analysis sequence listed in the sample table is correct. This instructs the data acquisition software what samples are being run and where to save the data files. The first and last discs must be properly noted in the sample table.
- 6.2.5 Using stainless steel forceps, fold the spiked discs into eighths and place them into individual tin sample containers. Pack the spiked filters completely inside of the tin containers.
- 6.2.6 Load the calibration standards into the autosampler in the same order as the analysis sequence created in the sample table.
- 6.2.7 Start the instrument by selecting the "run" command in the menu. Nitrogen (N₂) will elute first, followed by carbon (CO₂). Confirm that the retention time listed in the component table of the data acquisition software is the same as the actual retention time. If the difference between the actual and listed retention times is too large, the data system will not identify the peaks properly. Enter the correct retention times in the table and re-start

the calibration if needed.

- 6.2.8 After all of the calibration standard discs have been analyzed, confirm that the correlation coefficient for the six-level calibration plot is 0.995 or better. If this condition is not met, repeat the calibration procedure (steps 6.2.1-6.2.8).
- 6.2.9 If the calibration plot is satisfactory, cut out three 12 mm discs from a muffled, 47 mm GF/F filter. Spike 50 mL of the two calibration check standards onto separate discs and 50 mL of organic-free water onto the third disc for a filter blank. Allow the discs to dry.
- 6.2.10 Enter the information for the two calibration checks and one filter blank in the sample table. Enter "unknown" under "sample type" and "100" for "sample weight". Verify that the analysis sequence is correct. The first and last discs must be properly noted in the sample table.
- 6.2.11 Load the two calibration check spikes and the blank in the autosampler. Analyze these filter discs and determine if they are within tolerances. The calibration checks must be within $\pm 20\%$ of the nominal spike mass. The blank must be less than 5.0 mg carbon, which corresponds to the lowest calibration level. If the calibration check spikes or filter blank are not within these tolerances, refer to section 9.0.
- 6.2.12 If the quality control spikes and blank are within tolerances, then proceed with the analysis of POC lake water samples.
- 6.3 Analysis of Samples
- 6.3.1 Open the aluminum foil envelope containing a POC filter sample. Remove a small piece of aluminum foil from the envelope. Place the piece of aluminum foil, with the dull side facing up, into a 50 x 9 mm plastic Petri dish.
- 6.3.2 Cut out two 12 mm discs from the filter, while it is folded in half, with a 12 mm diameter cork boring tool (#6). Take care to cut out the discs from the area of the 47 mm filter that is coated with particles. Separate the four resulting discs. Place the discs, particle side up, on the foil in the Petri dish. Cover the Petri dish to prevent dust particles from settling on them, but loose enough to allow the discs to dry. Label the cover of the Petri dish with the Great Lake sampling station identification number that is listed on the aluminum foil envelope.
- 6.3.3 Repeat steps 6.3.1 and 6.3.2 for 4 more POC filters for a total of twenty 12 mm discs. Cut out three more discs from a muffled, 47 mm GF/F filter and follow step 6.2.9 for the calibration check standards and the filter blank.
- 6.3.4 Fill in the data acquisition software sample table for the twenty sample discs followed by the three quality control samples. Enter "unknown" in the "sample type" column and "100" in the "sample weight" column. Verify that the information in the sample table is correct. The first and last discs must be properly noted in the sample table.
- 6.3.5 Allow the discs to dry, fold them into eighths, and place them into individual tin sample containers. Pack the filters completely inside of the tin containers. Load the tin containers into the autosampler in the same order as the analysis sequence created in the sample table.

- 6.3.6 Analyze the sample discs by selecting the "run" command in the menu. If tolerances for the two calibration check standards or the blank are not met, refer to section 9.0.
- 6.3.7 If quality control tolerances are met, then repeat steps 6.3.1-6.3.6 for another batch of sample filters.
- 6.4 Instrument Shut-down
- 6.4.1 Depress the furnace and filament stand-by push-buttons on the instrument control panel.
- 6.4.2 Turn off the oxygen and air at the tanks. Helium is turned off only for a long-term shut-down.
- 6.4.3 For a short-term shutdown, steps 6.4.1 and 6.4.2 are sufficient. For a long-term shutdown, continue with steps 6.4.4-6.4.6.
- 6.4.4 Remove the water trap and dispose of the contents properly. Connect the water trap inlet and outlet fittings together with an adapter.
- 6.4.5 Remove the chromatographic column and cap both ends of the column. Connect the column inlet and outlet fittings together with an adaptor.
- 6.4.6 Cap the three vents on the lower front panel of the instrument. Turn off the main power switch.

7.0 Calculations

POC samples are collected by filtering the lake water through a 47 mm diameter glass fiber filter. The glass filtering apparatus exposes only a 38 mm diameter area of the 47 mm filter to the sample water. From this 38 mm diameter area, four 12.065 mm diameter discs (#6 cork boring tool) are cut out and analyzed for organic carbon. The resulting mass of carbon from the analysis of four discs per sample must be summed, multiplied by an area correction factor (x), and divided by the volume of water filtered to calculate the measured POC concentration in mg/L. The area correction factor is calculated by a ratio of the effective filter and disc areas:

$$\text{AREA}_{38} = 3.14159(19 \text{ mm})^2 = 1134 \text{ mm}^2$$

$$\text{AREA}_{12} = 3.14159(6.0325 \text{ mm})^2 = 114.33 \text{ mm}^2$$

$$\text{CORRECTION FACTOR (X)} = 1134/4(114.33) = 2.48$$

8.0 Maintenance and Trouble-Shooting

- 8.1 The copper metal in the reactor tube will oxidize and must be replaced periodically. If the detector response for nitrogen increases significantly in consecutive chromatograms, this is a symptom of complete oxidation of the copper. Generally, about 75 analytical runs can be made from one 80 mm packing of copper in the reactor tube.

