ANALYSIS OF LOW-LEVEL TOTAL NITROGEN (TN) AND DISSOLVED NITROGEN (DN) USING SHIMADZU TOC INSTRUMENTS EQUIPPED WITH TOTAL NITROGEN MODULES (TNM-L)

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1. SCOPE AND APPLICATION

1.1. This SOP details the analysis Total Nitrogen (TN) and Dissolved Nitrogen (DN) in surface waters, ground waters, effluents and influents. The tests covered by this SOP are W-TN and W-DN.

Key method parameters:

	W-TN/W-DN
Calibration range	0.0 – 0.50 mg N/L
Minimum Detection Limit (MDL)	0.020 mg N/L
Practical Quantitation Limit (PQL)	0.050 mg N/L

1.2. Results are reported without qualification in the range from the practical quantitation limit (PQL) 0.050 mg N/L up to 0.50 mg N/L. Samples with higher concentrations are analyzed following SOP NU-106 or after dilution into the range.

2. SUMMARY OF THE METHOD

2.1. The described procedure is based on ASTM D8083-16. The method allows for either use of no sparge or external sparging as described in 2.2.

Note: *Only the **TOC-N-CPH** instrument can be 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 no sparge or external sparging. In the external sparging procedure, the samples are purged with ultra-high purity (UHP) air using an external sparge kit for a duration of 360 seconds prior to injection. In the no sparging procedure, the samples are purged with ultra-high purity (UHP) air for the duration of 90 seconds in the syringe/plunger prior to injection.

The external sparge kit 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.

2.3. This method quantitatively measures nitrogen from organic and inorganic nitrogen compounds. The sample (300 µl) is combusted at 720 ºC in the presence of a platinum catalyst. Under these conditions all nitrogen containing compounds are converted to nitrogen oxide (NO). This is followed by a reaction 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 detector.

Note: *The combustion temperature and sample injection volume are the same for both no sparge and external sparging methods. See APPENDIX I for further details.*

2.4. This procedure is applicable to nitrogenous matter in the sample that can be injected into the apparatus reproducibly. The method does not measure N_2 as gas.

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2.5. The method requires the analysis of at least **4 portions** of **TONCHCK** (Organic Nitrogen Test Solution) (6.8.4., 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. Interferences:

2.6.1. 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. Dissolved salts that are not volatized may result in devitrification or solidify in the catalyst tube resulting in decreased flow rates and decreased catalyst efficiency. Total organic carbon (TOC) up to 300 mg/L does not interfere with the Total Nitrogen analysis.

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

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation

Instrumentation Unique ID	Component	Manufacturer	Model/Product #
W-TOC-N-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
W-TOC-O-CSH	Total Organic Carbon Analyzer	Shimadzu	TOC-L-CSH
	Autosampler ASI-L for 24 mL, ROHS - compliant	Shimadzu	638-93199-58
	TNM-L Total Nitrogen Module, ROHS	Shimadzu	638-91151-43
	External Sparge Kit for ASI-L	Shimadzu	638-77183-41
	TOC Control L Software	Shimadzu	Ver. 1.09
W-TOC-P-CSH	Total Organic Carbon Analyzer	Shimadzu	TOC-L-CSH
	Autosampler ASI-L for 24 mL, ROHS - compliant	Shimadzu	638-93199-58
	TNM-L Total Nitrogen Module, ROHS	Shimadzu	638-91151-43
	External Sparge Kit for ASI-L	Shimadzu	638-77183-41
	TOC Control L Software	Shimadzu	Ver. 1.09

3.2. Instrument Accessories

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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[1]	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
Glass Funnel	Fisher (SR)	10-346-5B
Desiccator	Fisher Scientific	08-645-6
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom.		

3.4. Supplies & Consumables

Equipment	Manufacturer/ Vendor[1]	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 [1]**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, NU-042, and LB-030)

Purchased chemicals, reagents, and standards are processed according to the provisions in

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SOPs NU-041, NU-042 and LB-030. Before use they are entered into the laboratories LIMS system and labeled with a unique ID and expiration date. Remember that expiration dates on purchased materials can be found either on the container or the certificate of analysis, if available. If no explicit expiration dates are assigned by the manufacturer follow the protocol outlined in the aforementioned SOP's.

