# Non-Purgeable Organic Carbon (NPOC) Analysis Using the Shimadzu 680°C Combustion Catalytic Oxidation/Non-Dispersive Infrared method

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# Scope and Application

The following standard operating procedure (SOP) is used by the U.S. Geological Survey's Mercury Research Laboratory (MRL) to determine Dissolved Organic Carbon (DOC) or Total Organic Carbon (TOC) concentrations in water. This SOP describes the preparation of the sample and subsequent analysis. Sample analysis is conducted by the Shimadzu TOC-$V_{CSH}$ total organic carbon analyzer, using the NPOC analysis method. Quality assurance and control protocols are employed throughout sample analysis, including: laboratory practices to prevent sample contamination, method blanks, secondary standard checks, and analytical replication.

# Laboratory Safety

Analysts who use the MRL must have read, understood, and signed the Chemical Hygiene Plan for the MRL prior to potential exposure to any chemicals. The analyst must have a thorough understanding of the required safety protocols for the lab chemicals prior to their use of the lab. Adequate personal protection equipment such as safety glasses, gloves, and chemical resistant clothing must be worn when exposure to hazardous chemicals are possible. Caution should always be exercised as chemicals are present in the laboratory and often in use by other analysts. Hazardous chemicals should only be handled by adequately trained personnel under a high volume fume hood with extreme caution.

Detailed information is included for each reagent specific to the method described in this SOP and additional safety information can be found in the safety data sheets (SDS) located in the lab. Hazardous chemicals used in this method include concentrated acids (HCl and $H_3PO_4$). During analysis, the automated sample introduction system may begin moving without warning and presents a mechanical hazard.

# Sample Vials

Samples and standards should be prepared for analysis in glass amber vials. Vials should be sterile and previously unused or triple-rinsed in reagent-grade water, dried, and then heated to 550°C for 2 hours.

# Reagents and Standards

## Reagents

Upon receipt at the laboratory, containers will be marked with the date of receipt and stored in the appropriate areas. When reagents are mixed for use in this method, the person who mixes them will record the chemical contents and concentration, and initial and date the reagent container. Reagents and manufacture instructions follow below.

Reagent water: Ultra-pure reagent grade water containing less than 0.1 ng/L Hg with a resistance greater than 18 MW-cm. The water is delivered through a 0.2 µm filter, as obtained from a Millipore Academic water-purification system or equivalent.

Hydrochloric Acid (HCl): EM Science Omni Pure HCl or equivalent.

Phosphoric Acid ($H_3PO_4$): ACS Grade, 85% min. (w/w)

Zero Air: Grade 5.0 (ultra high purity).

## Analytical and Quality Control Standards

Upon receipt at the laboratory and on the day of preparation, standard solutions should be labeled with the date received/opened. Two organic carbon standard solutions from different sources are required for analysis: an “analytical standard” which is used to calibrate the instrument and a “quality control standard” which is purchased from a separate source and used to validate instrument performance throughout analyses. Additionally, an inorganic carbon standard is used to verify complete removal of inorganic carbon prior to analysis.

# Sample Preparation

Samples should be refrigerated or put on ice as soon as possible after they are collected. Samples should remain chilled until the time of analysis. Samples for DOC analysis should be filtered in the field or in the lab as soon as possible after collection and prior to analysis. Samples for TOC analysis should remain unfiltered. After initial processing is complete (e.g. filtering, splitting samples), samples should be stored in clean glass amber vials (see “Sample Vials”).

# Instrument Operation

The instrumentation for DOC/TOC analysis consists of two interconnected modules: the Shimadzu TOC-$V_{CSH}$ Analyzer, and the Shimadzu ASI-V Autosampler. The Autosampler is designed to operate on samples or standards prepared in septa sealed 40 ml glass vials. The Autosampler holds a removable 68-vial rotating sample rack.

## Initial Start Up

1. Ensure the tank of Zero Air is connected to the regulator leading to the instrument, sufficient pressure remains in the tank, the tank valve is open, and the regulator is adjusted to about 30 psi.

