DETERMINATION OF TOTAL KJELDAHL NITROGEN IN WATER USING THE SEAL AQ2 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. The Seal AQ2 Discrete Analyzer is used for the determination of total Kjeldahl Nitrogen (TKN) in ground, surface, and saline waters, and domestic and industrial wastes. The calibration range is from the method detection limit (MDL) 0.080 to 4.0 mg N/L. The range may be extended by dilution up to a limit of 10 mg N/L of organic N in the sample prior to digestion. The practical quantitation limit (PQL) is 0.20 mg N/L.

1.2. The tests covered by this SOP are W-TKN and W-TKN-F.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method 351.2, Rev. 2.0 (1993) and Seal AQ2 method EPA-111-A Rev. 4.

2.2. Prior to analysis, digestion converts free ammonia and organic nitrogen compounds to ammonium sulfate (DEP SOP NU-091).

2.3. Ammonium reacts with salicylate and hypochlorite in a buffered, alkaline solution in the presence of sodium nitroferricyanide (pH = 12.4-12.7) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

2.4. Interferences: The working buffer eliminates precipitation of calcium and magnesium hydroxides. Samples that are turbid must be filtered prior to analysis and either reported qualified or redigested. High nitrate concentrations (>10 mg N/L) interfere with the TKN preparation procedure. The interference can be either negative or positive. For any samples with a known nitrate concentration of 10 mg N/L or higher the TKN result should be qualified with a J qualifier, or the sample diluted sufficiently to decrease the nitrate concentration to below 10 mg N/L PRIOR to digestion.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation -

Instrument Unique ID	Component	Manufacturer	Model
Seal AQ2 1	Discrete Analyzer	SEAL Analytical	AQ2+
Seal AQ2 3	Discrete Analyzer	SEAL Analytical	AQ2

3.2. Equipment -

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Vortex Mixer	Corning	LSE
Analytical Balance	Mettler Toledo	MS204TS/00
pH Meter	Fisher Scientific	Accumet AE150
Water Bath	Fisherbrand	Isotemp GPD 10
Stirrer/Hot Plate	Corning	PC-420D
Stirrer	Corning	PC-210
Stir bars, Assorted	Fisher (SR)	14-513-61; 14-513-58; S68069

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Drying Oven	ThermoScientific	Lindberg Blue M
Desiccator Cabinet	Fisherbrand	08-645-6
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher	Research Plus, 3123000080
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-210-5B; 10-212B; 10-212-5L; 10-208M
Glass Beaker, Assorted	Fisher (SR)	02-540G; 02-540K; 02-555-20E; 20-540R
Filtering Flask	Fisher (SR)	10-180F
Magnetic Filter Funnel, 47 mm	Fisher	NC0006472
Glass Funnel	Fisher (SR)	10-346-5B
Watch Glass, 15 cm	Fisher	02-612F
Or Equivalent [1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom		

3.3. Consumable and frequently replaced supplies -

Supplies/Consumables	Vendor[1]	Catalog Number
Sample Cups, 1.2 mL	SEAL Analytical (SR)	5385
Sample Reagent Segments	SEAL Analytical (SR)	5000
Reagent Wedge Containers	SEAL Analytical	5010
Peristaltic Tubing	SEAL Analytical	5140
Roller Lubricant Pen	SEAL Analytical	AQLB
Roller and Wash Bath Cleaning Swabs	SEAL Analytical	AQCSW-20
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
Syringe, Polypropylene, 10 mL	Fisher (SR)	B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Glass Fiber Filters w/o Binders, 47 mm, 0.7 µm	Millipore Sigma	APFF04700
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Reagent Bottles, Polyethylene, 1 L	Fisher (SR)	05-719-88
Reagent Bottles, Nalgene Amber, 1L	Fisher (SR)	03-313-8F
Reagent Bottle, Amber, 125 mL	Fisher	03-313-3C
Parafilm	Fisher (SR)	13-374-12
Kimwipes, Assorted	Fisher (SR)	06-666A; 06-666C
Transfer Pipet, 5 mL	Fisher (SR)	13-711-5AM
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Before preparing reagents or standards, or running samples, read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

Note: Reagent and standard preparations are documented in the LIMS ‘Standard Preparation

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Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the prep worksheet in LIMS or in the Reagent and Standard Logsheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard, reagent, and the standard reference material is maintained in the REAGENT_PREP folder on the Nutrients drive. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

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.