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.

Note: All reagent and standard preparations must be documented in the Reagent and Standard Logsheet for W-TN analysis. This excel sheet (NU-RGTSTD) is located in the subfolder \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TOC-TN\Forms\. It needs to be updated whenever a reagent or standard is newly prepared. For every analysis file print the 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 subfolder\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TOC-TN\CertificatesofAnalysis\. All containers holding standards or reagents must be labeled with their unique ID and expiration date. In-House deionized water, used for reagent and standard preparation, stored in containers is replaced daily.

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	Purity	Storage
4.1.1.	Deionized (DI)	H_2O	In-House	N/A	N/A	N/A
4.1.2.	Air,	Air Ultra Zero	Airgas	AI UZ300	Ultra Zero	Room Temp
4.1.3.	Sulfuric Acid	H_2SO_4	Fisher (SR)	A300S-212	Certified ACS	Room Temp
4.1.4.	Hydrochloric Acid	HCl	Fisher (SR)	A144S-212	36.5 – 38.0% (w/w),	Room Temp
4.1.5.	Potassium	KNO_3	Fisher	P-383-500	Certified ACS	Room Temp
4.1.6.	Ammonium Sulfate	$(NH_4)_2SO_4$	Fisher	A702-500	≥99.0%, Certified ACS	Room Temp
4.1.7.	Glycine	$C_2H_5NO_2$	Sigma-Aldrich	G7126-100G	≥99% (HPLC), ReagentPlus®	Desiccator
4.1.8.	Simple Nutrients Certified	SIM NUT AMP (NOX ERA STOCK##)	ERA	505	N/A	Fridge
4.1.9.	Sodium Hydroxide	NaOH	Fisher (SR)	S318-3	≥97.0 %, Certified ACS	Room Temp

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4.1.10.	Drierite 10-20 Mesh, White	$CaSO_4$, Desiccant	W.A. Hammond Drierite Co.	14005/07-577-4B	≥99.5% $CaSO_4$	Room Temp
4.1.11.	Drierite 8 Mesh, Blue	$CaSO_4$ and $CoCl_2$ Desiccant	W.A. Hammond Drierite Co.	23005/S692-212	≥98% $CaSO_4$, <2% $CoCl_2$	Room Temp
4.1.12.	Platinum Catalyst, 20g	Catalyst	Shimadzu	638-60202-01	N/A	Room Temp
4.1.13.	Halogen Scrubber	Halogen Scrubber	Shimadzu	630-00992-00	≥99% Copper	Room Temp

Deionized water is replaced daily. All other purchased chemicals are given the expiration date from the CoA and/or chemical container. If there is not an expiration date listed, the expiration date is designated by following the protocol in SOPs NU-041 and NU-042.

4.2. Stock standards, spiking solutions, & reagents

4.2.1. **Stock Standard Solution (1000 mg N/L)** –

Potassium Nitrate (4.1.5.)	3.609 g
Ammonium Sulfate (4.1.6.)	2.360 g
Sulfuric Acid (4.1.3.)	2 mL
DI Water (4.1.1.)	to 1000 mL

Dry approx. 8 g of Potassium Nitrate (KNO_3) and approx. 5 g of Ammonium Sulfate ($(NH_4)_2SO_4$) at 120ºC for 2 hours. Cool in a desiccator.

Weigh out 3.609 g of dried KNO_3 into a weighing boat. Weigh out 2.360 g of dried $(NH_4)_2SO_4$ into a separate weighing boat. In a 1000 mL volumetric flask containing approx. 800 mL of DI water, add 2 mL of H_2SO_4. Swirl to mix. Next, rinse the contents of the weighing boats into the flask using DI water. Dilute to the mark and invert several times to mix thoroughly. Label this flask **'TN STOCK 1000 PPM'**.

This solution is used for the preparation of the calibration standards. Holding time, 180 days.

Note: This solution is also used for high-level TN analysis. See SOP NU-106.

4.2.2. **KNO_3 Stock (50 mg N/L) –**

Potassium Nitrate (4.1.5.)	0.3609 g
Sulfuric Acid (4.1.3.)	2 mL
DI Water (4.1.1.)	to 1000 mL

Dry approx. 1 g of Potassium Nitrate (KNO_3) at 120°C for 2 hours. Cool in a desiccator.

