Carbon Analysis – Inorganic and Organic Carbon

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Purpose: This procedure describes the steps to analyze total and dissolved inorganic and non-purgeable organic carbon in water. The units of total and dissolved carbon for this analysis are milligrams per liter of C.

Sample Holding Time:
≤ 28 days @ 4° C unpreserved

Materials Required:

- TOC-L Shimadzu Carbon Analyzer
- MilliQ water
- Dilute (0.5%) Hydrochloric Acid
- 25% Phosphoric Acid
- 1N Hydrochloric Acid
- Analytical balance
- Certified inorganic carbon stock solution
- Certified organic carbon stock solution
- Ultra Zero Compressed Air tank
- Glassware (when preparing standards and ICVs):
  - 4 x 100 mL volumetric flask
  - 3 x 50 mL volumetric flask
  - Ground glass stoppers for flasks or parafilm
  - 0.1-1 mL micropipette
  - 1-5 mL micropipette
  - Fisherbrand Class B clear glass threaded vials, 23 mm diameter, 85mm length, 20-400 thread size

Glassware Preparation: The Fisherbrand Class B clear glass threaded vials used in this method may either be acid washed (Refer to SOP "Acid washing") or rinsed with deionized water and muffled in a furnace at 500°C for 4.5 hours (Refer to SOP "Muffling Glassware"). The remaining glassware used in this method is washed in a 10% Hydrochloric Acid Bath, preceded, and followed by a series of MilliQ water rinses. (Refer to SOP "Acid washing").

Personal Protective Equipment / Waste Disposal: Nitrile gloves and a laboratory coat are always required during this procedure. This is not only for your protection, but also to prevent contamination of samples. Proper personal protective equipment is always required for safety and contamination prevention. Always use chemical resistant gloves (not latex), safety glasses, lab coat, and a fume hood while using concentrated acids; a lab coat and gloves are required at all other times during this procedure.

Quality Assurance/Quality Control:
Instrument runs are bookended with the following quality control samples:

- Reagent blank, plain MilliQ water
- Check standard
- The lowest concentration standard
- Calibration Verification (CV), standard(s) prepared from a different stock material than the calibration curve

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After each set of 10 samples, a duplicate sample, a blank, check standard, and calibration verification standard are run to monitor for drift or carryover. If the duplicate samples concentrations are greater than the laboratory reporting level (LRL) and each concentration is within 20% of the Relative Standard Deviation (RSD), the concentration of the analyte is reported as an average of the values.

Waste Disposal: Most of the reagent solutions used in this procedure can go down the drain; however the pH should be near neutral (pH 5 – 8). Flush during and after disposal by running tap water. Excess dry reagents from preparing the stock can go in the trash.

Consumable Ordering

Item	Catalog #	Item	Catalog #
Borosilicate vials	Fisher 03-338H	Inorganic Carbon Standard 1000ppm C	Fisher 18454
Caps	Fisher 03-392C	Inorganic Carbon Std 10.0 ppm	VWR 101224-466 (EA)
Septa	Fisher 03-394C	Inorganic Carbon Std 20.0 ppm	VWR 101224-468 (EA)
Air Ultra Zero Size 300 CGA 590	Air Gas AI UZ300	Organic Carbon Std 1000 ppm	VWR RC1847-4 (EA)
Optima HCl (500 mL)	Fisher A466500	Total Organic Carbon 10.0 ppm 500 mL	VWR 101226-832 (EA)
o-Phosphoric Acid; 85.0 w/w %	Fisher A242212	Total Organic Carbon 20.0 ppm 500 mL	VWR 101223-156 (EA)

Autosampler and Instrument Preparation

1.0 Double check the compressed air tank is open at the top of the tank. The tank is located next to the door in the WSEL lab. The left gauge should display ~300 psi. The right gauge displays how much air remains in the tank. Replace tank once below 400 psi to prevent impurities from entering system.