4.1. Chemicals -

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Sulfuric acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus ≥95%	See CoA	Room Temp
4.1.2.	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	ACS, ≥97%	See CoA	Room Temp
4.1.3.	Sodium Salicylate	$NaC_7H_5O_3$	Sigma-Aldrich	S3007-500G	≥99.5%	See CoA	Room Temp
4.1.4.	Sodium nitroferricyanide	$Na_2[Fe(CN)_5NO]$ *$2H_2O$	Fisher Chemical	S350-100	ACS, ≥99%	See CoA	Room Temp
4.1.5.	Sodium Hypochlorite, approx. 6%	NaOCl	Grainger (Any Generic)	56HN12	6.0% NaOCl, no additives	N/A	Fridge
4.1.6.	Sodium potassium tartrate tetrahydrate	$NaKC_4H_4O_6$ * $4H_2O$	Fisher Chemical (SR)	S386-500	USP, ≥99%	See CoA	Room Temp
4.1.7.	Sodium phosphate dibasic heptahydrate	HNa_2O_4P * $7H_2O$	Fisher Chemical (SR)	S373-500	ACS, ≥98%	See CoA	Room Temp
4.1.8.	Ethylenediamine tetraacetic acid disodium salt, EDTA	$C_{10}H_{14}N_2Na_2O_8$ *$2H_2O$, For cleaning	Fisher Chemical	S311-500	ACS, ≥99%	See CoA	Room Temp
4.1.9.	Cuvette Cleaning Solution	For cleaning	SEAL Analytical	5676	N/A	N/A	Room Temp
4.1.10.	Hellmanex III	For cleaning	Hellma	Z805939	N/A	N/A	Room Temp
4.1.11.	Hydrochloric Acid	HCl, For cleaning	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	See CoA	Room Temp
4.1.12.	Bleach, Generic	NaOCl, For cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	N/A	Fridge
4.1.13.	DI Water	H_2O	In house	N/A	N/A	Daily	N/A

Or Equivalent

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

Note: Wash all glassware and bottles with 1:1 HCl and rinse at least 7 times with DI water before preparing reagents and standards. Make sure any containers that are used to store prepared reagents and standards are clean and dry.

4.2. Reagents:

4.2.1. Stock Sodium Hydroxide, 20 % Solution –

NaOH (4.1.2.) 200 g

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DI water (4.1.13.)	to 1000 mL

In a 1 L beaker, add 200 g NaOH to approx. 800 mL DI water while stirring slowly. Quantitatively transfer solution to a 1 L volumetric flask, bring to volume with DI water and mix well. Do not filter.

Store at room temperature. Holding time, 180 days.

4.2.2. Stock Buffer Solution –

Sodium phosphate dibasic heptahydrate (4.1.7.)	254.0 g
NaOH (4.1.2.)	20 g
DI water (4.1.13.)	to 1000 mL

Using a 1 L beaker and magnetic stir plate, add 254.0 g sodium phosphate dibasic heptahydrate and 20 g NaOH to approx. 700 mL DI water. Stir until dissolved, approx. 60 minutes. Warm solution slightly if needed to dissolve completely. If heated, let solution come to room temperature. Quantitatively transfer solution to a 1 L volumetric flask, bring to volume with DI water and mix well.

Prone to precipitation with cooler temperatures.

Store at room temperature. Holding time, 60 days.

4.2.3. Stock Sodium Potassium Tartrate, 10% Solution –

Sodium potassium tartrate (4.1.6.)	200 g
NaOH (4.1.2.)	10-12 pellets
DI water (4.1.13.)	to 2000 mL
Sulfuric Acid (4.1.1.)	to pH 6-7

In a 2 L beaker containing approx. 1.2 L of DI water, dissolve 200 g of sodium potassium tartrate and 10-12 pellets of NaOH. While stirring, boil solution gently for 45 minutes to drive away any residual ammonia contamination. Cover with watch glass to cool. After solution has come to room temperature, quantitatively transfer to a 2 L volumetric flask and bring to volume with DI water. Invert to mix thoroughly.