Weigh out 0.3609 g of the dried KNO_3 into a weighing boat. In a 1000 mL volumetric flask containing approx. 800 mL of DI water, add 2 mL of H_2SO_4. Swirl to mix. Next, rinse the contents of the weighing boat into the

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flask using DI water. Dilute to the mark and invert several times to mix thoroughly. Transfer to a 1 L storage bottle and label **'50 PPM KNO3'**.

This solution is used for the preparation of the CCV, Spikes, and LFB. Holding time, 90 days.

4.2.3. **Glycine ($C_2H_5NO_2$); Purity 99+%**, Organic Nitrogen Stock Solution (1000 mg N/L) –

Glycine (4.1.7.)	1.3392 g
Sulfuric Acid (4.1.3.)	0.5 mL
DI Water (4.1.1.)	to 250 mL

Dry approx. 3 g of glycine at 120°C for 2 hours. Cool in a desiccator.

Weigh 1.3392 g of the dried glycine into a weighing boat. In a 250 mL volumetric flask containing approx. 150 mL of DI water, add 0.5 mL of H_2SO_4. Swirl to mix. Next, rinse the contents of the weighing boat into the flask using DI water. Dilute to the mark and invert several times to mix thoroughly. Label this flask **'ORGANIC NITROGEN STOCK 1000 PPM'**.

This is used for the preparation of the Total Organic Nitrogen Check solution. Holding time, 180 days.

Note: This reagent (ORGANIC NITROGEN STOCK 1000 PPM) is also used for simultaneous TOC/TN analysis SOP NU-106.

4.2.4. **Acid Rinse (ACID RINSE)** for Catalyst Regeneration –

Hydrochloric Acid (4.1.4.)	164 mL
DI Water (4.1.1.)	to 1000 mL

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 invert several times to mix thoroughly.

Holding time, 90 days.

4.3. Preparation of QC and Calibration Standards –

4.3.1. **TN ALTQC (ALTQCTNLO##)** –

NOX ERA STOCK (made from 4.1.8.)	See CoA
Sulfuric Acid (4.1.3.)	to 0.2%
DI Water (4.1.1.)	to volume

This solution is used as **ICV/IRM** (Initial Calibration Verification/Independent Reference Material). Currently there are no certified reference standards available for TN. The ALTQCTNLO is prepared by diluting the "NOX ERA STOCK ##" in appropriate volume of acidified (0.2% H_2SO_4) DI Water to a working concentration close to the midpoint of the calibration range. Alternatively, the ALTQC prepared for high level TN may be diluted to the proper low level range, using the naming format ALTQCTN##_DIL (See NU-106).

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NOX ERA STOCK is prepared from an ERA "Simple Nutrients" ampule that is obtained from an independent (secondary) source where the sum of the certified values for Ammonia and Nitrate/Nitrite is the concentration for TN.

Holding time of the TN ALTQC, 30 days.

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

4.3.2. **Blank Standard/Diluent (0 mg N/L)** –

Sulfuric Acid (4.1.3.)	4.0 mL
DI Water (4.1.1.)	to 2000 mL

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 invert several times to mix thoroughly.

Used for the method blank (MBLK) and continuing calibration blank (CCB). This solution is also used for W-TOC analysis (see SOP NU-106).

Holding time, 30 days.

4.3.3. **(PQCK, 0.050 mg N/L) PQL** and Calibration Standard –

TN STOCK 1000 PPM (4.2.1.)	0.050 mL
Diluent (4.3.2.)	to 1000 mL

To a 1 L volumetric flask containing approx. 900 mL of diluent, add 0.050 mL of the TN STOCK 1000 PPM. Fill up to the mark with diluent and invert several times to mix thoroughly. Transfer to a 1 L storage bottle and label as **'PQCK 0.050 PPM N'**.

Holding time, 30 days.

4.3.4. **CCV3, 0.20 mg N/L** (Continuing Calibration Verification Standard) –

50 PPM KNO3 Stock (4.2.2.)	4.0 mL
Diluent (4.3.2.)	to 1000 mL

To a 1 L volumetric flask containing approx. 800 mL of diluent, add 4.0 mL of the 50 PPM KNO3 stock standard. Fill up to the mark with diluent and invert several times to mix thoroughly. Transfer to a 1 L storage bottle and label as **'CCV/0.2 PPM N'**.

