Standard Operating Procedure for:

Dissolved Organic Carbon (DOC)

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1 Identification of the method

1.1 Measurement of DOC via high temperature combustion on a Shimadzu TOC-VCPH. (APHA 5310 B).

2 Applicable matrix or matrices

2.1 This method is suitable for the analysis of environmental samples.

3 Detection limit

3.1 Method Detection Limit: 0.2 mg/L.

3.2 This Method Detection Limit was determined by taking the calculated concentrations of the lowest calibration standard from multiple analytical runs (using seven or more individual values taken from at least three analytical runs) and calculating a standard deviation from those concentrations. This standard deviation is then multiplied by the one-sided t-statistic at the 99% confidence level for the appropriate degrees of freedom (n – 1).

4 Scope of the method

4.1 This standard operating procedure is intended to provide MU Limnology technicians and analysts with guidance on the analysis of DOC with the Shimadzu TOC-VCPH. This document is not intended to replace individual training in this method by an experienced MU Limnology technician.

5 Summary of the method

5.1 Environmental samples are analyzed for DOC by the removal of carbonate and bicarbonate through acidification and purging with purified gas. This results in the loss of volatile organic carbon or POC leaving DOC remaining. This remaining carbon is oxidized to CO2 and measured via non dispersive IR.

5.2

- Operating range: 0.2–3500 mg/L.
- Sample volume: 60 ml
- Sample injection volume: 350–20400 µL.
- Run Time: 12–17 hrs
- Samples per Run: 37 samples

6 Interferences

6.1 Sample acidification invalidates any inorganic carbon determination.

6.2 The removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile organic carbon.

7 Health and Safety

7.1 This method involves handling freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing hands frequently.

7.2 Wear protective gloves, lab coats, and other appropriate PPE when handling all chemical substances used in this method. All technicians performing this method should review the MSDS for additional information and safety concerns regarding the chemical substances used throughout these procedures.

7.3 Keep all loose clothing away from mechanical moving parts associated with the carousel and sample tubes.

7.4 The following chemicals used in this method are considered especially hazardous and should be handled with extra care:

- Sulfuric Acid

8 Personnel qualifications

8.1 This method is considered advanced. Lab personnel should be trained to the technician level in a number of other lab protocols before being trained in this method. They must also be familiar with all standard MU Limnology sample handling and labeling procedures and appropriate SOPs. There is no operator designation for this method. The lowest level of certification is as a technician.

8.2 New technicians learning to operate this method should perform 4 runs before being certified.

- Run 1: The trainee should watch an experienced technician carry out all parts of a run (including reagent preparation, data analysis, etc.).
- Run 2: The trainee should carry out a run with close supervision.
- Run 3: The trainee should carry out a run independently with occasional check-ins.
- Run 4: The trainee should carry out a run fully independently. An experienced technician should check the results of this run after its finished.
- If a trainee completes all 4 runs without significant issues (poor sample replication, bad calibration, drifting base lines), they may be certified as a technician.

9 Equipment and supplies

9.1 Shimadzu TOC-VCPH analyzer with TNM-1 and ASI-V autosampler.

9.2 Halogen scrubber (Shimadzu PN 630-00992)

9.3 Sulfate scrubber (Shimadzu PN 220-95268-01)

9.4 Membrane filter (Shimadzu PN 046-00042-12)

9.5 CO2 scrubber (Shimadzu PN 630-00999)
9.6 Combustion chamber regular catalyst (Shimadzu PN 638-60116)
9.7 Organic Carbon Standard 1000PPM C, RICCA, 1847-16
9.8 Syringe plunger (Shimadzu PN 638-59213-01)
9.9 Oxygen tank
9.10 Cling wrap
9.11 Sulfuric Acid
9.12 1000 ppm Organic Carbon Standard, Ricca, 1847-16

10 Reagents and standards

10.1 Calibration Standards

10.2 Prepare standards as shown below in Table 1 in clean volumetric flasks. All stock solutions should be added quantitatively using a calibrated micropipette. Rinse all glassware three times with UPDI and then fill ~75% of the way to the line. Then add appropriate stock solution, fill to the line with UPDI, cover with parafilm and invert three times to mix. Standards may be stored in plastic at 4 deg C for up to 100 hours after preparation.

