ANALYSIS OF TOTAL NITROGEN (TN), DISSOLVED NITROGEN (DN), TOTAL ORGANIC CARBON (TOC), AND DISSOLVED ORGANIC CARBON (DOC) USING THE SHIMADZU TOC-L2 INSTRUMENT EQUIPPED WITH A TOTAL NITROGEN MODULE (TNM-L)

TABLE OF CONTENTS:

Page

1. SCOPE AND APPLICATION 2
2. SUMMARY OF THE METHOD 2-3
3. EQUIPMENT AND SUPPLIES 4-5
4. CHEMICALS 5-7
5. PREPARATION OF STANDARDS, REAGENTS, AND CALIBRATION 7-12
6. SAMPLE HANDLING AND PRESERVATION 12
7. SAMPLE PREPARATION AND ANALYSIS 12-17
8. DATA ARCHIVAL 17-18
9. MAINTENANCE/TROUBLESHOOTING 18-19
10. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES 19-21
11. SAFETY/WASTE MANAGEMENT/POLLUTION PREVENTION 21
12. REFERENCES 21
13. APPENDIX I: INTRUMENTS SETTINGS-PARAMETERS 22-23

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1. SCOPE AND APPLICATION

1.1. This SOP details the analysis of Total Nitrogen (TN), Dissolved Nitrogen (DN), Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) in surface waters, ground waters, salt water, effluents and influents. The tests covered by this SOP are: W-TN, W-DN, W-TOC, and W-DOC.

Key method parameters:

	W-TOC/W-DOC	W-TN/W-DN
Calibration range	0.0 – 100.0 mg C/L	0.0 – 5.0 mg N/L
Minimum Detection Limit (MDL)	0.50 mg C/L	0.10 mg N/L
Practical Quantitation Limit (PQL)	1.0 mg C/L	0.5 mg N/L

1.2. Samples with concentration of TN/DN greater than 5.0 mg N/L are reported after dilution into the range or qualified appropriately. Samples with TN concentrations below the PQL will be re-analyzed following SOP NU-105. Samples with concentration of TOC/DOC greater than 100.0 mg C/L are reported after dilution into the range or qualified appropriately.

2. SUMMARY OF THE METHOD

2.1. The described procedure is based on ASTM D8083-16 for TN/DN analysis and Standard Methods 5310 B-2011 for DOC/TOC analysis. These methods allow for either use of internal or external sparging as described in 2.2.

Note: *Only the **TOC-L2-CPH** instrument is equipped with the external sparge kit for mixing. See APPENDIX I for further details.*

2.2. The analyst has the option of analyzing the samples with internal or external sparging. In the external sparging procedure, the acidified samples are purged with ultra-high purity (UHP) air using an external sparge kit for a duration of 360 seconds prior to injection in order to remove the inorganic carbon fraction. In the internal sparging procedure, the acidified samples are purged with ultra-high purity (UHP) air for the duration of 90 seconds in the syringe/plunger prior to injection in order to remove the inorganic carbon fraction. Samples (50 ul) are introduced by the sample injector into a combustion tube.

2.3. The organic carbon and nitrogen components of the sample are combusted at 720ºC in the presence of a platinum catalyst to form carbon dioxide and nitrogen oxide (NO), respectively. Carbon dioxide is detected by infrared analysis. Nitrogen oxide reacts with ozone to form excited state NO_2. As the NO_2 returns to the ground state, the emitted radiation is detected using a chemiluminescence

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detector.

2.4. The method measures nonpurgeable organic carbon (NPOC). In many surface and ground waters, the purgeable organic carbon contribution to TOC is negligible. Therefore, in practice, the nonpurgeable organic carbon determination is substituted for TOC. Elemental carbon may not be oxidized at the furnace temperature, but it is not expected to be a constituent of the water samples analyzed. In addition, the method does not measure N_2 as gas.

2.5. The method requires the analysis of at least **4 portions** of **TONCHCK** (Organic Nitrogen Test Solution) (6.12.4., 9.4.) at the same volume as samples to be analyzed. To satisfy this requirement, **TONCHCK** is put on the run sequence at least twice (for a minimum total injection of 6 and up to 10 injections).

2.6. This procedure is applicable only to nitrogenous and organic matter in the sample that can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. An external sparging procedure may be used as a mixing mechanism to suspend small particles that may otherwise settle over time. The openings of the autosampler needle and injection syringe limit the maximum size of particles included in the analysis.

2.7. Interferences:

2.7.1. Acid preservation of samples minimizes interferences that can cause results to be low. Gases of halide components may interfere with the detection system. They are eliminated by passing the reaction gas through a halogen scrubber. Samples with a high particulate loading may need to be homogenized prior to analysis. Samples containing high total suspended solids (TSS) may cause TN analysis to be biased low. Dissolved inorganics in the sample that are not volatized may result in devitrification or solidify in the catalyst tube resulting in decreased flow rates. Total organic carbon (TOC) up to 300 mg/L does not interfere with the TN analysis.

2.7.2. Saline samples may impair the effectiveness of the catalyst and reduce its lifespan. Samples with a conductivity of ≥15,000 umhos/cm and ≤30,000 umhos/cmand/or a salinity of 9 PPTh or greater are batched as salt samples and are diluted 1:2. Salt samples with a conductivity of >30,000 umhos/cm are diluted 1:4. HCL and/or H_2SO_4 rinses are added between the samples and at the end of the run in order to minimize residue build up on the catalyst (see 6.12.3).

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3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation

Instrumentation Unique ID	Component	Manufacturer	Model/Product #
W-TOC-L2-CPH	Total Organic Carbon Analyzer	Shimadzu	TOC-LCPH
	Autosampler ASI-L for 24 mL, ROHS - compliant	Shimadzu	638-93199-58
	TNM-L Total Nitrogen Module, ROHS	Shimadzu	638-91108-58
	External Sparge Kit for ASI-L	Shimadzu	638-77183-41
	TOC Control L Software	Shimadzu	Ver. 1.11

3.2. Instrument Accessories

Accessory	Manufacturer/Vendor	Model(s) / Catalog Number
Combustion Tube	Shimadzu	638-41323-00
Platinum Screen 2 Sheets TOC	Shimadzu	630-00105-01
O-Ring, 4D P 10AX5	Shimadzu	03-11209-84
O-Ring, PTFE Maintenance Parts	Shimadzu	638-15025

3.3. Equipment

Equipment	Manufacturer/ Vendor	Model(s)/Catalog Number
Analytical Balance	Mettler Toledo/Fisher	XSR105DU
Adjustable Pipette, 10-100 µL	Eppendorf/Fisher	3123000047/13690029
Adjustable Pipette, 100 - 1000 µL	Eppendorf/Fisher	3123000063/13690032
Adjustable Pipette, 1-10 mL	Eppendorf/Fisher	3123000080/13690034
Volumetric Flask, Class- A, Assorted	Pyrex/Fisher (SR)	10-208M, 10-212-5L, 10-240E; 10-212B
Graduated Cylinder, assorted	KIMAX/Fisher (SR)	08-564A