2.0 Turn on the instrument - button on front. If front button is dark/unresponsive- turn on main power on the back right side

3.0 Connect to the Shimadzu TOC Analyzer through the TOC-L software by creating a sample table:

3.1 Click on the ***TOC-L Sample Table Editor***

3.2 Click on ***Sample Table Editor*** and Click ***OK*** when asked for user name

3.3 Click ***New***. A pop-up window should show TOC-L CPH for the system and Normal as Table Type. Click ***OK.***

3.4 Click on ***Save, File*** and name the file without using spaces or slashes (ex: LTERdate.t32)

3.5 Click ***Connect*** to connect to the instrument.

4.0 If machine has been idle for > 1week perform following maintenance steps

4.1 Select Instrument, Maintenance, Mechanical Check.

4.2 Select Instrument, Maintenance, Replace Flowline Content.

4.3 Under Instrument, Maintenance, select Regenerate IC Solution

5.0 Verify a stable baseline by clicking the ***Monitor*** button on the top ribbon. 2

5.1 Click the x50 button to zoom in, and then wait to verify a stable baseline.

5.2 The instrument is ready to run when the baseline is stable between -6 and 6 mV and all six statements in the Monitor screen are shown with green check marks.

NOTE: The baseline can take multiple hours to stabilize. Plan to turn on instrument and connect to software prior to preparing standards and QC solutions.

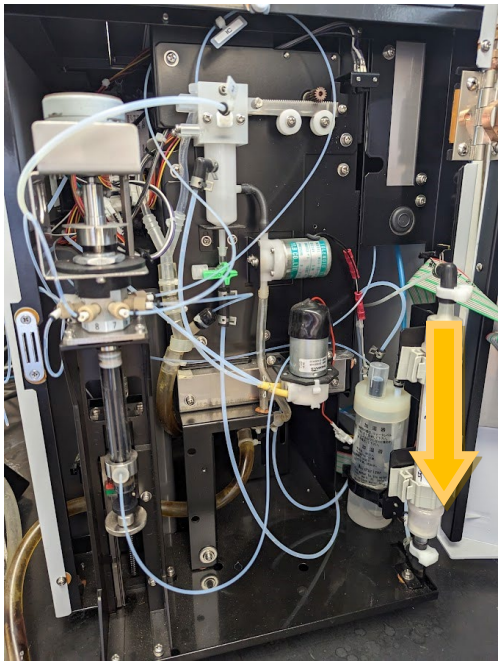
6.0 While waiting for instrument to stabilize

6.1 Replace and refill autosampler rinse reservoir (2L MQ). This is the large jug on the counter to the left of the sample tray. This should be dumped and refilled before every run.

6.2 Check waste container on the floor and empty into the sink if over half full.

6.3 Check reagent levels on the left side of the instrument including the: Diluent reservoir, HCl, and H_3PO_4

6.4 Check Water (MQ) level in humidifier. To do this, open front door of the instrument and check that the water level is to the mark in the bottle located in bottom right corner labeled "moisturizer"



7.0 Generating the sample table order: See Sample Table Guidelines on page 8

7.1 Open the most recent carbon run.

7.2 Highlight the entire sample table, including the far-left column of row numbers but NOT the first row of headers.

7.3 Right click and choose copy.

7.4 Go back to your new sample table, created in step 1.3. Click on row number 1 in the far-left column and Ctrl-V to paste run table from last run.

7.5 Delete sample IDs and Duplicates from sample table.

7.6 Repopulate table by clicking on a cell and then scan the sample vial barcode. Use the down arrow to go to the next cell and scan the next sample vial barcode until all four sets are populated with sample IDs. Copy and paste each set from the inorganic region of the sample table to the organic region of the sample table.

7.7 **NOTE: *YOU MUST CHANGE THE DUPLICATE SAMPLE AND VIAL POSTION FOR THE ORGANIC CARBON ANALYSIS.*** The vials don't have enough volume to be run for Inorganic carbon, organic carbon, AND two duplicates.

