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Standard Operating Procedures for Shimadzu TOC- Analyzer
MCRL- MSL-5 219

1. Purpose

Describe the procedure for operating the Shimadzu TOC-L Analyzer for NPOC/TN & DIC analysis at MCRL Sequim in MSL5 219. Document protocol to help streamline TOC workflow processes across PNNL campuses.

Carbon is measured by the TOC by combusting organic carbon in an oxygen rich environment to form CO2 which is then measured with a non-dispersive infrared (NDIR) detector. Inorganic carbon is measured by converting inorganic carbon to CO2 with phosphoric acid which is then also measured via NDIR. Carbon concentrations in samples are compared to a standard curve.

Nitrogen is measured by the TOC-L by combusting he sample to create nitrogen oxides which are then oxidized with ozone to nitrogen dioxide and detected with a chemiluminescence detector (light emission intensity). Nitrogen concentrations in samples are compared to a standard curve.

2. Scope

This procedure was written for the TOC Analyzer located in 219 MSL5 used for SBR campaigns. Specifically for dual NPOC/TN analyses with 40mL volumes. NPOC/TN procedures start on page 2. **DIC procedures start on page 11.**

3. Reagents & Standards

4.1 Purity of water – unless otherwise indicated, references to water will mean reagent water run through a MiliQ system (18.2 MΩ.cm (at 25°C) and a TOC value below 5 ppb). Freshly prepared water should be used when making standards for calibration and to refill the TOC reservoir before each run. If MiliQ is not available (i.e., at SERC) use DI water for preparations.

4.3 KHP primary standard for inorganic carbon (1000ppm stocks need to be remade every 6 months if kept refrigerated)

4.4 KNO3 primary standard for inorganic nitrogen (1000ppm stocks need to be remade every 6 months if kept refrigerated)

4.5 Hydrochloric acid (0.05 M & 1:12 M) for inside TOC machine & reservoir on side of instrument

4.6 Phosphoric acid (18%) in reservoir on side of instrument (50mL 85% ACS acid in 250 mL DI H2O)

4.7 Blanks – conditions blanks & instrument blanks are used with the reagent water (MilliQ) as described in 4.1

4. Sample Collection, Preservation, and Storage

5.1 Aqueous samples for NPOC/TN should be filtered at 0.2um or 0.45um and refrigerated within 1-2 days after being collected in the field. The sample should be stored in a container that has been adequately cleaned – acid washed and combusted (500C for 4 hours+) or a new EPA clean vial.

5.2 The holding time for refrigerated NPOC/TN samples is approximately 7 days. These samples should be analyzed within a week of being filtered.

5.3 The holding time for frozen NPOC/TN samples is 28 days. (According to UM Chesapeake Bay protocols "*It has been shown that frozen Quality Control samples up to a year old still fall well within the control limits, therefore if the frozen sample is stored for longer than the holding time, there is minimal degradation."*)

5.4 Sample containers should be filled to the brim with no head space if refrigerated*. If frozen, enough space for expansion should be left at the top of the container to prevent breakage. *If being stored in 40 mL amber glass sample vials and refrigerated, no more than 30mL of volume should be put into the vial to prevent splashing in the instrument. No more than 25 mL of volume should be filled if being frozen

5. Procedures (for a NPOC/TN run)

Preparing the TOC-L (daily checks)

1. Turn the compressed air tank on & make sure the regulator is set around 300kpa . Replace the tank when the pressure reads below 500 psi.
2. Push the button located on the front of the instrument to turn it on. (if nothing happens, switch the on/off switch on the right side of the instrument to on). The front indicator light will cycle through colors and then appear orange (not-ready state). The indicator light is green when the instrument is up to temperature & all parameters are OK. The light will be blue while it is running samples. If the indicator light is red, refer to the software and manual to determine the problem.