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Equipment	Manufacturer/ Vendor	Model(s)/Catalog Number
Glass Funnel	Fisher (SR)	10-346-5B
Desiccator	Fisher Scientific	08-645-6
Or Equivalent		
Note: (SR) designates supply is typically ordered through the in-house stockroom		

3.4. Supplies and Consumables

Equipment	Manufacturer/ Vendor	Model(s)/Catalog Number
Pipette Tips, 200 µL	Eppendorf/Fisher (SR)	05-403-41
Pipette Tips, 1000 µL	Eppendorf/Fisher (SR)	05-403-43
Pipette Tips, 1-10 mL	Eppendorf/Fisher (SR)	05-403-116
Weigh Dish, Hexagonal 1 ½ X 1	Fisher (SR)	02-202-100
Weigh Dish, Hexagonal 4 X 3	Fisher (SR)	02-202-102
Weigh Dish, Aluminum, 60 mm	Fisher (SR)	08-732-101
Transfer Pipet, 5 mL	Fisher (SR)	13-711-5AM
Parafilm	Fisher (SR)	13-374-12
Vial, Glass Shell	Fisher (SR)	03-339-30H
DWK Kimble Glass Screw-Thread Vials with PTFE/Silicone Septa**	Fisher**	03-340-47D
Sample Beakers, Polystyrene, Assorted	Fisher (SR)	08-732-120, 08-732-122
Kimwipes, SM 4.5” x 8.5”	Fisher (SR)	06-666A
Or Equivalent		
Note: (SR) designates supply is typically ordered through the in-house stockroom ** A substitute to these vials is available through Shimadzu (Cat. # 220-95316-01) in case of supply disruptions at Fisher.		

4. CHEMICALS, REAGENTS, AND STANDARDS (SEE DEP SOPs NU-041 and NU-042)

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

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Note: All reagent and standard preparations must be documented in the specific TOC-TN Reagent and Standard Logsheet for W-TN/W-TOC analysis. This excel sheet (NU-RGTSTD-TOCTN) is specific to TOC-TN analysis and located in the REAGENT_PREP folder on the Nutrients drive located in the subfolder \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\TOC\TN_TOC\TOC_TN. It needs to be updated whenever a reagent or standard is newly prepared. For every analysis file print the TOC-TN Reagent and Standard Logsheet and place it into the analysis folder.

Note: Reagent and standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the reagent and standard sheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent and standard is maintained in the REAGENT_PREP folder on the Nutrients drive located in the subfolder \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\TOC\TN_TOC\Certificates of Analysis. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: Prior to using the chemicals in this analysis, please read their MSDS and the safety notes found in Section 11 of this SOP.

4.1. Chemicals

	Chemical	Alias	Manufacturer[1]	Catalog Number	Concentration/ Purity	Expiration[2]	Storage Temperature
4.1.1.	Deionized (DI) Water	H_2O	In-House	N/A	N/A	Daily	N/A
4.1.2.	Air, Compressed	Air Ultra Zero	Airgas	AI UZ300	Ultra Zero Grade	N/A	Room
4.1.3.	Sulfuric Acid	H_2SO_4	Fisher (SR)	A300S-212	Certified ACS Plus	5 years (*)	Room
4.1.4.	Hydrochloric Acid	HCl	Fisher (SR)	A144S-212	36.5 – 38.0% (w/w), Certified ACS Plus	5 years (*)	Room
4.1.5.	Potassium Hydrogen Phthalate	KHP	Fisher (SR)	P243-100	≥98.5%, meets ACS	5 years (*)	Room
4.1.6.	Anhydrous Dextrose (Ground)	$C_6H_{12}O_6$	Fisher (SR)	D16-500	Certified ACS	3 years (*)	Desiccator
4.1.7.	Potassium Nitrate (Primary)	KNO_3	Fisher	P-383-500	Certified ACS	5 years (*)	Room
4.1.8.	Potassium Nitrate (Secondary)	KNO_3	Sigma-Aldrich	221295-500G	≥99.0%, Certified ACS	3 years (*)	Room
4.1.9.	Ammonium Sulfate	$(NH_4)_2SO_4$	Fisher	A702-500	≥99.0%, Certified ACS	5 years (*)	Room

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	Chemical	Alias	Manufacturer[1]	Catalog Number	Concentration/ Purity	Expiration[2]	Storage Temperature
4.1.10.	Glycine	$C_2H_5NO_2$	Sigma-Aldrich	G7126-100G	≥99% (HPLC), ReagentPlus®	6 years(*)	Desiccator
4.1.11.	Anhydrous Sodium Carbonate	Na_2CO_3	Fisher (SR)	S263-500	≥99.5% Certified ACS	5 years (*)	Desiccator
4.1.12.	WatR^{tm} Pollution Demand	ALTQC, ERA	Environmental Resource Associates	516	See CoA	See CoA	Fridge
4.1.13.	Simple Nutrients Certified Standard (NOX ERA STOCK ##)	SIM NUT AMP (NOX ERA STOCK ##)	ERA	505	Variable based on CoA	See CoA	Fridge (See CoA)
4.1.14.	Sodium Hydroxide	NaOH	Fisher (SR)	S318-3	≥97.0 %, Pellets/Certified ACS	5 years (*)	Room
4.1.15.	Drierite 10-20 Mesh, White	$CaSO_4$, Desiccant	W.A. Hammond Drierite Co. LTD/Fisher (SR)	14005/07-577-4B	≥99.5% $CaSO_4$	N/A	Room
4.1.16.	Drierite 8 Mesh, Blue	$CaSO_4$ and $CoCl_2$ Desiccant	W.A. Hammond Drierite Co. LTD/Fisher (SR)	23005/S692-212	≥98% $CaSO_4$, <2% $CoCl_2$	N/A	Room
4.1.17.	Platinum Catalyst, 20g	Catalyst	Shimadzu	638-60202-01	Normal Sensitivity	N/A	Room
4.1.18.	Halogen Scrubber (Copper)	Halogen Scrubber	Shimadzu	630-00992-00	≥99% Copper	N/A	Room

Or Equivalent

[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom

[2]Note: (*) designates manufacturer's suggested retest date. No expiration date given.

4.2. Stock standards, spiking solutions, & reagents

4.2.1. **Stock Standard Solution (1000 mg N/L)** - This solution is used for the preparation of the calibration standards. Holding time for the Stock standard is 180 days.

4.2.1.1. Dry 8g of Potassium Nitrate and 5g of Ammonium Sulfate at 120ºC for 2 hours. Cool in a desiccator.

4.2.1.2. Add approx. 800 mL of DI water to a clean 1 L flask. Add 2 mL

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of concentrated H_2SO_4 and mix.

