DETERMINATION OF TOTAL KJELDAHL NITROGEN IN WATER USING THE SEAL GAS DIFFUSION SEGMENTED FLOW ANALYZER

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1. SCOPE AND APPLICATION

1.1. The Seal AutoAnalyzer 500 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) 0.050 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 AA500 Method no. MK5-0154-23-Rev 0.

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

2.3. The sample is air-segmented and made alkaline to give a solution of about 0.1 M NaOH containing EDTA to eliminate the precipitation of the hydroxides of calcium and magnesium. Ammonia goes into the gas phase. This donor stream flows into a dialyzer holding a gas-permeable membrane, on the other side of which is an acidic acceptor stream. Ammonia gas diffuses through the membrane and is retained in the acceptor. Next, the ammonia reacts with salicylate, nitroprusside, which is used as a catalyst, and dichloro-isocyanuric acid at 37°C to produce a green-blue indophenol complex, which is measured at 660 nm.

2.. Interferences: EDTA is added to eliminate the precipitation of heavy metals. Samples with background absorbance do not interfere because interferents with color do not diffuse through the membrane. 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 – SEAL Analytical Segmented Flow Analyzer System:

Instrument Unique ID	Component	Manufacturer	Model	Application Notes
Bran Luebbe F	Autosampler	SEAL Analytical	AS-4	Probe speed: Medium
	Pump	SEAL Analytical	AA3 Pump 4	Pump speed: Normal
	Manifold	SEAL Analytical	AA500	6” Dialyzer Heater Assembly at 37°C

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	Colorimetric Detector	SEAL Analytical	AA500	Flowcell: 10 mm Filter: 660 nm
	Software	SEAL Analytical	AACE 8.04	

3.2. Equipment:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Vortex Mixer	Fisherbrand	02-215-414
Water Bath	Fisherbrand	Isotemp GPD 10
pH Meter	Fisher Scientific	Accumet AE150
Analytical Balance	Mettler Toledo	XSR105
Analytical Balance	Denver Instrument	XS-410
Stirrer/Hot Plate	Corning	PC-420
Stir bar	Fisher (SR)	14-513-61
Drying Oven	ThermoScientific	Lindberg Blue M
Desiccator Cabinet	Fisherbrand	08-645-6
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
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)	10-212B; 10-210-5D; 10-212E; 10-212-5L; 10-208M
Graduated Cylinder, Class A, Assorted	Fisher (SR)	08-553A; 08-548D
Glass Beaker, Assorted	Fisher (SR)	02-540E; 02-540G; 02-540K; 02-555-20E
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
Pump Tubing, ORN/WHT (0.64mm ID)	Oregon Manufacturing Support	170-0596-06
Sampler Tube Assembly (Small)	SEAL Analytical	168+9420-01
Sampler Tube Assembly (Large)	SEAL Analytical	168+9422-01
Wash Pump Model B4 Supply	SEAL Analytical	168+9423-01
Wash Pump Model B06 Drain	SEAL Analytical	168+9424-01
Pump Tubing, BLK/BLK (0.32 mL/min)	SEAL Analytical	116-0549-07
Pump Tubing, GRY/GRY (1.00 mL/min)	SEAL Analytical	116-0549-11
Pump Tubing, Airbar	SEAL Analytical	117+0540-07
Membrane, PTFE, 6”	SEAL Analytical	170+B810-01
Pump Lubricant and Oil	SEAL Analytical	590-0161-01; 538+9000-01
Pump and Autosampler Cleaning Swabs	SEAL Analytical	AQCSW-20
Pipette tips, Eppendorf, 200 µL	Fisher (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
Sample Tubes, 13 x 100 mm	Fisher (SR)	14-961-27
Standard Rack Tubes, 16 x 100 mm	Fisher	14-962-10D

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Centrifuge Tubes, Polypropylene, 15 mL	Fisher (SR)	05-538-59A
Centrifuge Tubes, Polypropylene, 50 mL	Fisher (SR)	05-538-67
Syringe, Polypropylene, Assorted	Fisher (SR)	22-204-500; B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
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, Assorted	Fisher (SR)	05-716-151; 05-719-146; 05-719-145; 05-719-88
Reagent Bottles, Amber Glass, Assorted	Fisher (SR)	05-719-75; 05-719-183
Parafilm	Fisher (SR)	13-374-12
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: 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: Reagent and standard preparations are documented in the LIMS 'Standard Preparation 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 \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TKN\CertificatesofAnalysis\. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