10.3 Table 1: Nominal Concentration and Preparation of Ammonium Standards

Standard Concentration (mg C/L)	20	10	5	0
Total Standard Volume (ml)	100	100	100	100
Volume 1000 ppm Carbon Standard	2	1	0.5	0.1

10.4 Sulfuric acid

Sulfuric acid may be used as purchased. No further preparation is necessary.

11 Quality Control

11.1 Check Standards

For every 16 samples (64 measurements), a full range of check standards, identical to the calibration standards, must be run.

12 Sample analysis

12.1 Set up

- Place samples in a room temperature water bath until completely thawed.
- Samples should be acidified (pH < 3) with 100 mL of 1 M H_2SO_4 per 60 ml of sample to convert inorganic carbon to CO2 gas. Mark the lids with a red line to show that sample has been acidified. When done, rinse the pipette tip with tap water before putting it in an appropriate disposal box.
- After acidification, samples are air sparged for 7.5 minutes to remove the inorganic carbon. Each sample is measured in two pre-combusted vials, and each of the two vials are measured twice (quadruplicate).

12.2 Shimadzu instrument operation:

- Turn on Shimadzu (button on lower right corner of machine front, not the green on/off button), turn on computer, and open the valve on the oxygen tank all the way then rotate back a turn or so. In order to do a full overnight run, the tank pressure (gage on the right) should be above 500 psi. If it is not, replace the oxygen tank.
- Open the TOC table editor
- Open the appropriate file. For a full carousel of DOC run overnight, “Limno NPOC Full 7.5” can be used.
- Select “save as” and rename the file so the template is not overwritten using the File name convention.
- Click the button that says “connect” to connect the computer program to the Shimadzu. While the machine is connecting do not remove the carousel cover. This will result in an alarm that cannot be bypassed. If this occurs, close down the program and start over.
- After the connection has been made, the carousel can be removed for filling.
- The Shimadzu takes 30 minutes to an hour to warm up. If after an hour it is not ready (signified by the icon in the top right corner of the screen), get help from an analyst.

12.3 Prepping the Samples/Filling the Carousel

- Calibration Standards should be acidified (pH < 3) with 100 mL of 1 M H_2SO_4 per 60 ml.

- Fill the carousel as follows for a typical run. Sample vials need to be filled at least half full, Calibration Standards can be filled to the shoulder of the vial if doing two consecutive runs. The first two slots in the carousel, the last slot, and slots 31, 55, 73, 86, and 93 are zero Calibration Standards made with UPDI. Slots 3 thru 6, 40 thru 43 and 89 thru 92 are filled with the Calibration Standards from high to low, as indicated on the spreadsheet open on the Shimadzu computer or the template for entering the results.
- Fill the sample vials, two per sample, in a spiral fashion following the slot numbers. Record the project and sample ID (usually bottle numbers) in the appropriate spots on the Shimadzu spreadsheet and set each sample vial to be read twice. This should result in 4 total measurements per sample (2 measurements of two sample vials).
- Once the carousel has been filled, cover it with plastic cling wrap and tuck in the edges to make sure they won't catch in the autosampler as it rotates. Be sure that the notch on the carousel is not covered by the plastic cling wrap. If it is, the sensor will not be able to find slot one which will cause an error.

12.4 Running the Shimadzu

- Place the carousel back on the Shimadzu and replace the cover.
- Fill the large bottle to the left of the autosampler with DI. This is the sipper rinse water.
- Verify the unwanted materials container below the computer is at least one fourth empty for an overnight run.
- Open the front door to the Shimadzu and verify that the IC chamber is about 1/3 full and bubbling.
- Verify the humidifier level is between the lines.
- Verify the drain vessel is filled to near the line and is bubbling gently. No bubbles may indicate a leak, rapid bubbling may indicate a plug.
- Verify that the oxygen pressure on the Shimadzu is 150 psi.
- Verify TNM is on even if it is not being used.
- Verify the TNM oxygen pressure is at 50 psi.
- Click the start button on the monitor. This will bring up a new window. Select the appropriate option, keep running if another run will be done the next day, shut down if not, and start the run.
- Watch to make sure that the carousel is turning and not catching. Verify the rinse containers inside the autosampler fill with water. Verify the right-hand needle bubbles while in a sample.
- It's a good idea to return after a half hour or so to verify all is well and the curve for the first high standard is typical.
- If the Shimadzu errors during the run it will make a very loud beeping sound. If this happens, get help from an analyst.

- If the machine behaves oddly (no peaks, won't get ready, etc.) get help from an analyst.