Holding time, 30 days.

4.3.5. **TN Low Level Calibration Standards** –

TN STOCK 1000 PPM (4.2.1.)	See Table
Diluent (4.3.2.)	to 1000 mL

In a 1000 mL volumetric flask containing 900 mL of diluent, add the appropriate volume of TN STOCK 1000 PPM as outlined in the table below for each standard. Fill up to the mark with diluent and invert several times to mix thoroughly. Transfer to a 1 L storage bottle and label

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appropriately.

Holding time, 30 days.

Standard Name	Volume of 'TN Stock 1000 PPM' (mL) (4.2.1.)	Final Volume (mL)	TN conc (mg N/L)
0.05 PPM N PQCK	0.050	1000	0.05
0.1 PPM N	0.100	1000	0.10
0.2 PPM N	0.200	1000	0.20
0.4 PPM N	0.400	1000	0.40
0.5 PPM N	0.500	1000	0.50

Note: If using the same volumetric flask to prepare the standards, make sure to start from the lowest concentration, and ensure the volumetric flask is thoroughly rinsed between the preparation of each standard.

4.3.6. **Total Organic Nitrogen Check (TONCHCK2, 0.2 mg N/L)** –

1000 mg N/L Glycine Stock (4.2.3.)	0.050 mL
Diluent (4.3.2.)	to 250 mL

To a 250 mL volumetric flask containing approx. 100 mL of diluent, add 0.050 mL of the 1000 mg N/L glycine stock solution. Dilute to mark and invert several times to mix thoroughly. Label this flask **'TONCHCK2 0.2 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 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** for external sparging (TOC-N only) or for internal sparging, **TOC-L2-CPH** (TOC-N), **TOC-O-CSH,** or **TOC-P-CSH** (depending on instrument). To save the file, click *Save* and name the file YYMMDDN(/P/O)#_LTN (where # is a number 1, 2, etc., that indicates the 1st, 2nd, etc., run of the day.). → *Save*.

6.4. In some cases, it may be helpful to perform an optional rinse before the

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calibration. Analyst also has an option of running four rinses (if analyzing salt samples, 4 rinses are required) at the end of the run. 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.

6.5. To setup a calibration sequence, highlight line 2 and then select *Insert* → *Calibration Curve* → Select folder icon → *Data* → *OK* → 'LTN CALIBRATION.cal' → *Open*.

Note: Verify that you have the correct method and sparge parameters for the type of analysis:

a. 'LTN CALIBRATION.cal' → *Next*
b. 'LTN CALIBRATION EXSPARGE.cal' → *Next* (external sparge-duration 360 seconds)

6.6. After inserting the calibration, select the autosampler image in top right corner. Assign vial numbers as follows:

0 mg N/L standard = 1 (diluent, 4.3.2.)
0.05 mg N/L standard = 2 (PQCK, Table 4.3.5.)
0.1 mg N/L standard = 3 (0.1 PPM N, Table 4.3.5.)
0.2 mg N/L standard = 4 (0.2 PPM N, Table 4.3.5.)
0.4 mg N/L standard = 5 (0.4 PPM N, Table 4.3.5.)
0.5 mg N/L standard = 6 (0.5 PPM N, Table 4.3.5.)

Select *OK* to complete. The vial column numbers should read 1,2,3,4,5,6.

6.7. To setup a sample sequence, highlight line three, select *Insert* → *Multiple Samples*. This will open the Sample Group Wizard.

6.7.1. Page 1 Sample Source; Select Calibration Curve option and browse button. Then select calibration file that was just created (6.5) and open → Next.

6.7.2. Page 2 Sample Parameters; Set options as follows:

Number of Samples = number of lines needed to complete the run.
Start Vial = 7
Sample Name = Enter file name. (YYMMDDN(P/O)#_LTN)
Sample ID = Leave blank
→ Finish → OK.

6.8. 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.)

No sparge runs: Glass Shell Vials (Table 3.4.)

For No sparge runs, samples, QC standards, and calibration standards can be poured directly into Glass Shell Vials (Table 3.4.). For external sparge runs, all

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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.