Follow these steps to repack the copper:

- A. Depress the filament stand-by push-button on the instrument control panel.
 - B. Remove the coupling from the bottom of the reactor tube and disconnect the autosampler from the top of the reactor tube.
 - C. Remove the reactor tube from the furnace. Remove the quartz wool plug and the copper metal from the bottom of the reactor tube.
 - D. Repack the reactor tube with 80 mm of fresh copper wire pieces. Use fresh quartz wool to hold the copper in place.
 - E. Scrape out the tin dioxide residue from the top of the reactor tube. Replace the quartz wool if needed.
 - F. Reconnect the top of the reactor tube to the autosampler and the bottom to the coupling. Use new Viton O-rings on the reactor tube.
 - G. Perform a leak check of the pneumatic systems (section 5.3.13).
- 8.2 An unstable baseline may indicate that the tungstic anhydride in the reactor tube needs to be repacked. Follow the steps in section 8.1, except remove the upper contents (tungstic anhydride) of the reactor tube.
- 8.3 Baseline problems may also be due to water in the detector. Water can also cause the flow of the carrier gas (helium) to drop. Check the water trap (anhydron) and repack if necessary. If the water trap is not spent, check the gas lines and chromatographic column for blockages. Refer to the instrument operating manual.
- 8.4 If gas leaks are suspected, see section 5.3.13 for the leak testing procedures.

9.0 Quality Control

- 9.1 The six-level calibration plot must have a correlation coefficient of 0.995 or better. A new calibration plot must be generated at the start of each day of analysis.
- 9.2 An empty tin sample container is analyzed prior to calibration at the start of each day of analysis.
- 9.3 Calibration check standards and filter blanks are run immediately after the generation of a calibration plot and after the analysis of every twenty sample discs. If tolerances for quality control standards and blanks are not met, prepare and analyze another set of quality control standards and a filter blank. If the tolerances are not met again, the instrument must be recalibrated.
- 9.4 Stock and working calibration (KHP) and calibration check (EDTA) solutions must be prepared at the beginning of each Great Lakes survey.
- 9.5 POC filters are analyzed promptly, in a ship-board laboratory, during the course of a survey.

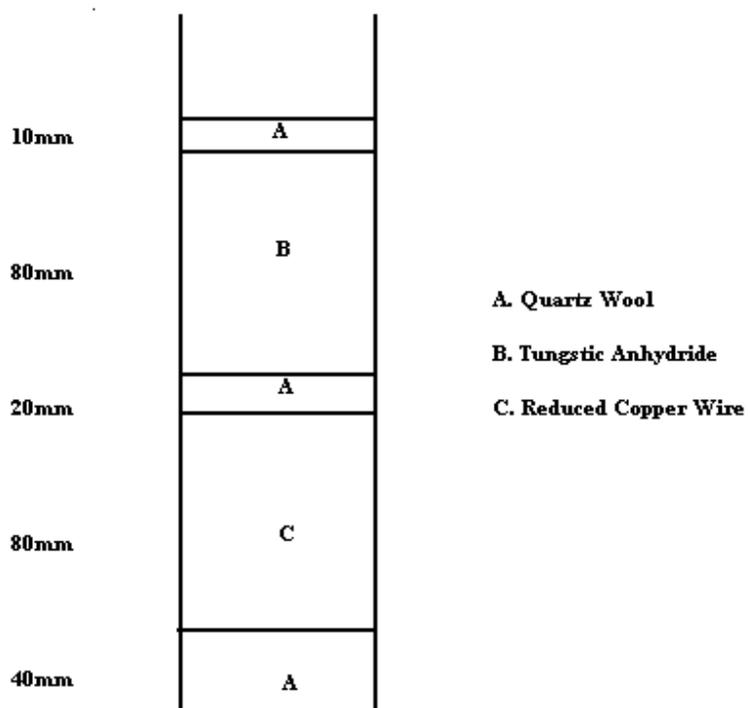


Figure 1: CHNS Reactor Tube Configuration

Table 1: List of Equipment

(most quantities depend on number of samples)

<u>EQUIPMENT</u>	<u>SOURCE OR EQUIVALENT</u>
Tin sample containers	Fisons 24006400 (100/pack)
Reactor tube	Fisons 46820000 (set of 2)
Anhydrone	Fisons 33821900 (100 grams)
Quartz wool	Fisons 33822200 (5 grams)
Copper wire pieces	Fisons 33835310 (40 grams)
Viton O-ring for water trap	Fisons 29013603 (set of 2)
Viton O-ring for reactor tube	Fisons 29032910 (set of 10)
Tungstic anhydride	Fisons 33835420 (25 grams)
EDTA (di-sodium salt)	J.T.Baker 8993-01(500 grams)
Potassium hydrogen phthalate	Fisher P243-100 (100 grams)
Concentrated sulfuric acid	Fisher A510-500 (500 mL)
47 mm GF/F filters	Whatman 182547 (100/pack)
50 mL micro-pipettor	Daigger Scientific G20537F
OMI- I indicator tube	Supelco 2-3900

Miscellaneous

- 50 x 9 mm plastic Petri dish
- 400 mL beaker
- 12 mm punch
- stainless steel forceps
- Class A volumetric flasks and pipets
- permanent markers
- aluminum foil