4.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Storage
4.1.1.	Dichloroisocyanuric acid sodium salt, DIC	$C_3Cl_2N_3NaO_3*2H_2O$	Acros Organics	436450500	≥97%	Desiccator
4.1.2.	Potassium sodium tartrate tetrahydrate	$C_4H_4KNaO_6*4H_2O$	Fisher Chemical	S387-500	ACS, ≥99%	Room Temp
4.1.3.	Sodium Hydroxide	NaOH	Fisher Chemical	S318-3	ACS, ≥97%	Room Temp
4.1.4.	Sodium phosphate dibasic heptahydrate	$HNa_2O_4P*7H_2O$	Fisher Chemical	S373-500	ACS, ≥98%	See CoA
4.1.5.	Sodium nitroferricyanide	$Na_2[Fe(CN)_5NO]*2H_2O$	Fisher Chemical	S350-100	ACS, ≥99%	Room Temp
4.1.6.	Sodium salicylate	$NaC_7H_5O_3$	Sigma Aldrich	S3007-500G	≥99.5%	Room Temp

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4.1.7.	Sulfuric acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus, ≥95%	Room Temp
4.1.8.	Tetrasodium EDTA dihydrate	$Na_3C_{10}H_{12}O_8N_2*2H_2O$	Acros Organics	46085000	≥99%	Room Temp
4.1.9.	Triton X-100	Surfactant	Fisher Bioreagents	BP151-500	Electrophoresis	Room Temp
4.1.10.	Simple Nutrients, WasteWatR	ALTQC, ERA	ERA	505	See CoA	Room Temp/Fridge
4.1.11.	Hydrochloric Acid	HCl, For cleaning	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	Room Temp
4.1.12.	Bleach, Generic	NaOCl, For cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	Fridge
4.1.13.	DI Water	H_2O	In House	N/A	N/A	N/A
4.1.14.	Sodium phosphate dibasic dodecahydrate	$HNa_2O_4P * 12H_2O$	Acros Organics	448160010	≥99%	Room Temp
Or Equivalent **[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom.**						

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.

Note: Wash all glassware and bottles with 1:1 HCl and rinse with DI water sufficiently to remove all traces of hydrochloric acid before preparing reagents and standards. Make sure any containers that are used to store prepared reagents and standards are clean.

4.2. Reagents:

4.2.1. Triton X-100, 30% Solution –

DI water (4.1.13.)	70 mL
Triton X-100 (4.1.9.)	30 mL

In a 100mL beaker with a stir bar, add 70 mL of DI water. Use a graduated cylinder to measure 30 mL of Triton-X-100. Place the beaker on a stir plate then add the Triton-X to the DI water very slowly while stirring. Continue stirring overnight covered with parafilm to dissolve.

Do not apply heat to dissolve.

Store at room temperature. Holding time, 90 days.

4.2.2. Stock Sodium Potassium Tartrate, 20% Solution –

Sodium potassium tartrate (4.1.6.)	400 g
Sodium Hydroxide (4.1.3.)	3.0 g
DI water (4.1.13.)	to 2000 mL
Sulfuric Acid (4.1.7.)	to pH 7.5 ± 0.5

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In a 2 L beaker containing approx. 1.2 L of DI water, dissolve 400 g of sodium potassium tartrate and 3.0 g of NaOH. While stirring, boil solution gently for 60 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 by adding concentrated sulfuric acid dropwise until desired pH (approx. pH 7.5) is reached.

May be stored at room temperature or in the refrigerator. Holding time, 6 months.

4.2.3. System Wash Solution –

Triton X-100, 30% (4.2.1.)	2 mL
DI water (4.1.13.)	to 1000 mL

In a 1000 mL volumetric flask containing approx. 800 mL of DI water, add 2 mL of Triton X-100, 30% solution. Invert to mix, then bring to volume with DI water.

Store at room temperature. Holding time, 7 days.

4.2.4. Sampler Wash Solution –

Sulfuric acid (4.1.7.)	54 mL
DI water (4.1.13.)	to 2000 mL

In a 2 L volumetric flask containing approx. 1.8 L of DI water, add 54 mL of concentrated Sulfuric acid. Dilute to mark and invert to mix.

Store at room temperature. Holding time, 7 days.