6.8.1. For each batch: Select one sample from a batch of 20 or less samples as QC sample for the batch. Pour this sample in duplicate to assess precision.

6.8.2. Prepare a 0.20 mg N/L matrix spike (MS, spike code T1) by adding 80 µL of the 50 PPM KNO3 stock (4.2.2.) to one 20 mL portion of the selected sample. Prepare a laboratory fortified blank (LFB) following the same procedure as the 0.20 mg N/L matrix spike by substituting blank standard (4.3.2.) for the sample. Effluent samples are batched separately.

6.8.3. Samples with a conductivity of ≥15,000 µmhos/cm and ≤30,000 µmhos/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 µmhos/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.

6.8.4. Each run begins with the following sequence:

Rinse (4.3.2.,6.4.) (optional)
Calibration (6.5.)
ALT#(ALTQC, 4.3.1.) – This serves as **ICV**

PQCK (PQL, 0.05 mg N/L, Table 4.3.5.)

TONCHCK2 (4.3.6.)
TONCHCK2 (4.3.6.)
CCV3 (0.2 mg N/L, 4.3.4.)
CCB (Blank Standard, 4.3.2.)
These continuing calibration checks (CCV3 and CCB) are scheduled to **bracket every 10th sample on the run**.
MBLKs (4.3.2.), **LFBs** (6.8.2.), sample duplicates (6.8.1.), and spiked samples (MS,6.8.2.)
Final **CCV3** and **CCB**

Note: The **ALTQC** (4.3.1.) analyzed immediately after the calibration is considered **ICV** (Initial Calibration Verification).

6.9. 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*.

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6.10. 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.

6.10.1. *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…".

6.10.2. To export the ASCII file to the correct folder in the Nutrients Drive: *File* → *ASCII Export* → *Detail* (Include Injection Data) →\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\TotalOrganicCarbonAnalyzers\ → *Filename* → *Save.*

6.10.3. The ASCII file is automatically exported to \data\ folder on local hard drive.

6.10.4. 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 in the current year's subfolder located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\TotalOrganicCarbonAnalyzers\

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).

6.10.5. 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.*

6.11. Quantitation/Calculations:

6.11.1. To upload the run file, log into LIMS, select QC Manager (QCM), and select the appropriate instrument. Click the 'Convert File" button and select the appropriate folder (current year). The Text File that was exported (6.10.1.) should be available for conversion. Select the appropriate file and follow the QC Manager's (QCM) instructions.

6.11.2. The instrument will give the values for nitrogen in mg N/L. The value given by the instrument is the average of three injections for a given sample that have a Max CV (coefficient of variation) of 5%.

The calibration consists of five standards plus a blank. The regression model applied is linear.

6.11.3. 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.

6.11.4. The calibrants are all prepared manually (see section 4.3.2. and 4.3.5). The correlation coefficient for the calibration is calculated by the instrument

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software. Use the NU-TN form located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TOC-TN\Forms\CalibrationCalc.Forms\to calculate the recoveries of the calibrants. Make sure you use the latest version of the form. Open the form, enter the header information, 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. The acceptance limits for blank calibration point results are below the MDL and above the negative value of the MDL (-MDL < blank < MDL).

7. DATA ARCHIVAL/VALIDATION

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-TN), filename/s (YYMMDDN(/P/O#_LTN), 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, 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 in the appropriate instrument folder in the current year's subfolder located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\TotalOrganicCarbonAnalyzers\.

7.5. TN instrument data text transfer files for upload to QC manager are stored in electronic format in the appropriate instrument folder in the current year's subfolder located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\TotalOrganicCarbonAnalyzers\

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

\\floridadep\data\labs\Lab5\Chemistry\Inorganic\Nutrient\Validations\TOC-TN_N_O_P\.

8. MAINTENANCE/TROUBLESHOOTING

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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 sufficient 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 (4.3.2.) at least once per month.

8.1.6. Make sure the HCL Intake is changed with fresh 2N HCL (4.2.4.) 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 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* →

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Highlighted → *Yes*, then click *View Vial Settings* → Assign the vial numbers for rerun samples.

8.2.4. Autosampler lid must be in place for run to proceed properly.

8.2.5. 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.