3. Open the software by clicking on the TOC-L sample table icon in the center of the desktop. There is no password, just hint enter when the screen appears. Entering your initials is optional.
4. Open a previous sample table (by clicking on File, Open, Sample Table and navigating to the appropriate project folder) Then hit CONNECT located in the toolbar above the sample table to the right. A sample table needs to be open to connect the instrument. The furnace automatically turns on. The correct sample table will be connected to the instrument before it begins the run.
5. Refill the rinse water container with fresh MilliQ/DI water (located behind the autosampler). Carefully rest the cap with thin plastic tubing carefully into a clean beaker (which has had contact with nothing else). Deposit old rinse water down the drain & refill with fresh MilliQ/DI all the way up to the rim. If the tube touched any surfaces or gloves during this process, get a clean chem wipe, squirt it with Milli Q water and carefully wipe the outside of the tube below the cap. Once the tubing and cap is screwed back on tightly, make sure the tip of the tube is resting close to the bottom of the container. (this is especially important for large sample runs...don't want the tube to be exposed to any air as the water level lowers during the run.)
6. Refill dilution water (the large reservoir nested on the direct left side of the instrument) at least once every week or two. Check if the water level is getting low and top-off before a run.
7. 1:12 HCl is used in NPOC analysis (not TN). Check the volume of the acid bottles in use. This volume should be just below the arm. Usually this is plenty for several weeks of analysis. See manual for making this solution.
8. Check the level of liquid in the Halogen Scrubber (the long glass tube next to the syringe which contains the rolled stainless mesh). If there are no bubbles, need to add more acid so the level is an inch or so above the mesh screen (volume should be just between the white text markings on the tube). Remove the Halogen scrubber cap and make sure the drain line does not touch anything in the machine. Carefully remove the tube and take it to the lab hood. Fill the tube to the desired level with the green squirt bottle (stored under the hood) with 0.05 M HCl acid. Place the glass tube back into the instrument and put the cap back on. When recapping the scrubber ALWAYS check to be sure the small tubing valve is inside the wire mesh and not outside of it. Bubbles should be visible immediately. Check for any acid drips and wipe up with a clean wipe to prevent corrosion inside the instrument.
9. Check the reservoir for the mist-catcher behind the halogen scrubber. Be sure the water level is between the two dark lines. If looking low, remove the plastic cap and top off with fresh Milli-Q water.

10. Before running blanks or beginning a sample run, from the program select at the top of the screen click “Instrument”, then “Maintenance” and select “Replace Flow Content”. In the window that pops up [insert what exactly happens here] and then click “start”. Once finished, close the window. Then go to “Instrument”, “Maintenance” and select “Washing.”. Check all boxes but the “IC”. Select start and close when finished.
11. Once flowline content has been flushed, click on the monitor icon in the top right corner of the program. In the window that pops up, select on the 50x spot to the right of the graph. Let the baseline monitor sit for about 15-20min. Do not start the run until the red and blue lines remain stable and straight, should be close to zero.

Standard Preparation for Calibration Curve

1. Daily calibration must be performed before sample analysis can begin (i.e., a new curve must be made at the beginning of each instrument run). Shimadzu TOC-L requires a minimum of a 4-point calibration.
2. Decide how many points (aka specific concentrations) and what range of concentrations are appropriate for the NPOC and TN standard curves based on samples being run. (Note: usually 8-9 points in a single curve is ideal). Choose desired standard concentrations to be made by hand. Record appropriately in lab notebook.
3. See following instructions for an example of a 0-30mg/L NPOC curve and 0-3mg/L TN curve. *Note: Recommend not auto-diluting standards more than 10x in the instrument.*
    a. NPOC Calibration Curve: the first column represents the concentrations of the points that will make up the standard curve. The second column represents the concentration of the stock solution those points will be auto-diluted from in the instrument. Use this table to determine how many different standard solutions will need to be made:

NPOC Calibration Curve Point:	Standard Stock Solution Concentration:
0 mg/L C	Reagent H_2O (milliQ/ DI)
0.5 mg/L C	1 mg/L C
1.0 mg/L C	1 mg/L C
5 mg/L C	10 mg/L C
10 mg/L C	10 mg/L C
50 mg/L C	100 mg/L C

80 mg/L C	100 mg/L C
100 mg/L C	100 mg/L C

b. TN Calibration Curve: the first column represents the concentrations of the points that will make up the standard curve. The second column represents the concentration of the stock solution those points will be auto-diluted from in the instrument. Use this table to determine how many different standard solutions will need to be made:

TN Calibration Curve Point:	Standard Stock Solution Concentration:
0 mg/L N	Reagent H_2O (milliQ)
0.25 mg/L N	1 mg/L N
0.50 mg/L N	1 mg/L N
1.0 mg/L N	1 mg/L N
2.0 mg/L N	3 mg/L N
3 mg/L N	3 mg/L N

4. Remove 1000ppm KNO_3 and 1000ppm KHP 1000ppm stock solutions from the fridge (*need to make new stock solutions approximately every 6 months. See Appendix 1 for instructions on making standards*) and let them come to room temperature.
5. Once at room temperature, shake the stock solutions vigorously for at least 1 minute. Mark in permanent marker on two clean, combusted beakers "1000ppm C" and "1000ppm N". Pour approximately between 5-10mL of KNO_3 into the beaker labelled N, and 5-10mL of KHP into the beaker labelled C. Keep covered with the combusted foil.
6. Determine how much of the concentrated 1000ppm stock will be needed to get each of the standards in your curve by using the following equation:
    a. V2 = (C1*V1)/C2; where V2 = volume (mL) needed of concentrated stock to pipette into flask, C1 = target concentration of standard solution needed to be made (i.e., 1 mg/L KHP), V1 = volume of flask holding C1 (i.e., 100mL volumetric flask), and C2 = concentration of stock solution being diluted (i.e., 1000 ppm KHP).