Transfer solution to a clean 2 L beaker with a stir bar/plate. Using a calibrated pH meter (see DEP SOP NU-026), adjust the pH to that of DI water by adding concentrated sulfuric acid dropwise until desired pH (approx. pH 6-7) is reached.

Store in refrigerator. Holding time, 180 days.

4.2.4. Stock Color Solution –

Sodium nitroferricyanide (4.1.4.)	3 g
DI water (4.1.13.)	to 100 mL

In a 100 mL volumetric flask containing approx. 50 mL of DI water, dissolve 3 g of sodium nitroferricyanide. Bring to volume with DI water.

Transfer to amber bottle. Store in refrigerator. Holding time, 30 days.

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Re-prepare if solution develops a blue-green tint.

Caution: Can produce cyanide gas if in contact with acid. Refer to safety section for handling and disposal (10.6.).

4.2.5. Stock Salicylate Solution –

Sodium salicylate (4.1.3.)	150 g
NaOH (4.1.2.)	20 g
DI water (4.1.13.)	to 1000 mL

Fill a large beaker with approximately 700 mL of DI water, add 150 g of sodium salicylate. Add 20 g of NaOH and stir until dissolved. Quantitatively transfer the solution to a 1 L volumetric flask, bring to volume with DI water and invert to mix thoroughly.

Using a vacuum filtration apparatus, filter solution with a glass fiber filter.

Transfer to amber bottle. Store in refrigerator. Holding time, 60 days.

4.2.6. Working Color Reagent –

Stock Salicylate Solution (4.2.5.)	40 mL
Stock Color Solution (4.2.4.)	0.25 mL

In a clean reagent wedge, pipet 40 mL of the Stock Salicylate Solution and 0.25 mL of the Stock Color Solution. Aspirate with pipette to mix thoroughly.

Store at room temperature. Holding time, 2 days.

For best results, prepare fresh daily.

4.2.7. Working Buffer Reagent –

Stock Sodium Potassium Tartrate, 10% (4.2.3.)	50 mL
Stock Buffer Solution (4.2.2.)	20 mL
Stock Sodium Hydroxide, 20% (4.2.1.)	18.4 mL
DI water (4.1.13.)	to 100 mL

In a clean and empty 100 mL volumetric flask, add 50 mL of the Stock Sodium Potassium Tartrate 10% Solution, then 20 mL of the Stock Buffer Solution. **Mix together**, then add 18.4 mL of the Stock Sodium Hydroxide, 20% Solution. Bring to volume with DI water and invert to mix thoroughly.

The order and steps in which the reagents are added is important!

Store at room temperature. Holding time, 3 days.

For best results, prepare fresh daily.

4.2.8. Working Hypochlorite Reagent –

Sodium Hypochlorite, 6.0% (4.1.5.)	10.0 mL*
DI water (4.1.13.)	to 50 mL

In a 50 mL volumetric flask containing approx. 30 mL of DI water, add

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10.0 mL of sodium hypochlorite. Bring to volume with DI water and invert to mix thoroughly.

Store at room temperature. Holding time, 3 days.

For best results, prepare fresh daily.

***Note:** Please check the hypochlorite concentration of stock solution and adjust the volume added as necessary. The volume of sodium hypochlorite added above (10.0 mL) is for 6.0% NaOCl.

4.2.9. Alkaline EDTA Cleaning Rinse (Control 1) –

Sodium EDTA (4.1.8.)	10 g
NaOH (4.1.2.)	20 g
DI water (4.1.13.)	to 1000 mL

In a 1 L volumetric flask containing approx. 600 mL of DI water, dissolve 10 g of sodium EDTA and 20 g NaOH. Dilute to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

Note: Safe for phosphate analysis.

4.2.10. Hellmanex Cleaning Solution –

DI water (4.1.13.)	40 mL
Hellmanex (4.1.10.)	0.8 mL

In a clean reagent wedge, pipet 40 mL of DI water and 0.8 mL of Hellmanex. Aspirate to mix.

Store at room temperature. Used for cleaning only.

Caution: Do not use if running phosphate analysis on same instrument.

4.3. Calibration and Quality Control Standards –

Refer to DEP SOP NU-091 for the preparation of all standard solutions.

5. SAMPLE HANDLING AND PREPARATION

5.1. Refer to DEP SOP NU-091 for preparation, sample preservation, and handling details.

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

Note: Digested solutions containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer and then redigested with dilution. Add "_F" to the sample ID to document the filtration.