2. Press the power button on the front of the Shimadzu TOC-$V_{CSH}$ Analyzer to power on the instrument.

3. Open the TOC-V Sample Table Editor software and click “OK”.

4. Start a new sample table by clicking “New” in the upper left toolbar. Select TOC and click OK.

5. Save the sample table by clicking “Save” in the upper left toolbar and save the file with a name that includes the date the run will be started.

6. Click “Connect” in the upper right hand toolbar. You will hear the instrument initialize and gas will flow to the instrument. Ensure the instrument is receiving gas by checking the rotameter near the power button on the front of the instrument.

7. Refill the rinse vessel and the dilution vessel with reagent water after triple rinsing with reagent water. Ensure the caps are securely replaced and the intake tubes are fully submerged.

8. Ensure the water line is within the proper range on the Moisturizer container inside the Shimadzu TOC-V. Open the front of the instrument by pulling the lever on the left side. If water needs to be added, add reagent water either by removing the Moisturizer container and filling directly or with the designated squirt bottle after triple rinsing and filling with fresh reagent water.

9. Check that both the HCL and $H_3PO_4$ containers have sufficient volume and are relatively fresh (see “Maintenance”).

10. Check that the screws under the top cover of the instrument are tight (open the front and then pull open the lid on top of the TOC-V).

## Starting a Run

1. Once you have standards and/or samples (see “Initial Instrument Calibration” and “Sample Analysis”) prepared in clean 40 ml glass amber vials (see “Sample Vials”), place them in the desired positions in the Autosampler rack, place the rack back on the Autosampler and secure the ASI cover (the rack will begin to rotate once the ASI cover is in place).

2. To add a sample, multiple samples, or a calibration curve to the sample table, click “Insert” on the top toolbar and select the appropriate option. You can also copy and paste lines in the sample table to add new samples.

3. For inserting a single sample:
    a. Select the appropriate method and click “Next”. Use LOW_npoc.met if sample concentrations are expected to fall in the 0-15ppm range. Select NPOC.met for samples up to 50 ppm.
    b. Enter the “Default Sample Name” and click “Next”.
    c. Make sure the correct calibration curve(s) is entered. For the LOW_npoc method, “Calibration Curve 1” should be C:\TOC3201\CalCurves\low_cal.cal. For the NPOC method, high_cal.cal should also be entered for “Calibration Curve 2”. Click “Next”.
    d. Select the desired “No. of Injections”. Default is 3 of 5. We use this setting for everything except the initial “blanks” sample, for which we select 7 of 7. Click “Next”.
    e. “Use default setting” should be selected. Click “Finish”.

4. For inserting multiple samples:
    a. Select the appropriate method and click “Next”. Use LOW_npoc.met if sample concentrations are expected to fall in the 0-15ppm range. Select NPOC.met for samples up to 50 ppm.
    b. Make sure the correct calibration curve(s) is entered. For the LOW_npoc method, “Calibration Curve 1” should be C:\TOC3201\CalCurves\low_cal.cal. For the NPOC method, high_cal.cal should also be entered for “Calibration Curve 2”. Click “Next”.
    c. Enter the “No. of Vials” to analyze and the “Start Vial” and click “Finish”.

5. For inserting a calibration curve:
    a. Browse for and select the desired calibration curve. For the LOW_npoc method, you need only enter the low_cal.cal curve. Click “Open”.
    b. If using the NPOC method, after entering the low_cal.cal curve, you must next enter the high_cal.cal curve.

6. Once all samples/standards are inserted into the sample table, ensure that the corresponding vial numbers are entered into the “Vial” column. You can change entries in this column by clicking the “View vial settings” button on the top right toolbar of the sample table, clicking into the desired cell, and typing the appropriate number.

7. Ensure that the proper names are entered in the “Sample Name” column. You can click directly into these cells in the sample table and type or scan in the desired name/ID.