i. Example for making 100mL of 1 mg/L C standard:
V2 = [(1 mg/L C)*(100 mL)]/1000 mg/L C
V2 = 0.1 mL (100 uL) of 1000 ppm C

7. Make 100mL (in clean, acid washed volumetric flask) of each of the following standards: 1 mg/L KHP, 10mg/L KHP, 30 mg/L KHP, 1 mg/L KNO_3, and 3 mg/L KNO_3
   a. **1 mg/L KHP**: fill the volumetric flask half-way with milliQ. With a clean pipette tip, pipette in 100uL of 1000ppm KHP from the small beaker into the flask. Cap and invert at least 40-50 times. Fill the rest of the way up with milliQ until the bottom of the meniscus is right at the etched line. (if you go past the line, dump the solution into the waste bin and start again).
   b. **10 mg/L KHP**: Same instructions as above, but pipette in 1 mL of 1000ppm KHP into a clean volumetric flask. Fill the rest up with milliQ
   c. **100 mg/L KHP**: Same instruction as above, but pipette in 10 mL of 1000 ppm KHP into a clean volumetric flask. Fill the rest up with milliQ.
   d. **1 mg/L KNO_3**: Same instruction as above, but pipette in 100uL of 1000 ppm KNO_3 into a clean volumetric flask. Fill the rest up with milliQ.
   e. **3 mg/L KNO_3**: Same instruction as above, but pipette in 300uL of 1000 ppm KNO_3 into a clean volumetric flask. Fill the rest up with milliQ.
8. Make the check standard.
   a. Determine how many check standards will be needed:
      i. Total # of samples/10 + 2 (for the initial and final check standard) = # of check standards needed.
         1. *Example*: 20 samples/10 + 2 = 4 check standards
   b. Determine how much enough check standard volume is needed (each check standard vial will need aprox. ~20-25mL)
      i. # of check standards needed * 20 mL = Total volume
         1. *Example*: 4 check standards * 20 mL = 80mL
   c. For a 100mL of 1 mg/L KHP + 1 mg/L KNO_3 check standard fill a 100mL volumetric flask up halfway with milliQ. Pipette in 100uL of 1000ppm KNO_3 and 100uL of 1000ppm KHP. Invert at least 40-50x. Fill the flask the rest of the way up with milliQ until the bottom of the meniscus is aligned with the etched line.
   d. We also sometimes use a 50C and 2N check std depending on the range

Creating a New Method (*only necessary if creating a new method with different parameters than described below or for different study/project; if not skip to "Building Calibration Curve" below*)

1. Click "File", "new method"
   a. On system click the down arrow and select "TOC-L with TNM"

   b. Click Next
   c. For Analysis select “NPOC/TN”. Leave everything else at default value unless relevant for a specific project..
   d. For file name, be sure to input a name that includes the project code, injection volume, and date it was made: i.e.,: SBR_150uL_Method_April_2021
2. For NPOC calibration curves (page 3) leave calibration curve 1 blank. The calibration curve used in the method will be the one created each time you do a different run.
3. Input the following information on page 4 Injection Parameters for NPOC analysis (this will change only if decided otherwise) then click next:
   a. Units: mg/L
   b. Injection Volume: 50 uL
   c. No. of Injections: 3 of 5
   d. SD Max: 0.1000
   e. CV Max: 2.00
   f. No. of Washes: 1 *changed for volume issues
   g. Auto Dilution: 1
   h. Sparge Gas Flow: 80
   i. Sparge Time: 02:30 min
   j. Acid Addition: 1.5 %
   k. Multiple Injections: No
4. Peak time parameters (Page 5) make sure use default settings is checked for NPOC analysis then click next
5. Leave Calibration Curve 1 file name for Analysis TN blank, then click next
6. Input the following information on page 4 Injection Parameters for TN analysis (this will change only if decided otherwise) then click next:
   a. Units: mg/L
   b. No. of Injections: 3 of 5
   c. SD Max: 0.1000
   d. CV Max: 2.00
7. Peak time parameters ~~(Page 5) make sure use default settings is checked for TN analysis then click next~~ Set time to 3:30 and 8:50 because peaks are coming out later with instrument age
8. Pharmaceutical water testing make sure “None” is selected then click “Finish”