8.0 Make calibration and quality control standards and blanks as described in **Section 4.0** of this document**.**

8.1 Fill the sample rack with samples, standards, and blanks according to the "Sample Rack Layout" Table on page 8.

8.2 Assign each row in the Sample Table a position using the **Vial Settings** button in the top right corner. This icon looks like a birthday cake.

8.2.1 Click the first row in the **Vial** column.

8.2.2 Using the order from the "**Sample Run Set Up**" Table on page 10 of this document, assign each sample a vial for the machine to pull from by typing the Vial number into the cell. This order should generally be the same for each run, so the table can normally be transcribed directly.

NOTE: If the tray has been set up in a different order than described in the "Sample Rack Layout" table or duplicates selected above do not correspond with the same samples described in the "Sample Run Set Up" table, make sure to also change the assigned Vial # in this step.

8.2.3 As you assign each sample a vial number, the corresponding position in the autosampler diagram on the right should turn blue.

8.2.4 Once each sample has been assigned a vial, click OK.

9.0 To start the run:

9.1 Check to ensure the instrument has reached a stable baseline, the combustion furnace has warmed up to 680 degrees C, and all six statements in the Monitor pane are shown with a green check mark.

9.2 Analysis per sample takes ~8 minutes, samples will have time to warm while MQ and Cal curve is analyzed.

9.3 Double check tray/vial settings to make sure it matches the actual rack.

9.4 Press Start to start run. Select Standby to leave the instrument running after your analysis is complete or Shutdown.

NOTE: Four sets of samples run for both inorganic and organic carbon with QC and two calibration curves run for roughly 27 hours.

Exporting Data and Clean-up

10.0 Data Export

10.1 Before data is exported, check to make sure that the calibration curves' R^2 values are ≥ 0.995.

10.2 Go to- File → ACSII Export-Normal onto flash drive or file server. This will save as a text file.

10.3 Press the print button and "Print to PDF" the entire sample table.

10.4 On a computer with printer access, open text file using Excel. Print file and enter LTER data into chemlab3 and email non LTER data to appropriate end user.

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11.0 Clean-up

11.1 After the Instrument is finished, remove the autosampler cassette.

11.2 Remove the samples, set aside in racks to have labels removed, sample poured out, DI rinsed, and muffled.

11.3 Remove glass vials holding standards and QC solutions. Rinse with MQ three times and set upside down to dry on wypall lined rack.

11.4 Return empty autosampler cassette and secure the lid.

Preparing Reagents

Note: The reagents for carbon analysis do not need to be changed frequently.

Stock Solutions	Mass/Volume	Volume Water	Vessel	Storage
Optima grade, Concentrated Hydrochloric Acid (HCl)	26.4 mL Hydrochloric Acid	Fill to mark with MilliQ	250 mL volumetric flask	25°C, 1 year
Conc. (85%) Phosphoric Acid (H_3PO_4)	73.5 mL Phosphoric Acid	Fill to mark with MilliQ	250 mL Volumetric flask	25°C, 1 year
Optima grade, Concentrated Hydrochloric Acid (HCl)	1 mL Hydrochloric Acid	Fill to mark with MilliQ	1000 mL Volumetric flask	25°C remake as needed

1.0 1N Hydrochloric Acid solution (CORROSIVE) – store in bottle connected to instrument, remake as needed

1.1 Obtain a 250 mL volumetric flask. Label the flask with contents (Hydrochloric Acid mixture), date, and initials.

1.2 Fill the flask partially with MilliQ water. ***In fume hood wearing proper PPE***, using a graduated cylinder, measure 26.4 mL of concentrated HCl and pour slowly into the volumetric flask with MilliQ water. Carefully swirl the flask to mix. **This reaction produces heat**. Allow flask to cool in fume hood. Fill to mark with MilliQ water and cap with ground glass stopper. Invert slowly to mix. Store in HDPE bottle, labelled with contents, date, and initials.

2.0 25% Phosphoric Acid (CORROSIVE) – store in bottled connected to instrument, remake as needed

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2.1 Obtain 250 mL volumetric flask. Label the flask with contents (Phosphoric Acid solution), date, and initials.