4.2.1.3. Weigh out 3.609 g of the dried KNO_3 into a weighing boat.

4.2.1.4. Weigh out 2.360 g of the dried $(NH_4)_2SO_4$ into a separate weighing boat.

4.2.1.5. Rinse the contents of the weighing boats into the flask using DI water. Dilute to the mark and mix thoroughly. Label this flask **'TN STOCK 1000 PPM'**.

Note: This solution is also used for low-level TN analysis. See SOP NU-105.

4.2.2. **KNO_3 Stock (1000 mg N/L)** - This solution is used for the preparation of the Initial Calibration Verification/Continuing Calibration Verification (ICV/CCV) standard for TN/DN analysis. It is prepared from a different source or lot number as the KNO_3 used to prepare the calibration standards. Holding time, 180 days.

4.2.2.1. Dry 4g of Potassium Nitrate at 120 ^oC for two hours. Cool in a desiccator.

4.2.2.2. Add approximately 100 mL of DI water to a clean 250 mL flask. Add 0.5 mL of concentrated H_2SO_4 and mix.

4.2.2.3. Weigh out 1.803 g of the dried KNO_3 into a weighing boat.

4.2.2.4. Rinse the contents of the weighing boat into the flask using DI water.

4.2.2.5 Dilute to the mark and mix thoroughly. Label this bottle **'1000 PPM KNO3'**.

4.2.3. **Stock Standard Solution (1000 mg C/L)** - This solution is used for the low spike (V1) for W-TOC, laboratory fortified blank (LFB_V1) for W-TOC, and the continuing calibration verification (ICV/CCV) standard. Holding time for the Stock standard is 180 days.

4.2.3.1. Dry 5g of primary grade potassium hydrogen phthalate at 120 ^oC for 12 hrs. Cool in a desiccator.

4.2.3.2. Add approx. 800 mL of DI water to a clean 1 L flask. Add 2 mL of concentrated H_2SO_4 and mix.

4.2.3.3. Weigh out 2.1254 g of the dried potassium hydrogen phthalate into a weighing boat.

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4.2.3.4. Rinse the contents of the weighing boat into the flask using DI water. Dilute to the mark and mix thoroughly. Pour this solution into the reagent bottle labeled **'TOC STOCK: 1000 PPM'.**

Note: This solution is also used for W-TOC analysis on TOC-V and TOC-L instruments. See SOP NU-062.

4.2.4. **Stock Standard Solution (10000 mg C/L)** - This solution is used for the calibration standards and the practical quantitation limit (PQCK4). Holding time for the Stock standard is 180 days.

4.2.4.1. Dry 5 g of primary grade dextrose at 120ºC for 12 hrs. Cool in a desiccator.

4.2.4.2. Add approx. 20 mL of DI water to a clean 100 mL flask. Add 0.2 mL of concentrated H_2SO_4 and mix.

4.2.4.3. Weigh out 2.505 g of the dried dextrose into a weighing boat.

4.2.4.4. Rinse the contents of the weighing boat into the flask using DI water. Dilute to the mark and mix thoroughly. Pour this solution into the reagent bottle labeled '**STOCK DEX 10000'**.

Note: This solution is also used for W-TOC analysis on TOC-V and TOC-L instruments. See SOP NU-062.

4.2.5. **Mixed Spiking Solution (500 mg N/L, 10000 mg C/L):** Used for the mixed W-TOC/W-TN spike (N1) and mixed Laboratory Fortified Blank (LFB_N1).

4.2.5.1 Dry about 6 g of dextrose and 1 g of potassium nitrate at 120ºC for 2 hours. Cool in a desiccator.

4.2.5.2. Add approx. 50 mL of DI water and 200 uL H_2SO_4 to a clean 100 mL flask.

4.2.5.3. Weigh out 0.3609 g of the dried KNO_3 into a weighing boat.

4.2.5.4. Weigh out 2.505 g of the dried dextrose into a weighing boat.

4.2.5.5. Rinse the contents of the weighing boats into the flask using DI water. Dilute to mark and mix thoroughly. Label this flask **'MIXED SPK'**. Holding time, 180 days.

4.2.6. **Inorganic Spiking Solution (1000 mg C/L):** Used for W-TOC inorganic

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spike (IS).

4.2.6.3. Dry about 5 g of Sodium Carbonate Anhydrous (Na_2CO_3) at 120 ^oC for 12 hrs. Cool in a desiccator.

4.2.6.4. Add approx. 150 mL of DI water to a clean 250 mL flask.

4.2.6.5. Weigh out 2.2125 g of the dried sodium carbonate into a weighing boat. Rinse the contents of the weighing boat into the flask using DI water. Dilute to the mark and mix thoroughly. Pour this standard into a reagent bottle labeled **'IC SPK 1000 PPM'**. **Do not acidify this standard**. Holding time, 180 days.

Note: This solution is also used for W-TOC analysis. See SOP NU-062.

4.2.7. **Glycine ($C_2H_5NO_2$); Purity 99+%,** Organic Nitrogen Stock Solution (1000 mg N/L)-This is used for the preparation of the Total Organic Nitrogen Check solution.

4.2.7.3. Dry about 3 g of glycine at 120 ^oC for 2 hours. Cool in a desiccator.

4.2.7.4. Add approx. 150 mL of DI water and 0.50 mL H_2SO_4 to a clean 250 mL flask.

4.2.7.5. Weigh out 1.3392 g of the dried glycine into a weighing boat.

4.2.7.6. Rinse the contents of the weighing boat into the flask using DI water. Dilute to the mark and mix thoroughly. Label this flask **'ORGANIC NITROGEN STOCK 1000 PPM'**. Holding time, 180 days.

Note: This solution is also used for low-level TN analysis. See SOP NU-105.

4.2.8. **Acid Rinse (HCL RINSE)** for Catalyst Regeneration - To a 1 L volumetric flask containing approx. 800 mL of DI water, carefully add 164 mL of concentrated HCL. Dilute to the mark and mix thoroughly. Holding time, 90 days.

4.3. Preparation of QC and Calibration Standards- Holding time, 30 days

4.3.1. **ALTQC** – Two ALTQC standards are prepared to check/verify the TOC and TN calibration standards respectively. The TOC ALTQC (**ALTQC##_TOC**) is a certified quality control standard purchased from ERA. For the expiration date of the purchased stock ampule, refer to the manufacturer's certificate of analysis.

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4.3.2. Prepare ALTQC standard to the "certified concentration" as instructed by the manufacturer. If the "certified concentration" is ≤ 40 PPM, then this standard is the working ALTQC standard, and can be used directly for analysis. If the "certified concentration" is >40 PPM, the prepared solution is an intermediate ALTQC. Name/label it in LIMS as "**ALTQC##_TOC Intermediate".** Dilute the intermediate appropriately so that the working concentration is within 10 PPM-40 PPM range. The working solution will be named/labeled **ALTQC##_TOC** in LIMS. Holding time of the diluted standards, 30 days.