Note: Samples with organic N concentrations above 10 mg N/L must be diluted prior to digestion in order to report results without a J qualifier. Subtract the ammonia concentration, if known, from the TKN result to calculate the organic N concentration. Add a comment on the run report to document this observation.

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6.1. Initial Preparation:

6.1.1. Remove reagents from the refrigerator. Check the expiration dates on all standards and reagents. Discard expired standards and/or reagents and re-prepare if necessary.

6.1.2. Select digested trays to be analyzed and vortex samples and standards thoroughly. Record worksheet ID/tray number on the Reagent and Standard Logsheet.

6.1.3. Identify any samples that still contain precipitate after vortexing. Place these samples, and all other tubes in the batch, into the water bath rack. Set the water bath to 60 °C. Once the bath reaches temperature, record the temperature in the log and place the rack with the tubes into the bath. Heat the tubes for approximately seven minutes. After heating, vortex the tubes and let them cool before pouring. Denote any batches that were heated on the bench sheet.

Note: Tubes must be individually covered with parafilm during the heating process to prevent moisture loss through evaporation.

Note: Do not use the water bath cover when heating tubes, as this can cause condensation buildup that could drip down onto the tubes.

6.2. Calibration:

6.2.1. The instrument is calibrated using the digested 4.0 mg N/L working CAL STD (DEP SOP NU-091 Section 4.3.6.).

6.2.2. All dilutions for calibration standards and samples are prepared with a digested blank (see DEP SOP NU-091) as the diluent.

6.2.3. The auto calibration feature of the AQ2 is used in the analysis of TKN. The standards in the calibration are as follows (C2 to C6 are prepared by the instrument from the digested working calibration solution (6.2.1.)).

STD	Conc. (mg N/L)	Source
C1	0.00	Digested blank (6.2.2.)
C2	0.20	Instrument dilution of C7
C3	0.40	Instrument dilution of C7
C4	1.0	Instrument dilution of C7
C5	2.0	Instrument dilution of C7
C6	3.0	Instrument dilution of C7
C7	4.0	Digested CAL STD (6.2.1.)

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following: Working Color (4.2.6.), Working Buffer (4.2.7.) and Working Hypochlorite (4.2.8.).

6.3.2. Reagent Location - The reagent tray, with plastic wedge-shaped containers

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is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. The buffer is in position 4, the color reagent in position 5, and the hypochlorite reagent is in position 6.

6.3.3. Controls - Fill one reagent vessel with the digested 1.0 mg N/L CCV (DEP SOP NU-091 4.3.7.) and place it in position 10. Fill another vessel with digested blank (CCB, 6.2.2.) and place it in position 11.

6.4. Analysis Setup/Sequence setup:

6.4.1. Turn on the AQ2 Analyzer (large button on the right side at the back of the instrument) and the computer. To start the software, double-click on the AQ2 Icon on the screen. The username is 'Instrument 1' for AQ2 1 and 'Instrument 3' for AQ2 3. The password for both instruments is 'password'.

Note: Make sure that the cooling of the compartment that holds the plastic wedge-shaped containers is turned off. Cooling of the reagent containers can cause precipitation of the stock buffer solution.

6.4.2. Proceed to the daily maintenance (Section 9.1.) if it is the first run of day. Otherwise continue to 6.4.3.

6.4.3. Check that the DI water reservoir is full and empty the waste container. Replace all used reaction segments.

6.4.4. In the software, go to the 'Setting' menu and select maintenance. Zero the reaction segment counter.

6.4.5. Setup of the analytical run –

Click on the scheduling icon on the left side of the screen. Choose **Reagent Set 3**. Select a free tray and name the run using the format TKNYYYYMMDD00#K for AQ2 1 or TKNYYYYMMDD00#R for AQ2 where the # refers to the first (# = 1), second (# = 2), etc. runs of the day.

The sequence screen should appear. Starting at **line 3**, type the sample sequence in the 'Sample ID' column. The software will automatically schedule the CCV/CCB every ten cups on the run and thus these do not need to be typed into the sequence.

Highlight the typed lines and select "Auto Fill" to fill the sequence numbers, and the **TKN** test. The test should appear in the requested test column to the right of the sample ID's.