Building Calibration Curve

1. Click “File”, “new calibration curve”.
2. Select system “ TOC-L with TNM” and click next

3. Select normal and check “Use dilution from standard solution” box and then click next.
4. Analysis select “NPOC”
    a. Type in Default Sample Name with STD_, range of concentration, and ppmC: i.e.,: STD_0-100ppmNPOC. (for TN: STD_0-3ppmTN)
    b. Unselect Zero Shift
    c. Under file name, click the three dots. A window with past calibration curve files will pop up. Select a recent calibration curve and edit the name in the “File name:” box to include the analysis type, concentration range, and date. Format should be like this example: **NPOC_0-30mgL_2021_03_31.cal** (or for Nitrogen: **TN_0-3mgL_2021_03_31.cal**)
    d. Then click open. Make sure name showing up in File Name box matches the name and date you just created. Then hit next.
5. Be sure the following Calibration Measurement Parameters for NPOC analysis match as shown below and then click next.
    a. Units: mg/L
    b. No. of Injections: 3 of 5
    c. SD Max: 0.1000
    d. CV Max: 2.00
    e. No. of Washes: 1
    f. Auto Dilution: 1
    g. Sparge Gas Flow: 80
    h. Sparge Time: 02:30 min
    i. Acid Addition: 1.5 %
    j. Multiple Injections: No
6. Add in calibration points.
    a. For each point (including zero point), click and highlight the number in the No. column. Then click “Add”. Input in the pop-up window what the standard solution concentration is in (mg/L) the “Standard Solution Conc” box and then what the desired calibration point concentration (mg/L) is in the Ca. Point Conc box. See section 5.3.2 starting on page 126 in the digital manual for more info.
    b. Make sure the No. of injections, SD Max and CV max match calibration parameters. Then click ok.
    c. Do this for all points on your calibration curve.
    d. BEFORE CLICKING NEXT be sure that the Injection Volume in the top right hand corner reads 50uL! Then click next.
7. ~~Select “Use default settings” and~~ Set time to 3:30 and 8:50 because peaks are coming out later with instrument age click Finish

8. Repeat steps 1-7 above but for your TN curve. Select Analysis “TN” instead of NPOC.

Building Sample Table with Method

1. Click “File”, “new sample table”
    a. Select “TOC-L with TNM” and Table Type “Normal”
2. Click “File”, “Open”, “Method”. Open the Method file going to be used for the run. (most often it should always be the SBR_150uL_Method_April_2021).
    a. Go over to the NPOC tab. Click on the 3 dots next to the Calibration Curve 1 and open the NPOC cal curve file you just made.
    b. Click on the “TN” tab and select the 3 dots next to the Calibration Curve 1 box. Select and open the TN cal curve file you just made.
    c. Hit OK
3. How to insert multiple samples into sample table:
    a. Insert a new sample by highlighting a row in the sample table and right clicking. For multiple samples select “Insert - Multiple Samples”.
    b. Window pops up and under Method click the three dots.
    c. Select your method that you just inserted your newly created cal curves in. Click open. Then click next.
    d. Choose the number of samples you want to insert.
    e. Fill the rest of information out if known. Then click finish.
4. Click a row in the sample table, right click and select “Insert - Calibration Curve” Do this for both the NPOC and TN curves.
5. Finishing adding the rest of your samples in the run to the sample table.
6. The general order of sample table run should be:
    a. 3 conditioning blanks (vials filled with 25-30mL milliQ) (Ex Sample name: CondBlanks)
    b. NPOC standard curve (insert correct calibration file created above)
    c. TN standard curve (insert correct calibration file created above)
    d. Blank (ex sample name: Blank1, Blank2)
    e. Initial Check Standard (ex sample name: CKSTD1_1ppm)
    f. Sample vials
    g. Check standard vial every 10 samples
    h. Random blanks every 10-20 samples
    i. Always end the run with a check standard and 1-2 blanks
7. Once all samples, blanks and standards are entered into the sample table, click File, Save As.
    a. Click on the SBR or COMPASS folder, and then the appropriate project activity folder (if its a mixed run, don't put it in a study folder).

   b. Name the sample table in the following format: "YYYYMMDD_Data_Raw_NPOC_TN_[Project]_[RCX]_STUDYCODE_[# of samples or range of samples]
      i. Ex: **20210331_Data_Raw_NPOC_TN_SBR_RC2_RC2_R1-36**
   c. Click Save.
8. Setting correct vial positions:
   a. Click the birthday cake icon in the top right corner of the sample table window
   b. In the Vial column go through and put the appropriate vial number associated with sample position. Double check this several times. Should match what's recorded in lab notebook. Be careful to check which vials in standard curves points are being used multiple times (Ex: the zero point for the NPOC and TN is from the same vial!)
   c. Once a position is chosen the graphic will highlight that vial position as blue.