8. Once your sample table is complete, start the run by clicking “Start” on the top right toolbar. Click “OK” and then select to either “Keep Running” or “Shut Down Instrument” once all the samples entered into the sample table have been analyzed. If you intend to analyze more samples later that day or have set up a full rack to run overnight and intend to set up more samples the next day, select “Keep Running”. Otherwise choose to shut down the instrument. Finally, click “Start”.

## Initial Instrument Calibration

1. Prepare and analyze initial “blanks” sample:
    a. Prepare a blank sample by filling a clean 40 ml glass amber vial with reagent water and secure the septa cap. Place the blank sample in position 1 of the Autosampler rack.
    b. Analyze this blank sample with the sample name “blanks”, setting 7/7 injections (see “Starting a Run”).
    c. After analysis is complete, ensure the average “blanks” concentration is low (<0.1 ppm) before moving on.

2. Prepare and analyze calibration curve(s):
    a. Set up a blank sample (BLANK) in position 1 with 5/5 injections. The initial “blanks” vial can be used again for this BLANK.
    b. Prepare calibration standards in the designated volumetric flasks, using the analytical standard and reagent water. Bring to volume with the designated squirt bottle after triple rinsing and filling the bottle with reagent water.
        i. For the LOW_npoc method, prepare analytical standard dilutions with concentrations of 1, 2.5, 5, 10, and 15 ppm.
        ii. For the NPOC method, prepare analytical standard dilutions with concentrations of 1, 2.5, 5, 10, and 100 ppm.
    c. Pour standards into analytical vials and secure the septa caps. Place the vials in ascending concentration order into positions 2-6 in the Autosampler rack.
    d. Insert the calibration curve(s) into the sample table (see “Starting a Run”). Note: if using the high_cal.cal curve (NPOC method), vial #6 (100 ppm) should be used for all points on that curve. The instrument dilutes the sample to the appropriate concentration.

2. Prepare and analyze QCS check standards:
    a. Prepare a blank sample.
    b. Prepare check standards in the designated volumetric flasks, using equal volumes of the quality control standard and the inorganic carbon standard. Bring to volume with the designated squirt bottle after triple rinsing and filling the bottle with reagent water.
        i. For the LOW_npoc method, prepare check standards with dissolved organic carbon concentrations of 1 and 10 ppm.

   ii. For the NPOC method, prepare check standards with dissolved organic carbon concentrations of 1 and 20 ppm.
c. Pour check standards into analytical vials and secure the septa caps.
d. Place the blank sample and check standards into the Autosampler rack in ascending order. If running immediately following a curve, the blank and check standards will be in positions 7-9. If a valid curve has previously been run and passed QA/QC, a blank and check standards must still be analyzed prior to analyzing samples, so should be run in positions 1-3.
e. Choose to insert multiple samples into the sample table (see "Starting a Run"), selecting 3 for the No. of Vials and setting the appropriate Start Vial. Change the Sample Name entries to "BLANK", "1 PPM CHECK", and "10 PPM CHECK", or "20 PPM CHECK", depending on the concentration of the QCS check standards.

3. Start the Analysis (see "Starting a Run").

4. Once the run is complete, ensure QA/QC protocols are met (see "Quality Assurance and Control Protocols").

## Sample Analysis

1. After ensuring instrument calibration (see "Initial Instrument Calibration"), select the samples to be analyzed. Samples should be poured into the appropriate sample vials for the Autosampler (see "Sample Vials"), if they haven't been stored in these vials. A full Autosampler rack will hold up to 59 samples and 3 sets of QCS blank and check standard vials.

2. Prepare QCS blank and check standard vials. A QCS blank and check standard set should be run after every 10 samples. Each set of checks may be used twice. Thus, 1 set of checks is needed for every 20 samples.

3. Choose to insert multiple samples into the sample table (see "Starting a Run") and enter the correct No. of Vials and Start Vial.