A typical run sequence includes the following:

CCV (DEP SOP NU-091 4.3.7.) – automatically scheduled
CCB (digested blank, 6.2.2.) – automatically scheduled
ERAQC# (ALTQC, DEP SOP NU-091 4.3.5.)
PQCK2 (DEP SOP NU-091 4.3.2.)

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MBLK
LFB
QC sample
Spiked QC sample (MS) (DEP SOP NU-091 4.3.3.) and **spike duplicate (MSD)/QC sample duplicate**
Samples
CCV
CCB

Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

Go to the "Maintenance and Setup" tab, click on the "Tests" button. In the "Select Test" list of methods select "TKN_SOP_NU092" and click on the "Print" button to print the AQ2 test parameters report.

Note: Multiple trays can be run on the same sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standard came from. Identify the tray QC in the sequence. The first ALTQC should be identified as the initial calibration verification (ICV). A failing ICV renders the calibration unusable.

6.4.6. Pouring the analytical run - Cup positions one and two are reserved for the calibration standards. Cup 1 is left empty. The TKN calibration solution (6.2.1.) is placed in cup 2. Pour the samples according to the cup position on the bench sheet.

6.4.7. Double-click on the 'Run' icon on the left side of the screen. A dialog box will appear. Select the tray that contains the sequence to be analyzed. Click 'OK'. Another dialog box will appear. Check the perform Auto-Calibration box and click 'OK'. (If the analysis should use the previous calibration, refer to the note below.) Select 'OK' on the following dialog box.

Note: Consecutive runs on the same day may use the same calibration. In this case, the Auto-Calibration option should not be checked. While not required, it may be beneficial to recalibrate the instrument if using new reagents.

6.4.8. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start; if not, the run will stop and display the reagent which has insufficient volume. Fill up the reagent and restart the run according to 6.4.7.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TKN in mg N/L.

6.5.2. The QC-Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see Section 5.9 of the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to DEP SOP NU-091

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Section 4.3.6. The other standards used in the second order calibration are auto-diluted by the instrument from the high standard. The percent error of the standards and correlation coefficient are calculated by the instrument software. (See 8.5.)

6.6. Post-Run Procedure:

6.6.1. Review water baseline values ("W" column) before accepting the results. Anomalous readings here may be indicative of the instrument performance problems. Any results associated with such readings should be questioned.

6.6.2. Click on the "Data Review" menu located on the left side of the screen.

6.6.3. If there are any pending injections in the run (marked "PNG"), highlight the results column for these injections and click on the "Cancel" button.

6.6.4. Make any corrections to the sample or standard IDs if needed before the next step.

6.6.5. Click on the 'Accept All' Icon in the "Apply to All" group. This adds 'ACC' to the results. Click "Yes" to permanently accept the results in the pop-up window that appears. The next pop-up will prompt you to save the file. Follow the naming convention described in 6.4.5. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder located in \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\DiscreteAnalyzers\AQ2-1\TKN\ (or AQ2-3). Exit out of the web browser window that opens.

6.6.6. Click on the 'Reporting' icon on the left side of the screen. Check the reporting format 'TKN/TP' and print the run report. It contains the calibration and all the data. Select the chronological order report function to print your report.

6.6.7. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 1' for AQ2 1 and 'GenChem: Seal AQ2 3' for AQ2 2.

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-TKN(-F)), filename(s) (e.g. TKNYYYYMMDD001K), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, Reagent and Standard Logsheet, digestion temperature data logger report, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test

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authorization. If pipettes were used for preparing sample digestates for analysis, the folder should also contain a copy of the NU-PIPETTE form documenting dilution recipes and pipettes used. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

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

7.4. AQ2 instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in: \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\Discrete Analyzers\AQ2-1\TKN\.

7.5. 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. QUALITY CONTROL/DATA ASSESSMENT (SEE NU-038 AND NU-043)

8.1. For 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 NU-043.

8.2. A CCV (DEP SOP NU-091 4.3.7.) and a CCB (digested blank, DEP SOP NU-091) are analyzed every 10 samples and at the beginning and end of each run. Recovery acceptance limits for the method blanks (MBLKs) and CCBs are < 0.080 and > -0.080 mg N/L. The check standard (CCV) recovery limits are 90 – 110%

8.3. Each tray of digested samples contains a digested PQL level standard (PQCK2, DEP SOP NU-091 4.3.2.) and a second source standard (ALTQC, DEP SOP NU-091 4.3.5.). Recovery acceptance limits are 70 -130% for PQCK2 and 90 – 110% for the ALTQC. The PQCK2 is also used for the ongoing MDL and PQL verification.