Note: Refer to the Troubleshooting flowcharts and sections in the user's manual. If unable to resolve the issue, contact Shimadzu for service.

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 (0.5 mg N/L, 0.4 mg N/L, 0.2 mg N/L, 0.1 mg N/L, and 0.05 mg N/L) and a blank. It is followed by a sequence of initial quality control standards as described in section 6.8.

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 limits for 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 a MBLK, an LFB, a matrix spike, and a duplicate sample or spike. The spikes are prepared according to section 6.8.2. Acceptance limits for the LFB and matrix spike recoveries are 85-115%.

9.4. On every analytical run, one organic nitrogen check (TONCHCK2) is run at least twice at the beginning of the run (6.8.4.). 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) (4.3.6., 6.8.4.) 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).

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9.5. The **ALTQC (ICV/IRM)** standard (ALTQCTNLO##, 4.3.1.) is a second/independent source certified standard with acceptance limits supplied by the manufacturer (Independent Reference Material, IRM). The acceptance limit can never be wider than 90 – 110%, however if the manufacturer has a narrower range, then the stricter acceptance limit range must be used. One ALTQC standard is analyzed with every analytical run immediately after the calibration.

Note: *Currently there are no certified reference standards available for TN. The 'Simple Nutrients' (ERA NOX STOCK ##) standard is analyzed adding the certified values for ammonia and nitrate/nitrite to reflect TN. The acceptance limit for the sum was taken from the more stringent limit of the individual components.*

9.6. One PQL (PQCK, 4.3.3.) level standard 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%.

The PQCK and MBLK analysis results are uploaded to LIMS whenever samples are reported and used for ongoing annual MDL and PQL verification.

9.7. A CCV3 (CCV, 4.3.4.) and a blank (CCB, 4.3.2.) standard are run at the beginning of every run, after every 10^{th} sample, and at the end of the run. Acceptance limits for the recoveries of the CCV standard is 85 – 115%.

9.8. The sample/matrix spike precision should be less than 20% RPD for results over the practical quantitation limit (PQL).

9.9. Acceptance limits for the blanks (MBLKs and CCBs) are < 0.020 and > -0.020 mg N/L.

9.10. 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 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 must be worn at all times while handling samples and reagents.

11. REFERENCES

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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. Method validation data is on file with the supervisor.

11.1. ASTM D8083-16.

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.

11.10. Laboratory Health and Safety Plan.

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

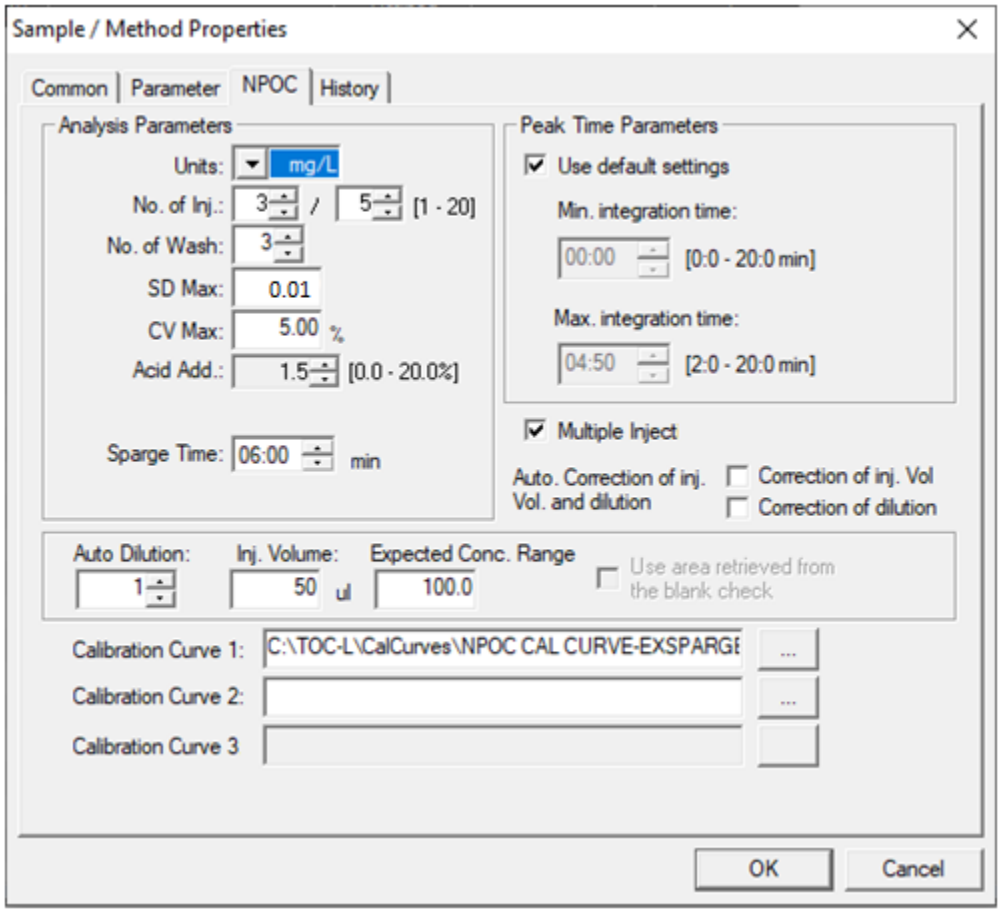
11.12 DEP SOP LB-030, Purchasing and Receiving Laboratory Chemicals and Supplies.