2.2 Fill the flask partially with MilliQ water. ***In fume hood wearing proper PPE***, using a graduated cylinder, measure 73.5 mL of concentrated H_3PO_4 and pour slowly into the volumetric flask with MilliQ water. Carefully swirl the flask to mix. **This reaction produces heat**. Allow flask to cool in fume hood. Fill to mark with MilliQ water and cap with ground glass stopper. Invert slowly to mix. Store in HDPE bottle, labelled with contents, date, and initials.

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Preparation of Calibration and Quality Control Standards

The Shimadzu TOC-L carbon analyzer auto-dilutes standards, therefore a low and high concentration standard are prepared for organic and inorganic carbon. QC standards (independent calibration verification) are diluted from a purchased stock solution to attain the appropriate concentration. For this analysis, the following standard solutions are prepared (note that the color corresponds to the tape on the stock solution):

Standard or ICV	Stock Solutions	Volume of Stock Solution	Volume Water	Flask Size	Number of Vials
Blank	MilliQ water	(none, fill vial with MilliQ directly)			15
2 ppm IC std	Aqua Solutions 100 ppm Inorganic Carbon Stock Solution	2 mL IC Stock Solution	Fill to mark with MilliQ	100 mL volumetric flask	3
100 ppm IC std	Aqua Solutions 100 ppm Inorganic Carbon Stock Solution	(none, pipette directly into carbon vial)			1
2 ppm OC std	RICCA 100 ppm Organic Carbon Stock Solution	2 mL OC Stock Solution	Fill to mark with MilliQ	100 mL Volumetric flask	3
100 ppm OC std	RICCA 100 ppm Organic Carbon Stock Solution	(none, pipette directly into carbon vial)			1
10 ppm IC ICV	RICCA 1000 ppm Inorganic Carbon Stock Solution	0.5 mL IC Stock Solution	Fill to mark with MilliQ	50 mL volumetric flask	1
20 ppm IC ICV	RICCA 1000 ppm Inorganic Carbon Stock Solution	2 mL IC Stock Solution	Fill to mark with MilliQ	100 mL Volumetric flask	2
5 ppm OC ICV	Aqua Solutions 100 ppm Organic Carbon Stock Solution	2.5 mL OC Stock Solution	Fill to mark with MilliQ	50 mL Volumetric flask	1
20 ppm OC ICV	Aqua Solutions 100 ppm Organic Carbon Stock Solution	20 mL OC Stock Solution	Fill to mark with MilliQ	100 mL Volumetric flask	2