8.4. An MBLK, an LFB, a matrix spike, and a duplicate sample and/or duplicate matrix spike are digested and analyzed with every batch of 10 or fewer samples (see DEP SOP NU-091). Acceptance limits for the LFB and matrix spike recoveries are 90 – 110%. The RPD of sample/matrix spike duplicates must be less than 20% for results above the PQL.

8.5. To verify the calibration range the following is assessed::

8.5.1. Linear Calibration Range (LCR) - See FDEP Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'.

8.5.2. The correlation coefficient of the second order calibration must be ≥0.995.

8.5.3. Acceptance limits for the blank level calibration standard (S1/S0) calculated results are <0.080 and > -0.080.

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8.5.4. Acceptance limits for the percent error of the PQL level calibration standard is ±30%.

8.5.5. Acceptance limits for the percent error of calibration standards above the PQL are ±10%.

8.5.6. Every day samples are analyzed a new calibration must be performed and verified at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

9. MAINTENANCE/TROUBLESHOOTING

9.1. Daily maintenance.

9.1.1. Empty and rinse the DI water reservoir at least three times. Refill to the top.

9.1.2. Empty the instrument rinse waste. Check that the instrument hazardous waste bottle isn't full.

9.1.3. In the SEAL AQ2 Program, go to 'Settings' then 'Maintenance.'

9.1.4. In 'Initialise,' press 'Zero Segments.' Do not press 'OK' yet.

9.1.4.1. Check to see if there are enough reaction segments from the day before to do maintenance. 2-3 unused is enough. Then press 'Cancel.' The probe will go to the first clean segment instead of Segment 1, spot 1.

9.1.4.2. If there are not enough clean segments, replace the segments. Double tap clean segments on the bench to knock out excess manufacturing particles, then place in the instrument. Press 'OK' to reset the used segments. The probe will go to Segment 1, spot 1.

9.1.5. Go to 'Diluter,' set the number of primes to 10, then press 'Start Primes.'

9.1.6. Watch the syringe piston to see if there are excess bubbles dragging from the top. A worn syringe piston can interfere with the calibration auto-dilution and analysis. Replace if necessary.

9.1.7. Clean the aspiration bath with a Q-tip or cleaning swab. Daily cleaning of the aspiration bath helps prevent particulate matter from entering the cuvette.

9.1.8. In a reagent wedge in position 1, place either the Cuvette cleaning solution (4.1.9.), Hellmanex Cleaning Solution (4.2.10.) or the Alkaline EDTA Rinse (4.2.9.). Go to 'Cuvette Functions,' then press 'Extra Wash.'

9.1.8.1. Watch the glass sampler probe cleaner. A water vortex should be visible when the sampler probe is being washed and liquid should not drip out.

9.1.8.2. Contamination between samples and reagents can occur if the

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probe cleaner is not operating properly. Liquid drips out of the glass probe cleaner when the probe creates a wider hole at the bottom of the glass (will look oval). This can occur over time or if the sampler probe is bent. Replace the probe cleaner and sampler probe if necessary.

9.1.9. After 'Extra Wash' is done, set the number of 'Auto Wash' to 5. Then press 'Auto Wash.' Make sure the aspiration bath is filling and emptying each time. Change the two pieces of peristaltic tubing going to the aspiration bath if it is not.

9.1.10. After 'Auto Wash' is done, press 'Extra Wash' again.

9.1.11. After the second 'Extra Wash' is done, press the 'Auto Wash' again.

9.1.12. To adjust the aspiration, remove the cover from the top of the cuvette/lamp. Press 'Test Aspiration' and observe where the water line begins. It should be around 2 inches out of the cuvette. Repeat 'Test Aspiration' 2 more times, then check that the 4th, 5th, and 6th repetition are in the same spot.

9.1.12.1. To make the water line come out of the cuvette further, increase the number of steps. Decrease the number of steps if it is too far out. Then repeat the above test.

9.1.12.2. If the water line is not around the same location in each repetition, random errors may occur in analysis. Replace the peristaltic tubing on the pump located next to the lamp.