Loading Blanks & Standards into the carousel

1. With gloved hands remove the ASI cover from the carousel and set aside on a clean surface. Carefully remove the carousel from the autosampler.
2. Following the sample table, determine how many blank vials are needed.
   a. Fill cleaned 9mL vials with ~9mL of milliQ for each blank. Mark as "MQ" in permanent marker on the vial. Cover and secure each vial with a septum cap.
   b. Place vials into the correct carousel position based on the sample table order.
3. Fill the appropriate numbers of 9 mL vials with the standards prepared earlier, about 9mL full. Mark on vial which standard is poured in. Cover and secure with a septum cap. Place into positions indicated by the sample table (see steps b & c below), with NPOC standard vials in consecutive order after the zero blank followed by TN standard vials. Document in lab notebook what vials positions are before placing vials to help check when creating the sample table.
   a. Note~~: If doing a standard curve with concentrations over 50 mg/L C or 5 mg/L N, recommend putting in 1-2 blank milliQ vials between the two curves to prevent carry-over.~~
   b. NPOC standard curve (with the points 0, 0.25, 0.5, 1, 2, 5, 10, 15, 20, 30)
      i. Fill vial with 30mL milliQ for 0 point
      ii. Fill 2 vials with 30mL of 1 mg/L C standard and place them in the positions for the 0.25, 0.5, and 1 points.
      iii. Fill 2 vials with 30mL of 10 mg/L C standard and place them in the positions for the 2, 5, and 10 points.

iv. Fill 2 vials with 30mL of 30 mg/L C standard and place them in the positions for the 15, 20, and 30 points.

c. TN standard curve (with the points 0, 0.1, 0.15, 0.2, 0.25, 0.5, 1, 1.5, 2, 3)
   i. Fill 2 vials with 30mL of 1 mg/L N standard and place them in the positions for the 0.1, 0.15, 0.2, 0.25, 0.5, and 1points.
   ii. Fill 2 vials with 30mL of 3 mg/L N standard and place them in the positions for the 1.5, 2, and 3 points.

4. Fill the appropriate numbers of 40 mL vials for check standards about 30mL full. Secure open septum cap on vial. Place into positions as indicated by the sample table.
5. Confirm the locations in the sample table software match the location of the vials in the carousel. Recommend double checking order twice.
6. Carefully place the now filled carousel back into the autosampler. Be sure you hear a click sound to ensure the carousel fits snug and secure. Place the ASI cover back and click it into place. Make sure "ASI error cover open" notification leaves on the computer screen.
7. Click Connect, disconnect and then reconnect the instrument to the appropriately created sample table for the current run.

Starting the Instrument Run

1. Before starting the run, be sure the status in the upper hand corner of the software says "Ready" and is lit up in green.
2. Check the "Monitor" screen to be sure the baseline flux is stable by zooming in all the way on the graph.
3. Once it says "Ready", flowlines have been rinsed, baseline flux looks steady, and the sample table is complete you can start the run.
   a. Click on or highlight the first sample in the sample table
   b. Click "Start" at the top of the software
   c. Then click "Shut down instrument" BE SURE TO ALWAYS SELECT THIS BEFORE STARTING INSTRUMENT RUN
   d. The click "Start"

6. Procedures (for a DIC run)

Preparing the TOC-L

1. Turn the compressed air tank on & make sure the regulator is set around 300kpa . Replace the tank when the pressure reads below 500 psi.
2. Push the button located on the front of the instrument to turn it on. (if nothing happens, switch the on/off switch on the right side of the instrument to on). The front indicator light will cycle through colors and then appear orange (not-ready state). The indicator light is green when the instrument is up to temperature & all

parameters are OK. The light will be blue while it is running samples. If the indicator light is red, refer to the software and manual to determine the problem.