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- **Low Inorganic Carbon Standard:** Pipet 2 mL of 100 ppm Aqua Solutions Inorganic Carbon standard into a 100 mL volumetric flask and diluted to the mark with MilliQ water. Stopper and invert several times to ensure good mixing. Do not over mix, to prevent IC from leaving solution as CO_2. Standard should be prepared directly before analysis. Pour prepared standard into 3 clean, labelled carbon vials and seal with cap and septa with no headspace.
- **High Inorganic Standard:** Fill one clean, labelled carbon vial with 100 ppm Aqua Solutions Inorganic Carbon Stock Solution and seal with cap and septa with no headspace.
- **Low Organic Carbon Standard:** Pipet 2 mL of RICCA 100 ppm organic carbon stock solution into a 100 mL volumetric flask and diluting to the mark with MilliQ water. Stopper and invert several times to ensure good mixing. Standard should be prepared directly before analysis. Pour prepared standard into 3 clean, labelled carbon vial and seal with cap and septa with no headspace.
- **High Organic Carbon Standard:** Fill one clean, labelled carbon vial with 100 ppm RICCA Organic Carbon Stock Solution and seal with cap and septa with no headspace.
- **10 ppm IC ICV:** Pipet 0.5 mL of 1000 ppm RICCA Inorganic Carbon standard into a 50 mL volumetric flask and diluted to the mark with MilliQ water. Stopper and invert several times to ensure good mixing. Do not over mix, to prevent IC from leaving solution as CO_2. Standard should be prepared directly before analysis. Pour prepared standard into 1 clean, labelled carbon vial and seal with cap and septa with no headspace.
- **20 ppm IC ICV:** Pipet 2 mL of 1000 ppm RICCA Inorganic Carbon standard into a 50 mL volumetric flask and diluted to the mark with Milli
- Q water. Stopper and invert several times to ensure good mixing. Do not over mix, to prevent IC from leaving solution as CO_2. Standard should be prepared directly before analysis. Pour prepared standard into 2 clean, labelled carbon vials and seal with cap and septa with no headspace.
- **5 ppm OC ICV:** Pipet 2.5 mL of 100 ppm Aqua Solutions Organic Carbon standard into a 50 mL volumetric flask and diluted to the mark with MilliQ water. Stopper and invert several times to ensure good mixing. Standard should be prepared directly before analysis. Pour prepared standard into 1 clean, labelled carbon vial and seal with cap and septa with no headspace.
- **20 ppm OC ICV:** Pipet 20 mL of 100 ppm Aqua Solutions Organic Carbon standard into a 100 mL volumetric flask and diluted to the mark with MilliQ water. Stopper and invert several times to ensure good mixing. Standard should be prepared directly before analysis. Pour prepared standard into 2 clean, labelled carbon vials and seal with cap and septa with no headspace.
- **Reagent Blanks:** Fill clean, labelled carbon vials with MilliQ water. Reagent blanks are run to flush the lines and monitor carryover or issues with the baseline. Blanks are run before and after calibration curves, after each set, and at the end of the method.

Sample Rack Layout

Sample Name	# Vials	Autosampler Position
Sample Set 1 (Samples 1-10)	10	9-18
Sample Set 2 (Samples 11-20)	10	21-30
Sample Set 3 (Samples 21-30)	10	32-41
Sample Set 4 (Samples 31-40)	10	44-53
Blanks	15	1, 2, 3, 4, 19, 31, 42, 54, 56, 57, 62, 64, 65, 67, 69
2 ppm IC Standard	3	5,20,55
100 ppm IC Standard	1	6
10 ppm ICV IC Standard	1	7
20 ppm ICV IC Standard	2	8,43
2 ppm OC Standard	3	58,63,68
100 ppm OC Standard	1	59
5 ppm ICV OC Standard	1	60
20 ppm ICV OC Standard	2	61,66

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Method	Sample Name	Vial
IC	MQ	1
IC	MQ	2
IC	MQ	3
IC	Calibration 0 mg/L	4
IC	Calibration 0.25 mg/L	5
IC	Calibration 0.5 mg/L	5
IC	Calibration 1.0 mg/L	5
IC	Calibration 2.0 mg/L	5
IC	Calibration 5 mg/L	6
IC	Calibration 10 mg/L	6
IC	Calibration 15 mg/L	6
IC	Calibration 25 mg/L	6
IC	Calibration 35 mg/L	6
IC	Calibration 50 mg/L	6
IC	2 ppm Check DIC	5
IC	10 ppm DIC ICV	7
IC	20 ppm DIC ICV	8
IC	Blank	4
IC	Sample 1	9
IC	Sample 2	10
IC	Sample 3	11
IC	Sample 4	12
IC	Sample 5	13
IC	Sample 6	14
IC	Sample 7	15
IC	Sample 8	16
IC	Sample 9	17
IC	Sample 10	18
IC	Sample 10 Duplicate	18
IC	Blank	19
IC	Blank	19
IC	2 ppm check DIC	20
IC	20 ppm DIC ICV	8
IC	Sample 11	21
IC	Sample 12	22
IC	Sample 13	23
IC	Sample 14	24
IC	Sample 15	25
IC	Sample 16	26
IC	Sample 17	27