Note: The initial ALTQC solution prepared from the ampule to the "certified concentration" must be acidified with the appropriate volume of H_2SO_4 to the concentration of 0.2% H_2SO_4. Any further dilution of this solution to the appropriate ALTQC working concentration must be done with diluent (4.3.4.).

4.3.3. **TN ALTQC (ALTQCTN##):** Currently there are no certified reference standards available for TN. The ALTQCTN is prepared by diluting the "NOX ERA STOCK ##" to a working concentration range of 1.0 – 4.0 mg N/L. NOX ERA STOCK is prepared from an ERA "Simple Nutrients" ampule, where the sum of the certified values for Ammonia and Nitrate/Nitrite is the concentration for TN.

Note: The ALTQCTN solution must be acidified with the appropriate volume of H_2SO_4 to the concentration of 0.2% H_2SO_4.

4.3.4. **Blank Standard/Diluent (0 mg C/L, 0 mg N/L)** - To a 2 L volumetric flask containing approx. 1.5 L of DI water, carefully add 4.0 mL of concentrated H_2SO_4. Dilute to the mark and mix thoroughly. Used for the method blank (MBLK) and continuing calibration blank (CCB) and rinses (RINSE).

Note: This solution is also used for W-TN and W-TOC analysis following SOP NU-105 (low level TN) NU-062, respectively.

4.3.5. **PQCK4 (1.0 mg C/L PQL) -** Check for W-TOC initial QC- To a 1 L volumetric flask containing approx. 900 mL of diluent (4.3.4.), add 0.1 mL of the 10000 mg C/L stock standard (4.2.4.), fill up to the mark with diluent and mix thoroughly.

Note: This solution is also used for W-TOC analysis. See SOP NU-062.

4.3.6. **ICV/CCV (20 mg C/L, 2 mg N/L)** - To a 2 L volumetric flask containing approx. 1 L of diluent (4.3.4.), add 40 mL of the 1000 mg C/L stock standard (4.2.3.), add 4 mL of the 1000 mg N/L KNO_3 Stock (4.2.2.), and fill up to the mark with diluent and mix thoroughly.

4.3.7. **Mixed TOC TN calibration standards**: Add about 250 mL diluent (4.3.4.) to each 500 mL volumetric flask. Add volumes of the stock

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solutions as described in the table below and make the volume up to the mark with diluent.

TABLE 4.3.7. Mixed Calibration Standards

Standard Name	Volume of 'STOCK DEX 10000' (mL) (4.2.4.)	Volume of 'TN Stock 1000 PPM' (mL) (4.2.1.)	Final Volume (mL)	TN conc mg N/L	TOC conc mg C/L
MDLSPK	0.04	0.25	500	0.50	0.80
CAL STD 1	0.05	0.40	500	0.80	1.0
CAL STD 5	0.25	0.625	500	1.25	5.0
CAL STD 20	1.0	1.0	500	2.0	20
CAL STD 100	5.0	2.5	500	5.0	100

4.4. **Total Organic Nitrogen Check** (**TONCHCK, 2 mg N/L**)-To a 250 mL volumetric flask containing approx. 100 mL of diluent (4.3.4.), add 0.5 mL of the 1000 mg N/L glycine stock solution (4.2.7.). Dilute to mark with diluent and mix thoroughly. Label this flask '**TONCHCK 2 PPM N**'. Holding time, 30 days.

5. SAMPLE HANDLING AND PRESERVATION

5.1. Samples are preserved with sulfuric acid at collection and stored on ice or at ≤ 6 °C (not frozen). Samples submitted to the laboratory for DN/DOC analysis must be filtered in the field prior to acidification All samples must be analyzed within 28 days of collection date for results to be reported unqualified.

5.2. Samples are stored in the Nutrient refrigerators in the receiving room of the FDEP laboratory. Allow samples to come to room temperature before processing.

6. SAMPLE PREPARATION AND ANALYSIS (SEE DEP SOP NU-033)

6.1. Open program TOC-CONTROL-L.

6.2. Choose Sample Table Editor icon →hit ok.

6.3. To start a new file and run: Click on the *New* button in the top left corner. Select the H/W settings on the drop-down menu → **TOC-L2-CPH-SPARGE** (External Sparge) or **TOC-L2-CPH** (Internal Sparge). To save the file, click *Save* and name the file YYMMDDLN# (where # is a number 1, 2, etc., that indicates the 1st, 2nd, etc., run of the day. Ex.: 120211N1). → *Save*.

6.4. In some cases, it may be helpful to perform an optional rinse before the calibration. To add the rinse sequence to the beginning of the run (the rinse blank result will be calculated from a previous calibration), highlight line 1, right click, and select *Insert*→ *Multiple Samples*. This opens the Sample Group Wizard.

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6.4.1. Page 1 Sample Source: Select *Method* option and browse button. Then select option a or b depending on the type of run:
a. 'NPOC_TN_EXSPARGE. met' → *Open* → *Next.* (external sparge run)
b. 'NPOC_TN.met' → *Open* → *Next.* (internal sparge run)

6.4.2. Page 2 Sample Parameters. Set options as follows:
Number of Samples = 1
Start Vial = 0
Sample Name = Enter file name (Ex.200101N1)
Sample ID = RINSE→ Finish

6.5. To setup a calibration sequence, highlight line two, right-click, and select *Insert-Calibration Curve* → Select appropriate cal file depending on the type of run:
a. 'NPOC CAL CURVE.EXSPARGE.cal' → *Open* (external sparge run)
b. 'NPOC CAL CURVE.cal'→ *Open* (internal sparge run)

6.6. After inserting the calibration, select the autosampler image in top right corner. Assign vial numbers as follows:
0 mg C/L standard = 1 (diluent, 4.3.4.)
0.8 mg C/L standard = 2 (MDLSPK, TABLE 4.3.7.)
1.0 mg C/L standard = 3 (CAL STD 1, TABLE 4.3.7.)
5.0 mg C/L standard = 4 (CAL STD 5, TABLE 4.3.7.)
20.0 mg C/L standard = 5 (CAL STD 20, TABLE 4.3.7)
100.0 mg C/L standard = 6 (CAL STD 100, TABLE 4.3.7)
Select *OK* to complete. The vial column numbers should read 1,2,3,4,5,6.

Note: Calibration sequence for both types of run external and internal sparge will be the same.