3. Open the software by clicking on the TOC-L sample table icon in the center of the desktop. There is no password, just hit enter when the screen appears. Entering your initials is optional.
4. Open a previous sample table (by clicking on File, Open, Sample Table and navigating to the appropriate project folder) Then hit CONNECT located in the toolbar above the sample table to the right. A sample table needs to be open to connect the instrument. The furnace automatically turns on. The correct sample table will be connected to the instrument before it begins the run.
5. Refill the rinse water container with fresh MilliQ / DI water (located behind the autosampler). Carefully rest the cap with thin plastic tubing carefully into a clean beaker (which has had contact with nothing else). Deposit old rinse water down the drain & refill with fresh MilliQ / DI all the way up to the rim. If the tube touches any surfaces or gloves during this process, get a clean chem wipe, squirt it with Milli Q water and carefully wipe the outside of the tube below the cap. Once the tubing and cap is screwed back on tightly, make sure the tip of the tube is resting close to the bottom of the container. (this is especially important for large sample runs...don't want the tube to be exposed to any air as the water level lowers during the run.)
6. Refill dilution water (the large reservoir nested on the direct left side of the instrument) at least once every week or two. Check if the water level is getting low and top-off before a run.
7. 18% Phosphoric acid is used in DIC analysis. Check the volume of the acid bottles in use. This volume should be just below the arm. Usually this is plenty for several weeks of analysis. See manual to create new solution.
8. Check the level of liquid in the Halogen Scrubber (the long glass tube next to the syringe which contains the rolled stainless mesh). If there are no bubbles, more acid needs to be added so the level is an inch or so above the mesh screen (volume should be just between the white text markings on the tube). Remove the Halogen scrubber cap and make sure the drain line does not touch anything in the machine. Carefully remove the tube and take it to the lab hood. Fill the tube to the desired level with the green squirt bottle (stored under the hood) with 0.05 M HCl acid. Place the glass tube back into the instrument and put the cap back on. When recapping the scrubber ALWAYS check to be sure the small tubing valve is inside the wire mesh and not outside of it. Bubbles should be visible immediately. Check for any acid drips and wipe up with a clean wipe to prevent corrosion inside the instrument.

9. Check the reservoir for the mist-catcher behind the halogen scrubber. Be sure the water level is between the two dark lines. If looking low, remove the plastic cap and top off with fresh Milli-Q water.
10. Before running blanks or beginning a sample run, from the program select at the top of the screen click “Instrument”, then “Maintenance” and select “Regeneration of IC solution”. In the window that pops up click start. This takes about 5-10minutes. Once finished, close the window.
11. Then at top of the screen click “Instrument”, then “Maintenance” and select “Replace Flow Content”. In the window that pops up [insert what exactly happens here] and then click “start”. Once finished, close the window. Then go to “Instrument”, “Maintenance” and select “Washing.”. Check boxes called “IC”. Select start and close when finished.
12. Once flowline content has been flushed, click on the monitor icon in the top right corner of the program. In the window that pops up, select on the 50x spot to the right of the graph. Let the baseline monitor sit for about 15-20min. Do not start the run until the red and blue lines remain stable and straight, should be close to zero.

Standard Preparation for Calibration Curve

1. Daily calibration must be performed before sample analysis can begin (i.e., a new curve must be made at the beginning of each instrument run). Shimadzu TOC-L requires a minimum of a 4-point calibration.
2. Decide how many points (aka specific concentrations) and what range of concentrations are appropriate for the DIC standard curves based on samples being run. (Note: usually 7 points in a single curve is ideal). Choose desired standard concentrations to be made by hand. Record appropriately in lab notebook.
3. See following instructions for an example of a 0-40mg/L DIC curve:
4. DIC Calibration Curve: the first column represents the concentrations of the points that will make up the standard curve. Unlike NPOC curve, auto-diluted in the instrument is not used here since vials have to be filled to the brim.

DIC Calibration Curve Point:	Standard Stock Solution Concentration:
0 mg/L C	Reagent H_2O (milliQ / DI)
1 mg/L C	1 mg/L C
5.0 mg/L C	5 mg/L C
10.0 mg/L C	10 mg/L C
40.0 mg/L C	40 mg/L C

80 mg/L C	80 mg/L C
100 mg/L C	100 mg/L C
200 mg/L C	200 mg/L C

9. Once at room temperature, invert the stock solution gently for at least 1 minute. Mark in permanent marker on a clean, combusted beakers "1000ppm C". Pour approximately between 5-10mL of DIC stock into the beaker. Keep covered with the combusted foil.
10. Determine how much of the concentrated 1000 ppm stock will be needed to get each of the standards in your curve by using the following equation:
    a. V2 = (C1*V1)/C2; where V2 = volume (mL) needed of concentrated stock to pipette into flask, C1 = target concentration of standard solution needed to be made (i.e., 1 mg/L DIC), V1 = volume of flask holding C1 (i.e., 100mL volumetric flask), and C2 = concentration of stock solution being diluted (i.e., 1000 ppm DIC).
        i. Example for making 100mL of 1 mg/L C standard:
            V2 = [(1 mg/L C)*(100 mL)]/1000 mg/L C
            <u>V2 = 0.1 mL (100 uL) of 1000 ppm C</u>
11. Make 100mL (in clean, acid washed volumetric flask) each of the needed standards for the DIC curve.
    a. Fill each volumetric flask half-way up with milliQ. With a clean pipette tip, pipette in necessary volume of 1000ppm KHP from the small beaker into the flask. Cap and invert gently at least 20-30 times. Fill the rest of the way up with milliQ until the bottom of the meniscus is right at the etched line. (if you go past the line, dump the solution into the waste bin and start again).
    b. Do this for each point on the standard curve.
12. Make the check standard.
    a. Determine how many check standards will be needed:
        i. Total # of samples/10 + 2 (for the initial and final check standard) = # of check standards needed.
            1. *Example*: 20 samples/10 + 2 = 4 check standards
    b. Determine how much enough check standard volume is needed (each check standard vial will need aprox. ~40mL)
        i. # of check standards needed * 40 mL = Total volume
            1. *Example*: 4 check standards * 40 mL = 160mL
    c. Make check standard