APPENDIX I: INSTRUMENT SETTINGS – Parameters

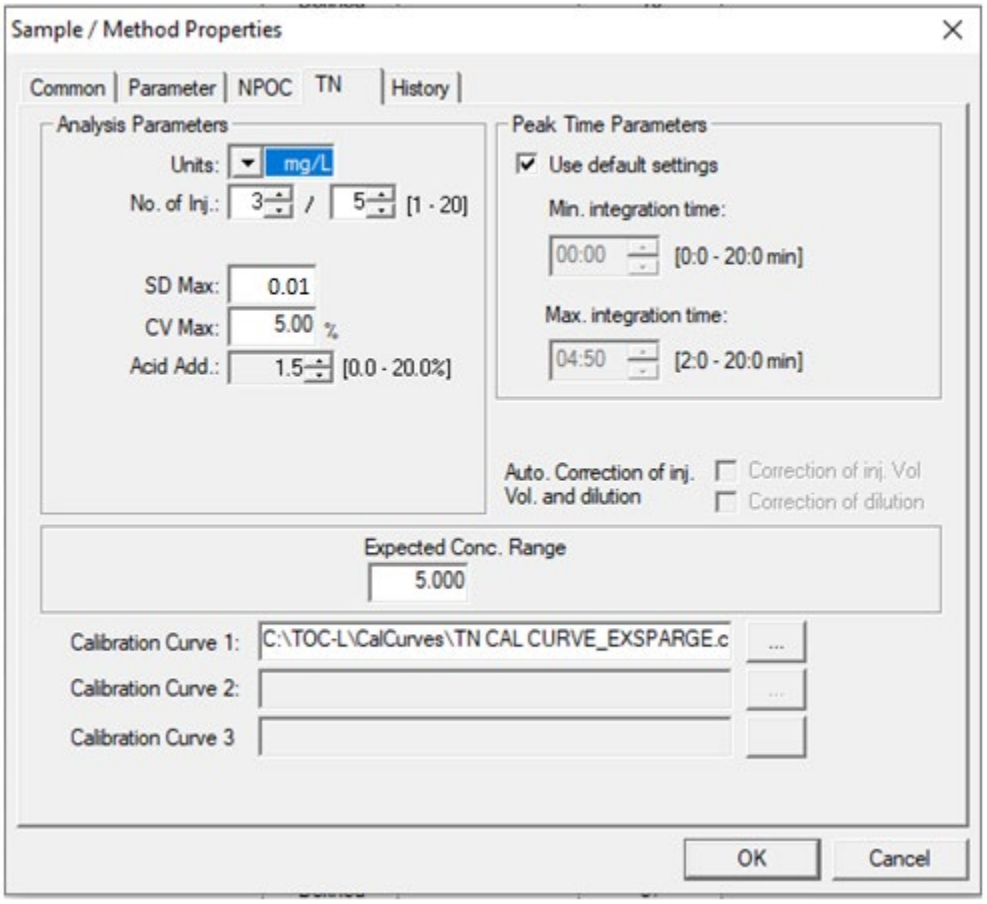
Note: Refer to Shimadzu Software User's Manual for details.