6.7. To setup the next calibration sequence, highlight line 2, right-click, and select *Insert- Calibration Curve* → Select appropriate cal file depending on the type of run:
a. 'TN CAL CURVE_EXSPARGE.cal' → *Open* (external sparge run)
b. 'TN CAL CURVE.cal' → *Open* (internal sparge run)

6.8. After inserting the calibration, select the autosampler image in top right corner. Assign vial numbers as follows:
0 mg C/L standard = 1 (diluent, 4.3.4.)
0.8 mg C/L standard = 2 (MDLSPK, TABLE 4.3.7.)
1.0 mg C/L standard = 3 (CAL STD 1, TABLE 4.3.7.)
5.0 mg C/L standard = 4 (CAL STD 5, TABLE 4.3.7.)
20.0 mg C/L standard = 5 (CAL STD 20, TABLE 4.3.7)
100.0 mg C/L standard = 6 (CAL STD 100, TABLE 4.3.7)
Select *OK* to complete. The vial column numbers should read 1,2,3,4,5,6.

Note: Calibration sequence for both types of run external and internal sparge will be the

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same.

6.9. To setup a sample sequence, highlight line four, select *Insert -Multiple Samples*. This will open the Sample Group Wizard.

6.9.1. Page 1 Sample Source; Select Method option and browse button. Then select method depending on the type of run:
a. 'NPOC_TN_EXSPARGE.met'→ *Next* (external sparge)
b. 'NPOC_TN.met'→ *Next* (internal sparge)
These methods have both calibration curves that were just created (6.5, 6.7).

6.9.2. Page 2 Sample Parameters; Set options as follows:
Number of Samples = 87
Start Vial = 7
Sample Name = Enter file name. (Ex.200101N1)
Sample ID = Leave blank
→ Finish → OK.

6.10. For sample and QC that only need to be analyzed for W-TN or W-TOC, highlight the row where you want to insert the sample, select *Insert-Calibration Curve* and select the corresponding file (6.5. or 6.7.) and add a single sample as before.

6.11. Template for both internal and external sparge runs have been created so that a new run sequence does not need to be performed for every analysis. Click *open* in the top left of the software. Select the appropriate template for High Level run. . Insert the sample IDs and save the file name. Then go to File→*print*. This will be the bench sheet.

6.12. Use appropriate vials for analysis based on the type of run:

External sparge runs: DWK Kimble Glass Screw-Thread Vials with PTFE/Silicone Septa 24 mL (Table 3.4.)
Internal sparge runs: Glass Shell Vials (Table 3.4.)
For internal sparge runs, samples, QC standards, and calibration standards can be poured directly into Glass Shell Vials (Table 3.4.). For external sparge runs, all samples and standards must be pipetted into labeled 24 mL Glass Screw-Thread Vials (Table 3.4.) according to the run sequence. Place them in the autosampler rack and return rack to turntable for realignment. Make sure that all vials are correctly seated.

6.12.1. For each batch (20 samples or less) : Select a quality control sample prepared in duplicate to assess precision for W-TN and W-TOC. Prepare a 2 mg N/L and 40 mg C/L matrix spike (spike code M1) by adding 80 µL of the MIXED SPK (4.2.5.) to one 20 mL portion of the selected sample.

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Prepare a laboratory fortified blank (LFB) following the same procedure as the 2 mg N/L/40 mg C/L matrix spike by substituting blank standard (4.3.4.) for the sample. Effluent samples are batched separately.

6.12.2. Inorganic spike (spike code IS): For each analytical run, prepare one inorganic sample spike. Add 400 µL of the 1000 mg C/L inorganic spiking solution (4.2.6.) to one 20 mL portion of a selected sample.

Note: Ensure appropriate vials are used based on the type of run for all batch QC samples (spike, LFB, and inorganic spike). All the mentioned batch QC samples are pipetted according to the appropriate volume.

6.12.3. Samples with a conductivity of ≥15,000 umhos/cm and ≤30,000 umhos/cm and/or a salinity of 9 PPTh or greater are batched as salt samples and are diluted 1:2. Salt samples with conductivity of >30,000 umhos/cm are diluted 1:4. A 2N hydrochloric (HCl) acid rinse (4.2.8.) is run every 5 cups in order to minimize residue build up on the catalyst. An additional diluent rinse (RINSE, 4.3.4.) may be added after every salt sample to further clean the catalyst. Salt batch is placed at the end of a run and no more than fifteen salt samples should be analyzed per run. The run should end with at least 1 HCL rinse and 4 diluent rinses to further minimize residue build up on the catalyst.

6.12.4. Each run begins with the following sequence:

- **Rinse** (4.3.3., 6.4.) (optional)
- **Calibration** (6.5., 6.7.)
- **ICV** (Mixed Check for TOC & TN, 4.3.6.)
- **MDLSPK** (Table 4.3.7.)
- **TONCHCK** (4.4.)
- **TONCHCK** (4.4.)

TONCHCK must be run twice. (see 9.4.)

- **PQCK4** (PQL 4.3.5.)
- **ALTQC##_TOC** (4.3.2.)
- **ALTQCTN##** (4.3.3.)
- **CCV** (CCV 4.3.6.) and **CCB** (Blank Standard 4.3.4.) – these continuing calibration checks are scheduled to bracket every 10th analysis on the run.
- **Batched samples** including **MBLKs** (4.3.4.), **LFBs** (6.12.1.), duplicate samples and spiked samples (6.12.1.)
- Final **CCV** and **CCB**

6.12.5. It is recommended to end each run with 8 rinses (RINSE 4.3.4., 6.4.) to clean the catalyst.

Note: The first CCV after a new calibration is called the Initial Calibration Verification standard (ICV). The first CCV is labeled as the ICV on each new run and all subsequent CCVs are labeled as CCV.

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6.13. Connect Instrument: Click *Connect.* Wait for connection and ready indicator (highlighted green when ready), then press *Start*→ Check Shut down instrument box → press Run to start the run.

Note: Print the run file that was just created by going to *file* → *print*→ *sample table*.

6.14. Post-run Procedure: Results are exported as an ASCII file and converted into a raw file in the QC manager for performing calculations and uploading to LIMS.

6.14.1. *File* → *ASCII Export Setting* → *Detail* → *Data.* Check the following export options; Sample ID, Dilution, Date/Time, Mean Conc., Result, and Vial. Also make sure the export data box is checked. Under the "Misc" tab that asks for the separator settings, choose "tabulator," and check the box for "Export strings in quotation marks…".

6.14.2. To export file: *File* → *ASCII Export* → *Detail* (Include Injection Data) → *Filename* → *Save.*

6.14.3. Export to C:\TOC-L2_TN\Data for TOC-L2 in the current years' subfolder. This is the local drive for the instrument workstation.

6.14.4. Open C:\TOC-L2 in Windows Explorer. Locate the text file you just created, as well as the peak (*.pkt), calibration (*.cal) and sample table (*.t32 or *.tlx) files associated with the analysis and copy all four files from the local drive to the appropriate instrument folder (TOC-L2) in the current year's subfolder located at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\Tocv\TOC-L2
Note: the exact location of the data files within instrument folder structure is determined by the software settings (Tools > Organize Files; please see User manuals for details).