Creating a New Method (*only necessary if creating a new method with different parameters than described below or for different study/project; if not skip to "Building Calibration Curve" below*)

1. Click "File", "new method"
    a. On system click the down arrow and select "TOC-L with TNM"
    b. Click Next
    c. For Analysis select "IC". Leave everything else at default value unless relevant for a specific project..
    d. For file name, be sure to input a name that includes the project code, injection volume, and date it was made: i.e.,: SBR_IC_Method_May_2021
9. For DIC calibration curve leave calibration curve 1 blank. The calibration curve used in the method will be the one created each time you do a different run.
10. Input the following information on page 4 (this will change only if decided otherwise) then click next:
    a. Units: mg/L
    b. Injection Volume: 150 uL
    c. No. of Injections: 3 of 4
    d. SD Max: 0.1000
    e. CV Max: 2.00
    f. No. of Washes: 2
11. Peak time parameters (Page 5) make sure use default settings is checked for IC analysis then click next
12. Pharmaceutical water testing make sure "None" is selected then click "Finish"

Building Calibration Curve

9. Click "File", "new calibration curve".
10. Select system " TOC-L with TNM" and click next
11. Select normal and then click next. (don't select check the box from "Use dilution from standard solution")
12. Analysis select "IC"
    a. Type in Default Sample Name with STD_, range of concentration, and ppmC: i.e.,: STD_0-40ppmDIC.
    b. Unselect Zero Shift
    c. Under file name, click the three dots. A window with past calibration curve files will pop up. Select a recent calibration curve and edit the name in the "File name:" box to include the analysis type, concentration range, and date. Format should be like this example: **DIC_0-40mgL_2021_03_31.cal**
    d. Then click open. Make sure the name showing up in the File Name box matches the name and date you just created. Then hit next.

13. Be sure the following Calibration Measurement Parameters for IC analysis match as shown below and then click next.
    a. Units: mg/L
    b. Injection volume: 150uL
    c. No. of Injections: 3 of 4
    d. SD Max: 0.1000
    e. CV Max: 2.00
    f. No. of Washes: 2
14. Add in calibration points.
    a. For each point (including zero point), click and highlight the number in the No. column. Then click "Add". Input in the pop-up window what the desired calibration point concentration (mg/L) is in the Ca. Point Conc box. See section 5.3.2 starting on page 126 in the digital manual for more info.
    b. Make sure the No. of injections, SD Max and CV max match calibration parameters. Then click ok.
    c. Do this for all points on your calibration curve.
    d. BEFORE CLICKING NEXT be sure that the Injection Volume in the top right hand corner reads 150uL! Then click next.
15. Select "Use default settings" and click Finish

Building Sample Table with Method

9. Click "File", "new sample table"
    a. Select "TOC-L with TNM" and Table Type "Normal"
10. Click "File", "Open", "Method". Open the Method file going to be used for the run.
    a. Go over to the DIC tab. Click on the 3 dots next to the Calibration Curve 1 and open the IC cal curve file you just made.
    b. Hit OK
11. How to insert multiple samples into sample table:
    a. Insert a new sample by highlighting a row in the sample table and right clicking. For multiple samples select "Insert - Multiple Samples".
    b. Window pops up and under Method click the three dots.
    c. Select your method that you just inserted your newly created cal curves in. Click open. Then click next.
    d. Choose the number of samples you want to insert.
    e. Fill the rest of information out if known. Then click finish.
12. Click a row in the sample table, right click and select "Insert - Calibration Curve".
13. Finishing adding the rest of your samples in the run to the sample table.
14. The general order of sample table run should be:

a. 3 conditioning blanks (vials filled with completely full milliQ) (Ex Sample name: CondBlanks)
b. NPOC standard curve (insert correct calibration file created above)
c. TN standard curve (insert correct calibration file created above)
d. Blank (ex sample name: Blank1, Blank2)
e. Initial Check Standard (ex sample name: CKSTD1_1ppm)
f. Sample vials
g. Check standard vial every 10 samples
h. Random blanks every 10-20 samples
i. Always end the run with a check standard and 1-2 blanks