12.5 Finishing the Run

- If one run has finished and another is to be begun, remove the data for the completed run as indicated below, verify there are no reruns, then set up the next run as indicated above.
- NEVER turn off the Shimadzu by pushing the on/off switch on the machine. It must go through a controlled cool down process to avoid damage.
- After all samples have been run and the Shimadzu has initiated a controlled cool down, completely shut down the Shimadzu.
- Turn off the oxygen at the tank.
- While the table editor is still open, export the data to a flash drive. Select export detail.
- Turn off the computer.
- Enter the data into the electronic data sheet before dumping vials in case water needs to be saved for reruns.
- Dump any liquid remaining in the vials into the acid water unwanted materials container.
- Rinse vials 3x with tap water and 3x with DI water. After drying inverted in a drying oven at least overnight, wrap the vials in aluminum foil and ash in the muffle furnace for at least an hour once it reaches temp (550 °C). After the vials have cooled, keep them in the foil and return them to the drawer.

13 Data acquisition, calculations, and reporting

13.1 Transferring Data to Excel Sheet

- Insert thumb drive with the exported data.
- Open the appropriate template.
- Import the data from the thumb drive to a raw data sheet.
- Copy the area column to the calculations page. Include only rows for which the "excluded" value is zero.

14 Computer hardware and software

14.1 Software TOC-Control V on Windows 7 computer.

15 Method performance

15.1 Desired Performance Criteria

- Method Detection Limit: 0.2 mg/L
- Precision: Sample CV < 5 % or sample range < 0.2 mg/L
- Calibration $r^2 > 0.99$

15.2 Maintenance

- Maintenance must be performed by an MU Limnology analyst.
- Refer to the owner's manual for more detailed instructions.
- Replace all parts at the same time. Write date of replacement on the new part. Record maintenance and repairs in the maintenance log.
- These maintenance procedures should be performed annually, or sooner if needed"
- Replace halogen scrubber, Shimadzu PN 630-00992. Replace early if copper windings start turning black.
- Replace sulfate scrubber, Shimadzu PN 220-95268-01. Replace early if media becomes discolored.
- Replace membrane filter, Shimadzu PN 046-00042-12. Note that this part number has changed since the owner's manual was printed.
- Replace CO2 scrubber, Shimadzu PN 630-00999.
- Repack the combustion chamber. For DOC use regular catalyst, Shimadzu PN 638-60116. See the manual for instructions on proper packing of the column, section 3.1.1.2.
- Inspect the syringe plunger for leakage. If any is noted, replace the plunger tip, Shimadzu PN 638-59213-01.
- The TNM also requires maintenance, but this is performed less frequently. See the manual.

16 Data assessment, acceptable criteria for quality control measures, and corrective actions for out-of-control or unacceptable data

16.1 Excel Cell References and Data Entry

Poor cell references or improperly entered data will lead to erroneous results. If a problem is noticed with a run, the data entry and the cell references should first be checked by a supervisor and pointed out to a technician if found.

16.2 Field Blank and Field Duplicates

When run, field duplicates should not vary in determined concentrations by more than 10%. Determined concentrations of field blanks should not exceed the method detection limit.

16.3 r^2 Value

The mandatory r^2 for valid results is a minimum of 0.99. In the event that a technician proceeds with a run which has a calibration r^2 less than 0.99, all samples from this rerun should be rerun.

16.4 Check Standard QC standards

Check standards must meet at least one of the following QC criteria:

- The CV of standard's concentration and its known concentration is less than 5

- The absolute difference of the standard's concentration and its known concentration is less than 0.2

If neither of these criteria are met the check standards are not considered valid.

16.5 Sample QC Standards

Quadruplicate replicates of each sample must meet at least one of the following QC criteria:

- The CV of standard's concentration and its known concentration is less than 5 %
- The absolute difference of the standard's concentration and its known concentration is less than 0.2 mg/L

If neither of these criteria are met the result is not considered valid and should be rerun.

17 Waste management

17.1 All waste generated is considered hazardous.

17.2 All analyzed standards, and reagents should be treated as waste upon completion of the run.

17.3 Waste should be kept in an approved container with proper labeling. Waste will not be held for longer than 6 months and MU Environmental Health and Safety (EHS) will be notified an appropriate time before this point so that waste can be collected and disposed of.

18 References

18.1 Standard Methods for the Examination of Water and Wastewater, 23rd Edition. 2017. Method 5310 B. American Public Health Association. Washington, DC.