6.14.5. Print a copy of the final report and data table containing injection peaks by selecting: *File* → *Print* → *Sample Report –All*, then *File* → *Print* → *Sample Table.*

6.15. Quantitation/Calculations:

6.15.1. To upload the file, log into LIMS, select QC Manager (QCM), and select the appropriate instrument. Click the 'Convert File" button, and the selection the appropriate folder (current year). The Text File that was exported (6.14.3.) should be available for conversion. Select the appropriate file, and follow the QC Manager's (QCM) instructions.

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6.15.2. The instrument will give the values for NPOC in mg C/L and Total Nitrogen in mg N/L. The results for TN and NPOC calculated by the instrument is the average of three out of five injections for a given sample with percent relative standard deviation of less than 5 %. The calibration consists of five standards plus a blank. The regression model applied is linear.

6.15.3. The QC Manager will calculate spike and standard recoveries and RPDs. For general calculations including RPDs and spike recoveries, see the FDEP Quality Manual Section 5.9.

6.15.3. The calibrants are all prepared manually (Section 4.3.4. and 4.3.7.). The correlation coefficient for the calibration is calculated by the instrument software. Use the current NU-TOC_TN form located at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\Tocv\ to calculate the recoveries of the calibrants. Open the form, enter the header information and the slope, intercept, and mean area results to calculate the recovery of calibrants. Acceptance limits for the recoveries of the calibration standards above the PQL are 90 – 110 % and 70 – 130 % for the PQL-level standard. For TOC, the 0.8 mg C/L standard (MDLSPK) acceptance limits are 62.5-150%. The acceptance limits for the blank calibration point results are below the MDL and above the negative value of the MDL (-MDL < blank < MDL).

7. DATA ARCHIVAL

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (W-TOC/W-TN), filename/s (YYMMDDN#), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data and calibration curve, calibration standard recovery sheet, results report method parameters, bench sheet, Reagent and Standard Logsheet (ensure TOC-TN specific Reagent and Standard Logsheet NU-RGTSTD-TOCTN is used), and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.3. The report folders are on file with the nutrients supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. Instrument raw data files, including peak (*.pkt), calibration (*.cal) and sample table (*.t32 or *.tlx) files, are backed up from instrument PCs and stored on the Nutrients drive under \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\Tocv\TOC-L2_TN.

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7.5. TOC/TN instrument data text transfer files for upload to QC manager are stored in electronic format in appropriate instrument folder (TOC-L2) in the current year's subfolder located at \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\Tocv\

7.6 Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. MAINTENANCE/TROUBLESHOOTING

Note: Make sure all maintenance performed is documented in the instrument's maintenance log.

8.1. Maintenance:

8.1.1. Before beginning sample analysis, conduct a general inspection of the instrument to ensure that everything is in order, and that there is enough UHP Air to complete the run.

8.1.2. The Humidifier and Dehumidifier vessels should be filled with DI water to the fill line on the vessel. Once a month, the humidifier vessel should be drained and refilled with fresh DI water. This prevents contamination.

8.1.3. Inspect the Drain Port (on the side of the instrument) weekly. Drain Port should be drained and refilled with fresh DI water once a month (Refer to user's manual for instructions and details).

8.1.4. Keep the rinse water bottle filled with DI water. If running samples overnight, fill the bottle with DI water before starting the run.

8.1.5. The diluent bottle should be changed with fresh Blank Standard/Diluent (4.3.4.) at least once per month.

8.1.6. Make sure the HCL Intake is changed with fresh 2N HCL (4.2.8.) at least once every three months.

8.1.7. Inspect the slider and O-rings before analysis and clean with DI water if needed.

8.1.8. Check the waste line daily to ensure proper flow. Waste backup will damage the autosampler. Dump waste down the drain as needed.

8.1.9. Regeneration of TC catalyst should be performed on a case by case basis TC catalyst regeneration has little effect on TN analysis. See instrument

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manual for regeneration procedure. Regeneration should be followed with a run of at least 20 CCBs and a few CCVs.

8.1.10. Other maintenance may be required periodically, such as replacing catalyst, replacing halogen-scrubber, maintaining syringe and 8-port valve. Refer to user's manuals for instructions and details.

8.2. Troubleshooting Notes:

8.2.1. To stop the run at any point and delete the calibration, Click *Stop* button → *Stop* (stop all processes immediately).

8.2.2. To interrupt the run at any point and save the calibration, Click *Stop* button → *Stop* (after current sample is completed).

8.2.3. In order to restart a run in progress (for example, if there is a QC failure at the beginning of a run) and keep the sample table set up for a new run, previous data gathered or attempted must be deleted prior to restart. Highlight the lines to delete, go to *Edit* → *Clear Measured Data* → *Highlighted* → *Yes*, then click *View Vial Settings* → Assign the vial numbers for rerun samples.

8.2.4. Treat samples suspected to contain humic acids as follows:
a. Split a well-mixed sample into two portions.
b. Keep one portion at pH ≤ 2 and adjust the other to pH 5-7 with 10 N Sodium Hydroxide to increase the solubility of humic acid.
c. Cap and allow both portions to sit at room temperature for ½ hour.
d. Analyze both splits and report the highest result.

9. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE NU-038 AND NU-043)

9.1. A new calibration must be performed at the beginning of the analysis and the linear calibration range (LCR) must be verified (see 9.2.). Consecutive runs on one day may use the same calibration. The linear calibration consists of five standards (CAL STD 100, CAL STD 20, CAL STD 5, CAL STD 1, MDLSPK) and a blank. It is followed by a sequence of initial quality control standards as described in section 6.12.

9.2. LCR: See the FDEP Quality Manual Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear regression (r^2) must be equal to or larger than 0.995. Acceptance limits for the recoveries of the calibration standards above the PQL are 90 – 110 % and 70 – 130 % for the PQL-level standard. The acceptance limit for the **TOC** MDLSPK standard (below the PQL) is 62.5 – 150 % recovery. The

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acceptance limits for the blank calibration point results are below the MDL and above the negative value of the MDL (-MDL < blank < MDL).

9.3. Each analytical run may contain a number of sample batches, with no more than 20 samples per batch. Each batch is required to have an MBLK, LFB, a matrix spike, and a duplicate sample. The spikes are prepared according to section 6.12.1. Acceptance limits for the LFB and matrix spike recoveries are 85-115 %.

9.4. TN: On every analytical run, one organic nitrogen check (TONCHCK) is run at least twice at the beginning of the run (6.12.14.). The result must be between 85-115 % recovery. If the recovery is outside the control limit the samples must be reanalyzed or reported qualified. The instrument should be checked to ensure that it is operating correctly.
Note: The method requires the analysis of at least **4 portions** of **TONCHCK** (Organic Nitrogen Test Solution) (4.4., 6.12.4.) at the same volume as samples to be analyzed. To satisfy this requirement, **TONCHCK** is put on the run sequence at least twice (for a minimum total injection of 6 and up to 10 injections).