External Sparge TN Settings:

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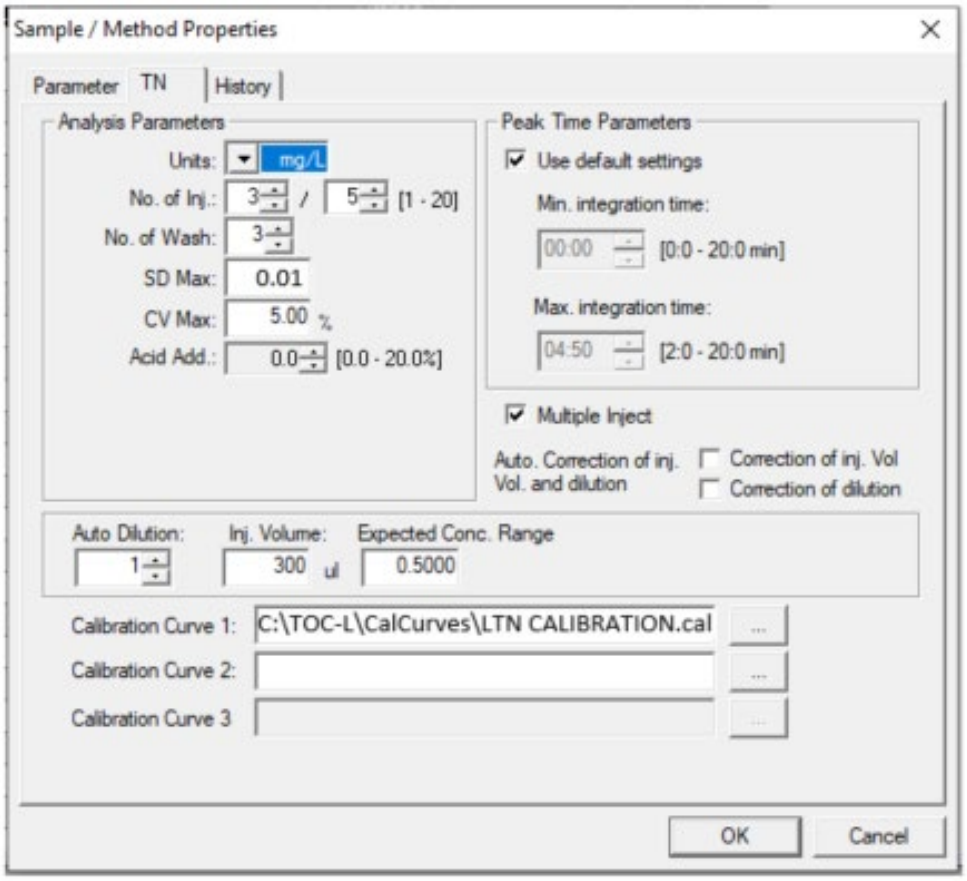


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No Sparge TN Settings:

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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.

01/26/2022 Following revisions were made to the SOP:

- Updated Table of Contents
- Added an option to run TN Analysis on Internal Sparge setting.
- Added tables to include instrumentation information, supplies & consumables, chemicals, and standards.
- Added instructions to run TONCHCK2 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

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setting and option.
- 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 Low Level TN parameters for both internal and external sparge.

4/15/2022 Following revisions were made to the SOP:

- Removed KNO3 secondary source requirement for CCV
- Assigned ALTQC as both the ICV and IRM in accordance with the Method, DEP QA Manual, and TNI requirements
- Added clarity to the acceptance limits for ALTQC (ICV/IRM)
- Changed CCV acceptance limit to 85-115%
- Added instructions on batching and handling saline samples
- Removed NPOC requirement from Low Level TN internal method which requires no sparging of samples prior to analysis – converted to NO Sparging
- Updated Appendix I to reflect changes to the Internal Sparge (No Sparge) settings

12/08/2023 Incorporated renaming of instrument TOC-L2 to instrument TOC-N. Added new instruments TOC-O and TOC-P.

11/14/2024 Updated file path links.

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