4.2.5. EDTA/NaOH Reagent –

Tetrasodium EDTA dihydrate (4.1.8.)	10 g
Sodium Hydroxide (4.1.3.)	8.5 g
DI water (4.1.13.)	to 500 mL
Triton X-100, 30% (4.2.1.)	1 mL

In a 500 mL volumetric flask containing approx. 200 mL of DI water, dissolve 10 g of tetrasodium EDTA dihydrate and 8.5 g of NaOH. Bring to volume with DI water. Next, add 1 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

4.2.6. Sulfuric Acid Reagent –

Sulfuric acid (4.1.7.)	6 mL
DI water (4.1.13.)	to 500 mL
Triton X-100, 30% (4.2.1.)	2.5 mL

In a 500 mL volumetric flask containing approx. 400 mL of DI water, add

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6 mL of sulfuric acid. Bring to volume with DI water. Next, add 2.5 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

4.2.7. Buffer Reagent –

Sodium phosphate dibasic (4.1.4. or 4.1.14.)	8 g
Sodium hydroxide (4.1.3.)	15 g
Potassium sodium tartrate tetrahydrate solution (4.2.2.)	60 mL
DI water (4.1.13.)	to 500 mL
Triton X-100, 30% (4.2.1.)	0.5 mL

In a 500 mL volumetric flask containing approx. 100 mL DI water, dissolve 8 g of di-sodium hydrogen phosphate-12-hydrate, 15 g of NaOH, and 60 mL of potassium sodium tartrate tetrahydrate solution. Bring to volume with DI water. Next, add 0.5 mL of Triton X-100, 30% solution and invert to mix thoroughly.

Store at room temperature. Holding time, 7 days.

4.2.8. Salicylate Reagent –

Sodium salicylate (4.1.6.)	32 g
Sodium nitroferricyanide (4.1.5.)	0.065 g
DI water (4.1.13.)	to 500 mL

In a 500 mL volumetric flask containing approx. 200 mL of DI water, dissolve 32 g of sodium salicylate and 0.065 g of sodium nitroferricyanide. Bring to volume with DI water and invert to mix thoroughly.

Transfer to dark bottle. Store in the refrigerator. Holding time, 7 days.

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

4.2.9. DIC Reagent –

Dichloroisocyanuric acid sodium salt (4.1.1.)	0.15 g
DI water (4.1.13.)	to 200 mL

In a 200 mL volumetric flask containing approx. 150 mL of DI water, dissolve 0.15 g of DIC using a stir bar and stir plate. Bring to volume with DI water and invert to mix thoroughly.

Store at room temperature. Prepare fresh daily.

4.2.10. 1:10 HCl Cleaning Solution–

Hydrochloric acid, conc. (4.1.11.)	100 mL
DI water (4.1.13.)	to 1000 mL

In a 1 L volumetric flask containing approx. 800 mL of DI water, add 100 mL of concentrated hydrochloric acid. Bring to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

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4.2.11. 1 N NaOH Cleaning Solution–

Sodium hydroxide (4.1.3.)	40 g
DI water (4.1.13.)	to 1000 mL

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 40 g of NaOH. Bring to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

4.2.12. 10% Bleach Cleaning Solution–

Bleach (4.1.12.)	20 mL
DI water (4.1.13.)	to 200 mL

In a 200 mL volumetric flask containing approx. 150 mL of DI water, add 20 mL of bleach. Bring to volume with DI water and invert to mix.

Do NOT use bleach with additives (incl. Clorox brand).

Store at room temperature. Used for cleaning only.

4.3. Standards:

4.3.1. Refer to DEP SOP NU-091 for the preparation of ERAQC, CCV, MDL SPK and PQCK2 standard solutions.

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

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

4.3.4. TKN working calibration and QC standards –

Digested 4 mg N/L TKN calibration standard (4.3.2.)	See table
Digested blank/diluent	See table

Combine two to three 4.0 mg N/L calibration standard tubes in a 50mL centrifuge tube. Do the same with two to three digested blank tubes. Using the table volumes below, prepare intermediate calibration standards by diluting the specified amounts of the 4 mg N/L TKN calibration standard into 15 mL centrifuge tubes with digested blank. Mix thoroughly.

Store at room temperature.

Standard Concentration (mg N/L)		QC Standard, Alias	Final Volume	Vol of 4 mg N/L standard added
Cal 1 (4.2.3.)	0	MBLK, CCB, BLK, CAR	100 mL	None
Cal 2	0.1	MDL SPK	10 mL	0.25 mL
Cal 3	0.2	PQCK2	10 mL	0.5 mL
Cal 4	1.0	CCV	10 mL	2.5 mL
Cal 5	2.0		10 mL	5.0 mL
Cal 6	3.0		10 mL	7.5 mL
Cal 7	4.0	Primer, Drift	100 mL	100 mL

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5. SAMPLE PREPARATION PROCEDURE (NU-033)

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

6. SAMPLE ANALYSIS AND QUANTITATION

Note: Digested solutions containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on Bran Luebbe F 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.