Method	Sample Name	Vial
IC	Sample 18	28
IC	Sample 19	29
IC	Sample 20	30
IC	Sample 20 Duplicate	30
IC	Blank	31
IC	Blank	31
IC	2ppm check DIC	20
IC	20 ppm DIC ICV	8
IC	Sample 21	32
IC	Sample 22	33
IC	Sample 23	34
IC	Sample 24	35
IC	Sample 25	36
IC	Sample 26	37
IC	Sample 27	38
IC	Sample 28	39
IC	Sample 29	40
IC	Sample 30	41
IC	Sample 30 Duplicate	41
IC	Blank	42
IC	Blank	42
IC	2 ppm check DIC	20
IC	20 ppm DIC ICV	43
IC	Sample 31	44
IC	Sample 32	45
IC	Sample 33	46
IC	Sample 34	47
IC	Sample 35	48
IC	Sample 36	49
IC	Sample 37	50
IC	Sample 38	51
IC	Sample 39	52
IC	Sample 40	53
IC	Sample 40 Duplicate	53
IC	Blank	54
IC	Blank	54
IC	2 ppm check DIC	55
IC	20 ppm DIC ICV	43

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Method	Sample Name	Vial
NPOC	Blank	56
NPOC	Blank	56
NPOC	Calibration 0 mg/L	57
NPOC	Calibration 0.5 mg/L	58
NPOC	Calibration 1.0 mg/L	58
NPOC	Calibration 2.0 mg/L	58
NPOC	Calibration 5 mg/L	59
NPOC	Calibration 10 mg/L	59
NPOC	Calibration 15 mg/L	59
NPOC	Calibration 25 mg/L	59
NPOC	Calibration 40 mg/L	59
NPOC	Calibration 50 mg/L	59
NPOC	2 ppm Check DOC	58
NPOC	5 ppm DOC ICV	60
NPOC	20 ppm DOC ICV	61
NPOC	Blank	57
NPOC	Sample 1	9
NPOC	Sample 2	10
NPOC	Sample 3	11
NPOC	Sample 4	12
NPOC	Sample 5	13
NPOC	Sample 6	14
NPOC	Sample 7	15
NPOC	Sample 8	16
NPOC	Sample 9	17
NPOC	Sample 10	18
NPOC	Sample 9 Duplicate	17
NPOC	Blank	62
NPOC	Blank	62
NPOC	2 ppm check DOC	63
NPOC	20 ppm DOC ICV	61
NPOC	Sample 11	21
NPOC	Sample 12	22
NPOC	Sample 13	23
NPOC	Sample 14	24
NPOC	Sample 15	25
NPOC	Sample 16	26
NPOC	Sample 17	27
NPOC	Sample 18	28
NPOC	Sample 19	29
NPOC	Sample 20	30
NPOC	Sample 19 Duplicate	29

Method	Sample Name	Vial
NPOC	Blank	64
NPOC	Blank	64
NPOC	2ppm check DOC	63
NPOC	20 ppm DOC ICV	61
NPOC	Sample 21	32
NPOC	Sample 22	33
NPOC	Sample 23	34
NPOC	Sample 24	35
NPOC	Sample 25	36
NPOC	Sample 26	37
NPOC	Sample 27	38
NPOC	Sample 28	39
NPOC	Sample 29	40
NPOC	Sample 30	41
NPOC	Sample 29 Duplicate	40
NPOC	Blank	65
NPOC	Blank	65
NPOC	2 ppm check DOC	63
NPOC	20 ppm DOC ICV	66
NPOC	Sample 31	44
NPOC	Sample 32	45
NPOC	Sample 33	46
NPOC	Sample 34	47
NPOC	Sample 35	48
NPOC	Sample 36	49
NPOC	Sample 37	50
NPOC	Sample 38	51
NPOC	Sample 39	52
NPOC	Sample 40	53
NPOC	Sample 39 Duplicate	52
NPOC	Blank	67
NPOC	Blank	67
NPOC	2pmm check DOC	68
NPOC	20 ppm DOC ICV	66
NPOC	Blank	69
NPOC	Blank	69

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