4. Scan the sample barcodes or type the sample name/ID into the appropriate cells in the "Sample Name" column as you place samples into the Autosampler rack.

5. After every 10 samples, copy and paste the first sample from the set to run as a duplicate and either enter a set of check standards or copy and paste the previous set of check standards, if it will have only been used once at that point (see "Quality Assurance and Control Protocols").

6. Set the vial numbers in the "Vial" column by clicking the "View vial settings" button on the top right toolbar of the sample table, clicking into the desired cell, and typing the appropriate number.

7. Place the Autosampler rack back on the Autosampler and secure the ASI cover (the rack will begin to rotate once the ASI cover is in place).

8. Start the analysis (see “Starting a Run”).

**Data Management**

1. When a run is complete, export the data by clicking File -> Ascii export -> Detail. Save as a text file with the date the run started as the name of the file.

2. Open the file in Excel, converting the format from tab delimited to columns. Save as an Excel file with the same name.

3. In a new tab, copy and paste the “Sample Name” and “Mean Conc.” Columns and remove duplicate data.

4. Calculate % recovery on the check standards by subtracting the previous blank concentration and dividing by the expected concentration. Calculate % differences for all of the duplicates. See “Quality Assurance and Control Protocols”.

# Quality Assurance and Control Protocols

Analytical Precision: One sample should be analyzed in duplicate for every 10 samples analyzed. The percent difference for duplicate samples should be less than 10%. In the case of failure, repeat the duplicate and bring to the attention of the quality assurance officer.

Instrumental Carryover: Instrumental carryover is assessed with instrument blank samples analyzed throughout the run. A blank sample consists of reagent water. Excessive instrument carryover (such that QCS check standards do not pass within 10% once blank values have been subtracted) indicates instrumental contamination with organic C.

Instrument Calibration: Calibrate the instrument with one 6-point standard curve, or two 4-point standard curves prior to sample analysis and conduct regular checks of instrument calibration throughout the run (QCS check standards). A standard curve should be created with masses of DOC that span that of the samples, and have an $R^2$ value greater than 0.995. A blank sample and two QCS check standards should be analyzed following every 10 samples, to verify instrument calibration, and have concentrations of 90 – 110% of its expected value (after subtracting the corresponding blank value). The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument. If a sample exceeds the instrument calibration, either the sample should be repeated at lower volume or the instrument recalibrated with the “high curve”. A new calibration curve should be analyzed prior to analyzing samples every 3-4 runs, or if check standards repeatedly fail. If a new calibration curve does not need to be analyzed at the beginning of the day,

a blank and QCS check standards should be analyzed prior to analyzing samples to check the instrument calibration.

# Maintenance

Regenerating the IC Solution:
Click Instrument -> Maintenance -> Regeneration of the IC Solution. This should be done prior to running a new calibration curve every time a new curve is created.

Regenerating the TC Catalyst:
Click Instrument -> Maintenance -> Regeneration of the TC Catalyst. This should be done every 1-2 weeks when in frequent use. It is also a good first troubleshooting step.

Changing the HCL Solution:
Perform a 1:4 dilution with concentrated HCl. Properly discard the old solution and add the new solution to the glass jar next to the instrument. Make sure the intake tube is fully immersed in the solution. This should be done whenever the volume is getting low or approximately every 6 months, while in use.

Changing the $H_3PO_4$ Solution:
Dilute ACS grade $H_3PO_4$ to a concentration of 17%. For 250 ml of solution, add 50 ml of 85% $H_3PO_4$ to 200 ml of reagent water. Properly discard the old solution and add the new solution to the glass jar next to the instrument. Make sure the intake tube is fully immersed in the solution. This should be done whenever the volume is getting low or approximately every 6 months, while in use.

Refilling the Combustion Tube with TOC standard Catalyst:
See the User Manual for instructions on removing and refilling the Combustion Tube with TOC Standard Catalyst to 100 mm. This should be done if low standard recoveries are observed or every few months, depending on the frequency of use.