9.5. The AltQC standards (ALTQC##_TOC, ALTQCTN##) are second source standards with acceptance limits supplied by the manufacturer. One AltQC standard for TN and one AltQC for TOC are analyzed with every analytical run. Acceptance limits are supplied by the manufacturer. (Refer to 4.3.2., 4.3.3.)

9.6. One MDLSPK (MDLSPK, TABLE 4.3.7.) is analyzed for every analytical run. MBLK and MDLSPK results are used for ongoing MDL and PQL verification. The acceptance limit for the MDLSPK standard for TOC (below the PQL) is 62.5 – 150 % recovery, for TN 70 – 130 % recovery.

9.7. One PQL (PQCK 4.3.5.) level standard for TOC is analyzed for every analytical run. For analytical runs that are longer than 24 hours, at least one PQL standard is analyzed every 24 hrs. Acceptance limits for the PQCK recovery are 70-130 %.

9.8. A CCV/ICV (CCV 4.3.6.) and a blank (CCB 4.3.4.) standard are run at the beginning of every run, after every 10^{th} analysis, and at the end of the run. Acceptance limits for the recoveries of the check standard are 90-110 %.

9.9. The sample duplicate results should be less than 20 % RPD for results over the practical quantitation limit (PQL).

9.10. Acceptance limits for the blanks (MBLKs and CCBs) are < 0.10 and > -0.10 mg N/L for TN and < 0.50 and > -0.50 mg C/L for TOC.

9.11. For general method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data, refer to DEP SOPs NU-038 and SOP NU-043. If

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there are any anomalous results (e.g., differing results for multiple analyses of same sample) or any unusual events (spilled sample, cannot locate sample, etc.), see supervisor for further instruction.

10. SAFETY/WASTE MANAGEMENT/POLLUTION PREVENTION

10.1. Refer to the MSDS notebook for information on safe handling of samples and reagents used with instrument.

10.2. Flush sample waste (as long as no other known hazards are present) down the sink with plenty of water.

10.3. Review the laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

10.4. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

11. REFERENCES

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

11.1. ASTM D8083-16 and Standard Methods Online, 5310 B-2011.

11.2 Shimadzu TOC-L/TOC-L2 Manual.

11.3. Quality Manual, Bureau of Laboratories, FDEP.

11.4. DEP SOP NU-062, Determination of Total Organic Carbon (TOC) and dissolved Organic Carbon (DOC) Using the Shimadzu TOC Analyzer

11.5. DEP SOP NU-033, Summary of Analysis Procedures for the General Chemistry and Nutrients Workgroups.

11.6. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.7. DEP SOP NU-041, Reagent Preparation Procedure.

11.8. DEP SOP NU-042, Standard Preparation Procedure.

11.9. DEP SOP NU-043, General Chemistry Quality Assurance and Data Authorization Guidelines.

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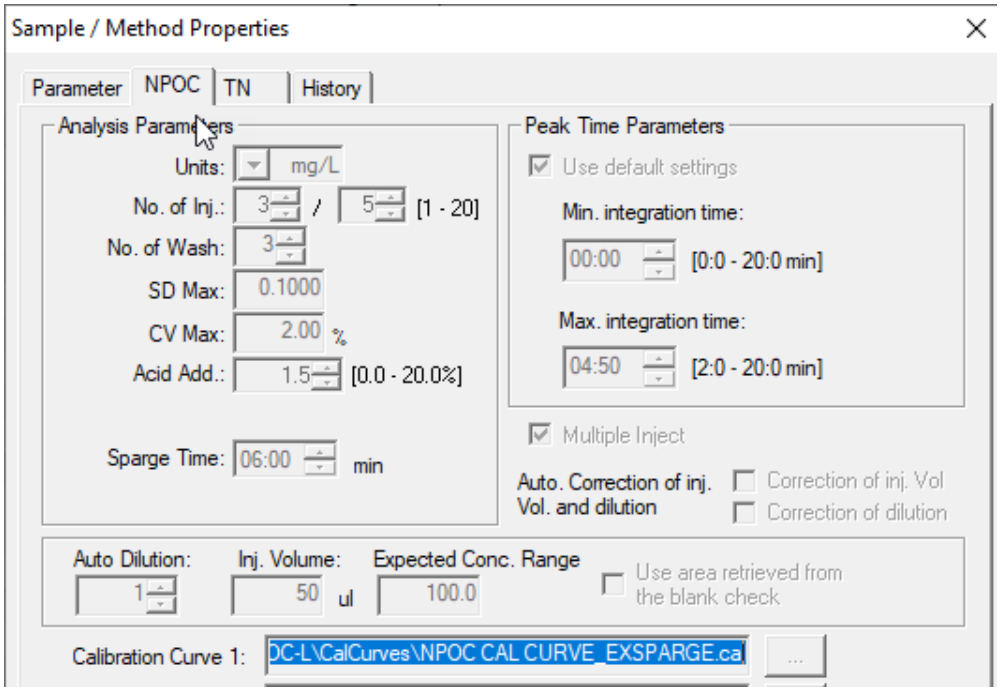
11.10. Laboratory Health and Safety Plan.

11.11. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

APPENDIX I: INSTRUMENT SETTINGS- Parameters

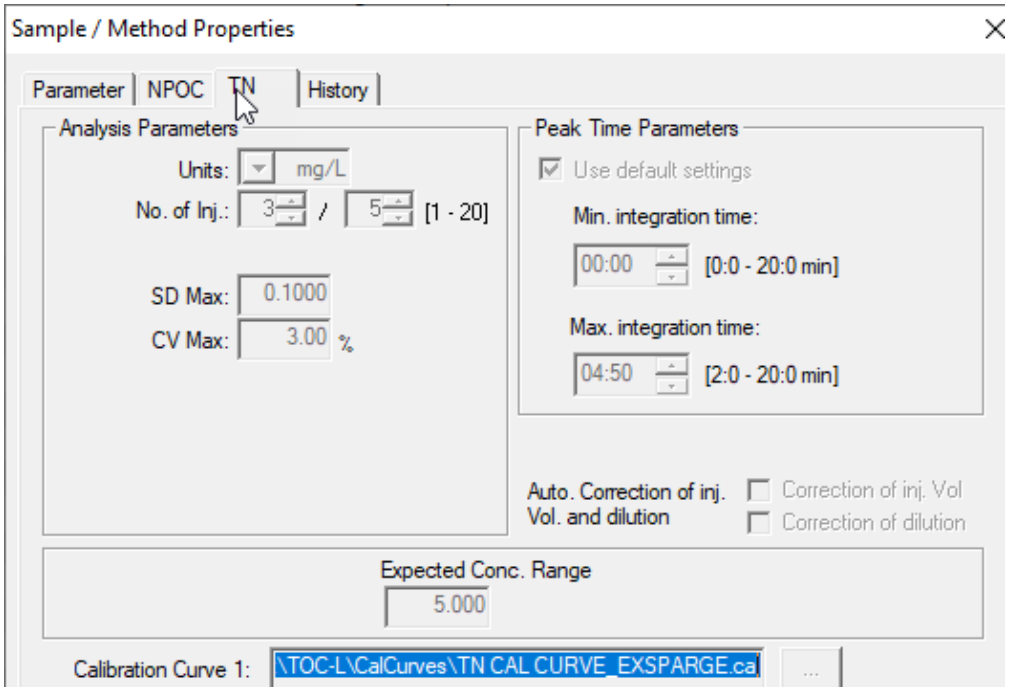
Note: Refer to Shimadzu Software User's Manual for details.