6.1. Initial Preparation:

6.1.1. Remove salicylate from the refrigerator. Check the expiration date 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. Instrument Startup:

6.2.1. Turn on the power to the sampler. The heater and colorimeter are kept plugged in. The colorimeter is ready for operation when the status light on the back is green.

6.2.2. Check the condition of the tubing and make sure all connections are made according to the following:

Path directly to waste:

ORN/WHT – Sample line (split sample line)

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Path into the top of the dialyzer (donor stream):
BLK/BLK – Air
ORN/WHT – Sample line (split sample line)
GRY/GRY – EDTA/NaOH Reagent* (4.2.4.)

Path into the bottom of the dialyzer (acceptor stream):
BLK/BLK – Air
ORN/WHT – Sulfuric Acid Reagent* (4.2.5.)

Path out of the dialyzer:
ORN/WHT – Buffer Reagent* (4.2.7.)
ORN/WHT – Salicylate Reagent (4.2.8.)
ORN/WHT – DIC Reagent (4.2.9.)

6.2.3. Flush the instrument by placing reagent tubing for reagents containing surfactant (designated with an '*' above) in the System Wash solution (4.2.2.) and reagent tubing without surfactant in DI water. Secure pump platen and turn pump switches to 'Run' and 'Normal.'

6.2.4. Open AACE software. Username is "Superuser" with no password.

6.2.5. Click Charting in the System window, make sure <<TKN GD>> analysis is selected, and click OK. The sample probe should move down into sampler wash receptacle.

6.2.6. Right-click the chart and click "Set Base/Offset" (normally 5%) once the DI water and System Wash have reached the detector.

6.2.7. After 15 minutes, check that a good bubble pattern is established and that the water baseline is stable.

6.2.8. Place the reagent tubing for the EDTA/NaOH, Sulfuric Acid, Buffer, and DIC reagents in the respective reagent bottles. Place the autosampler wash straw in the Sampler Wash. Leave the Salicylate tubing in the DI.

6.2.9. After 15 minutes, place the reagent tubing for the Salicylate reagent (that has been brought to room temperature) in the respective bottle.

Note: Salicylate should never be added at the same time as the other reagents. Otherwise, precipitation will occur in the system.

6.2.10. Allow reagents to pump for an additional 15 minutes, then check that a good bubble pattern is established, and the heater is at 37˚C.

6.2.11. Set the base once again and let the reagent baseline stabilize.

6.3. Create a new run sequence:

6.3.1. Click Set Up → Analysis/Run → TKN GD. Select "TKN GD.anl" so that it is highlighted, then select New Run.

6.3.2. Rename the run with the TKNYYMMDDX format, where YY is the year, MM is the month, DD is the day, and X indicates the instrument used ('F' for Bran Luebbe F). Postscripts 2, 3, etc. are used to denote the second, third, etc. runs of the day if necessary.

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6.3.3. Fill out the Tray Protocol, click Print in the "Main Page" tab, and then OK to save the sequence. This will print out the run sequence with the analysis parameters.

An example run sequence includes:

Primer (4.0 mg N/L DEP SOP NU-091 Section 4.3.6.)
Drift (4.0 mg N/L)
RINSE (Sampler Wash, 4.2.3.)
CAL1-7 (Calibration Standards, 4.3.2.)
High (4.0 mg N/L, to calculate carryover)
Low (Blank, 4.3.3.)
Low (Blank)
Baseline (Sampler Wash, 4.2.3.)
MDL SPK (DEP SOP NU-091 4.3.8.)
PQCK2 (DEP SOP NU-091 4.3.2.)
ERAQCXX (ALTQC, DEP SOP NU-091 4.3.5.)
CCB (Blank)
CCV (1.0 mg N/L DEP SOP NU-091 4.3.7.)
Drift (4.0 mg N/L)
Baseline (Sampler Wash)
Batched samples with **MBLK**, **LFB**, and **spikes** (DEP SOP NU-091 4.3.3.)
CAR (Blank)
CCB (Blank)
CCV (1.0 mg N/L)
Drift (4.0 mg N/L)
Baseline
End

6.4. Load the sampler tray with the benchtop diluted calibration standards (4.3.4.), the digested standards (see DEP SOP NU-091 4.3.) and batches (DEP SOP NU-091 4.3.3.) according to the corresponding positions in the Tray Protocol.