9.1.13. In 'Initialise,' press 'Zero Segments,' and replace all used segments. Then press 'OK.'

9.1.14. Close the maintenance window then go to 'Settings' → 'Daily Startup' → 'Continue.' Write down the highest voltage and the corresponding filter in the maintenance book. The highest voltage filter should remain the same for each instrument. A drastic change in voltage from day to day indicates a lamp going bad or an electrical issue.

9.2. Weekly maintenance: Turn off the instrument at the end of each week, unless running.

9.3. Monthly maintenance: Please see the instrument logbook.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Review the Laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

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

10.3. Read the MSDS information for safe reagent and sample handling.

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10.4. The AQ2 instrument has two streams of waste: copper-containing waste and rinse waste. Please see 10.5. for copper-containing waste collection and disposal. Rinse waste from the instrument can be disposed of in the sink is to be accompanied by tap water.

10.5. Copper-containing waste –

The solution used to digest samples contains copper, which is a hazardous waste and toxic. All copper-containing waste from the instrument, digested samples, and standards made with digestion solution should be collected in a hazardous waste container. These containers must have two labels: a hazardous waste label and a label to denote the start and fill date of the container.

Upon starting a waste container, denote the date in the 'Start Date' column of the Start/Fill label. Once a waste container is filled and removed from the instrument, denote the date under the 'Fill Date' column. A filled hazardous waste container can be stored for no longer than three working days within the hazardous waste storage of the analytical laboratory area. Further, no more than three full bottles of copper-containing waste should be stored in the analytical lab.

The waste will be collected by trained personnel and disposed as acidic metals waste in the TKN Copper Drum. After the empty waste container is returned, strike through the previous start and fill date. If the container was dumped prior to being full, strike through the previous start date.

10.6. The sodium nitroferricyanide (4.1.4.) used to prepare the color reagent produces cyanide gas if it encounters acid. Wear all personal PPE when preparing and thoroughly rinse flasks and containers after acid washing. Dispose excess sodium nitroprusside crystals by lab packing the chemical into a box labeled with the chemical name, approximate amount being discarded, and the date. Lab packed chemicals will be taken to the hazardous waste room by trained personnel for disposal. Excess color reagent can be disposed of down the sink when flushed thoroughly with water.

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. EPA Method 351.2, Rev. 2.0 (1993).

11.2. Seal AQ2 Method EPA-111-A, Rev. 4, Total Kjeldahl Nitrogen-N (Copper Catalyst) in Drinking, Ground and Surface Waters, Domestic and Industrial Wastes.

11.3. DEP SOP NU-026, Manual Determination of pH

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

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

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Quality Control.

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

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

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

11.9. DEP SOP NU-091, Preparation of Waters for Total Kjeldahl Nitrogen Analysis (Copper Catalyst).

11.10. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

11.11. Quality Manual, Bureau of Laboratories, FDEP.

11.12. Laboratory Health and Safety Plan.

Appendix of Significant Changes

10/20/2009 Some minor corrections were made in section 4.

08/20/2010 Procedural clarifications some corrections mainly to section 4 and 6.

08/09/2011 Preparations of the PQL standard and spiking TKN solution were modified and now include phosphorous (these solutions also used for TKN and TP analysis in soils).

12/10/2019 Clarified Section 3. Added procedures for preparation of Alkaline EDTA Rinse and Hellmanex Cleaning Solution to Section 4. Section 6 was modified to clarify running multiple trays on one sequence. Added ongoing MDL/PQL verification information and acceptance limits for calibration standards recoveries to Section 8. Section 9 maintenance and troubleshooting was added. Section 10 safety and waste disposal procedure was clarified.

12/22/2020 In Section 3, the instrumentation, equipment, and supplies were updated and inputted into table format. In Section 4, chemicals were updated and inputted into table format. Preparation procedures for reagents and standards were reformatted and clarified. The calibration curve blank level standard limits were added to Section 8.

02/27/2023 Pooled validation adding instrument 3 was completed.

04/04/2023 Added AQ2-3 information. Added water bath procedure in Section 6. Water bath procedure implemented on 10/03/2022. Moved standard preparation procedures to DEP SOP NU-091. Clarified hazardous waste procedure in Sections 10.5. and 10.6. Updated reference list in Section 11.

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