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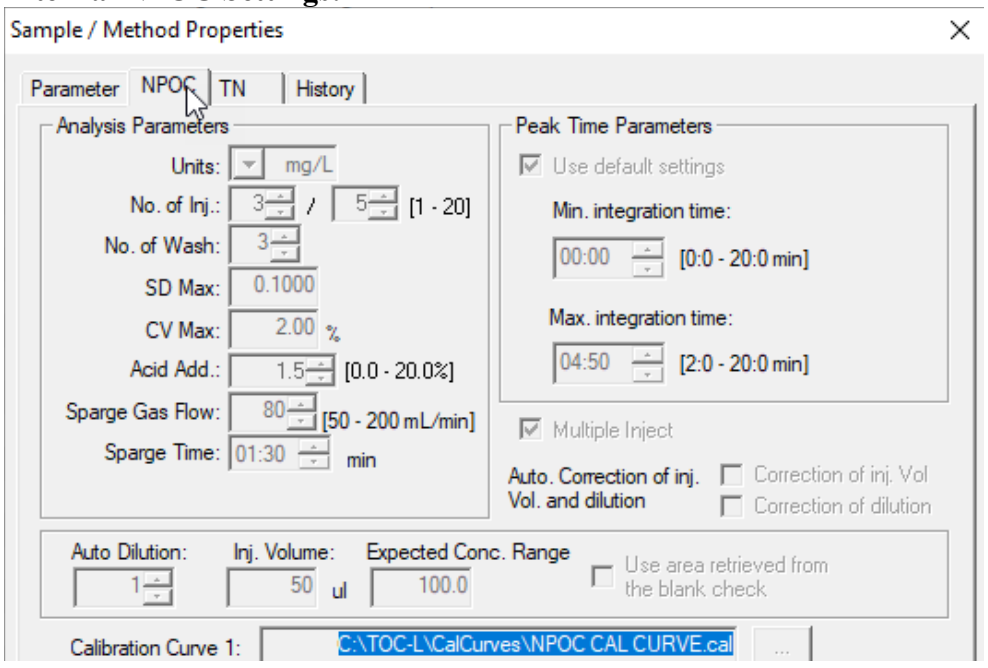


External TN Settings:

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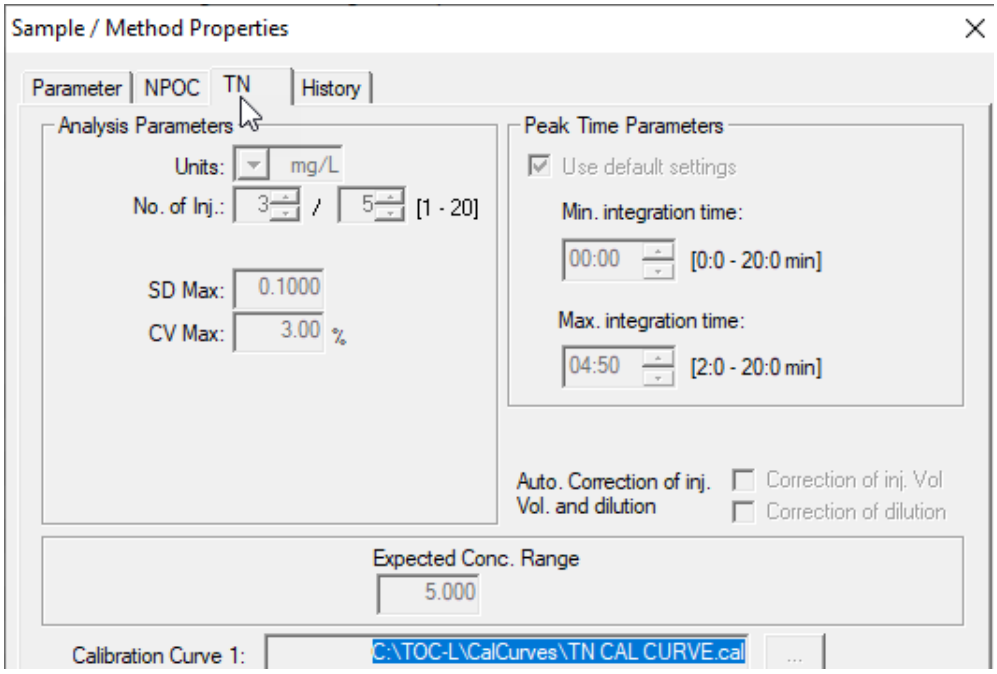


Internal NPOC Settings:



Internal TN Settings:

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APPENDIX OF SIGNIFICANT CHANGES

01/29/2020 SECTION 4: Added clarification that the ICV/CCV stock solution is prepared from a different source or lot of KNO_3 as used for the calibration standards.

02/14/2020 SECTION 6 AND SECTION 9: Clarified the acceptance limits for the blank calibration point in the calibration curve.

03/17/2021 Added instructions for analyzing high salinity samples.

01/25/2022 Following revisions were made to the SOP:

- Updated Table of Contents
- Added an option to run TOC/TN Analysis on Internal Sparge setting.
- Added tables to include instrumentation information, supplies & consumables, chemicals, and standards.
- Added instructions to run TONCHCK twice to fulfill method requirement.
- Rearranged the preparation of the stock standards, standards, calibration, and reagents sections to improve SOP flow.
- Updated the run/template set up sections to integrate the Internal Sparge setting and option.

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- Added brief instructions on uploading file to QCM.
- Updated the nomenclature of the methods and calibration files for run/template set up sections.
- Updated the maintenance section to include inspection and replacement of DI water of the Drain Port.
- Added the APPENDIX I section to include scanned tables (source of tables is the instrument software) that display the TOC/TN parameters for both internal and external sparge.

03/11/2022 Modified instructions for sample salt samples to allow for 1:4 dilutions of high conductivity samples >30,000 uhm/cm. Added and provided instructions on the use of the Reagent and Standard LogSheet (NU-RGTSTD-TOCTN) specific to TOC/TN analysis.

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