15. Once all samples, blanks and standards are entered into the sample table, click File, Save As.
    a. Click on the SBR or COMPASS folder, and then the appropriate project activity folder (if its a mixed run, don't put it in a study folder).
    b. Name the sample table in the following format: "YYYYMMDD_Data_Raw_DIC_[Project]_[RCX]_STUDYCODE_[# of samples or range of samples]
        i. Ex: **20210331_Data_Raw_DIC_SBR_RC2_RC2_R1-36**
16. Click Save.
17. Setting correct vial positions:
    a. Click the birthday cake icon in the top right corner of the sample table window
    b. In the Vial column go through and put the appropriate vial number associated with sample position. Double check this several times. Should match what's recorded in lab notebook. Be careful to check which vials in standard curves points are being used multiple times (Ex: the zero point for the NPOC and TN is from the same vial!)
    c. Once a position is chosen the graphic will highlight that vial position as blue.

Loading Blanks & Standards into the carousel

8. With gloved hands remove the ASI cover from the carousel and set aside on a clean surface. Carefully remove the carousel from the autosampler.
9. Following the sample table, determine how many blank vials are needed.
    a. Fill cleaned 40mL amber vials completely full of milliQ for each blank, so that there are no air bubbles when the cap is screwed on. Mark as "MQ" in permanent marker on the vial. Cover and secure each vial with a septum cap.
    b. Place vials into the correct carousel position based on the sample table order.

10. Fill the appropriate numbers of 40 mL vials with the standards prepared earlier, completely full with no air bubbles. Mark on vial which standard is poured in. Cover and secure with a septum cap. Place into positions indicated by the sample table, with DIC standard vials in consecutive order after the zero blank. Document in lab notebook what vials positions are before placing vials to help check when creating the sample table.
    a. Note: If doing a standard curve with concentrations over 50 mg/L C, recommend putting in 1-2 blank milliQ vials between the two curves to prevent carry-over.
11. Fill the appropriate numbers of 40 mL vials for check standards about completely full. Secure open septum cap on vial. Place into positions as indicated by the sample table.
12. Confirm the locations in the sample table software match the location of the vials in the carousel. Recommend double checking order twice.
13. Carefully place the now filled carousel back into the autosampler. Be sure you hear a click sound to ensure the carousel fits snug and secure. Place the ASI cover back and click it into place. Make sure "ASI error cover open" notification leaves on the computer screen.
14. Click Connect, disconnect and then reconnect the instrument to the appropriately created sample table for the current run.

Starting the Instrument Run

4. Before starting the run, be sure the status in the upper hand corner of the software says "Ready" and is lit up in green.
5. Check the "Monitor" screen to be sure the baseline flux is stable by zooming in all the way on the graph.
6. Once it says "Ready", flowlines have been rinsed, baseline flux looks steady, and the sample table is complete you can start the run.
    a. Click on or highlight the first sample in the sample table
    b. Click "Start" at the top of the software
    c. Then click "Shut down instrument" BE SURE TO ALWAYS SELECT THIS BEFORE STARTING INSTRUMENT RUN
    d. The click "Start"

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Instrument Naming Nomenclature for TOC-L – Project Dependent

~~Instrument Item~~	~~Name Format Description:~~	~~Example:~~
~~Instrument blanks~~	~~Blank[#]~~	~~Blank1, Blank2~~
~~Conditioning blanks~~	~~CondBlank[#]~~	~~CondBlank1, CondBlank2~~
~~Standard curve~~	~~STD_[range of concentrations][analysis type]~~	~~STD_0-30ppmNPOC~~ ~~STD_0-3ppmTN~~ ~~STD_0-40ppmDIC~~
~~Check standard~~	~~CKSTD[#]_[concentration][analysis type]~~	~~CKSTD1_1ppmNPOC~~ ~~CKSTD2_0.5ppmDIC~~
~~Sample name~~	~~[StudyCode]_[X][randomized run number]~~	~~RC2_A30~~
~~File name (from the "detail" Ascii export file type)~~	~~YYYYMMDD_Data_Raw_[analysis]_[Project]_[campaign]_STUDYCODE_[# of samples or range of samples]~~	~~20210801_Data_Raw_NPOC_TN_SBR_RC2_RC2_A1-36~~ ~~20210809_Data_Raw_DIC_SBR_RC2_RC2_A1-36~~
~~Summary file (from the "normal" Ascii export file type)~~	~~YYYYMMDD_Summary_Raw_[analysis]_[Project]_[campaign]_STUDYCODE_[# of samples or range of samples]~~	~~20210801_Summary_Raw_NPOC_TN_SBR_RC2_RC2_A1-36~~ ~~20210809_Summary_Raw_DIC_SBR_RC2_RC2_A1-36~~