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.

Note: When filling calibration standard tubes and sample tubes, only fill below the level of the wash pot. Otherwise, contamination between tubes may occur.

6.5. Once the baseline is stabilized, start the run by going to Run → Start → TKN GD → Double click the sequence → OK.

6.6. The pump and autosampler cannot be paused while running, but the sequence can be changed in the Tray Protocol.

Note: If the autosampler arm is hit while running, the autosampler error will flash and will no longer move. If not, the run will need to be restarted. All samples and QC affected by the autosampler error must be reran accordingly.

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Note: Before accepting changes made to the Tray Protocol, check the system window for the autosampler's peak number position ("No: #"). Any changes must be at least four peaks numbers **after** the number on the system window before pressing "OK." Ensure that changes are made according to peak numbers and not cup numbers!

6.7. When ready to stop the run, go to the Tray Protocol and add the "End" function to the cup where the run should stop. There should be a CAR, CCB, CCV, Drift, and baseline prior to the "End."

6.8. **Delete all unused lines after the "End" and click "OK" to save the changes.**

6.9. Once analysis is complete, the analyst will print the final report containing the calibration and the chart of the peaks.

6.10. Print the raw data file in Retrieve → Raw Data. Run files are automatically exported to the Nutrients drive (see 7.4.).

6.11. Instrument Daily Shutdown:

6.11.1. Place the reagent tubing for the Salicylate reagent into DI water. Pump on fast for at least 10 minutes to flush the salicylate out of the system.

6.11.2. Place the reagent tubing for the EDTA/NaOH, Sulfuric Acid, Buffer reagents into the System Wash solution. Place the reagent tubing for the Sampler Wash and DIC reagent in DI water. Pump on fast for 10-15 minutes.

6.11.3. Place all reagent tubing that was in the System Wash Solution into DI water and continue pumping on fast for 15 minutes.

6.11.4. Raise probe out of the wash receptacle and let the wash pot empty. Remove all reagent tubing from the DI water. Pump air through the system on fast for 10-15 minutes to dry off the membrane.

6.11.5. Set the pump from fast to normal and from run to stop. Release the pump platen, pump tubing and turn off the autosampler.

Note: The AA500 manifold for Bran Luebbe F is always on and does not need to be turned on/off.

6.12. Quantitation/Calculations:

6.12.1. The instrument reports results for TKN analysis in mg N/L.

6.12.2. 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 the FDEP Quality Manual.

6.12.3. The high calibration standard is prepared according to DEP SOP NU-091 Section 4.3.6. The other standards used in the second order calibration are prepared according to section 4.3.4. The quadratic calibration and percent error of the standards are calculated by the instrument software and are part of the analysis report. It includes the correlation coefficient to evaluate the fit (see 8.2.).

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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(s) (W-TKN(-F)), filename/s (TKNYYMMDDX), tray ID number, and initials of the analyst.

7.2. Each report folder should contain the following items – Result report, calibration curve, chart of the peaks, bench sheet with analysis parameters, raw data printout, digestion backlog, Reagent and Standard Logsheet, Pipette Logsheet (NU-PIPETTE) 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 authorization. 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. SEAL Bran Luebbe instrument run and text files for upload to QC manager are stored in electronic format in the current year's subfolder in the instrument's subfolder (BranLuebbeF) located at \\floridadep\data\dear\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\SegmentedFlowAnalyzers\BranLuebbe F\TKN-GD\.

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 \\floridadep\data\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. QUALITY CONTROL/DATA ASSESSMENT (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 < 0.050 and > -0.050 mg N/L. The check standard (CCV) recovery limits are 90 – 110%

8.3. Each tray of digested samples contains a digested MDL verification spike standard (MDL SPK, DEP SOP NU-091 4.3.8.), a 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 50-150% for the MDL SPK, 70 - 130% for PQCK2 and 90 – 110% for the ALTQC. The MDL SPK is also used for the ongoing MDL and PQL verification.

Note: An MDL SPK should be prepared with every tray, if possible. At minimum, an MDL SPK must be prepared twice a quarter for yearly statistics calculations, as required by our

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Quality Manual.

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 calculated results are <0.050 and > -0.050.

8.5.4 Acceptance limits for the percent error of the MDL SPK level calibration standard is ±50%.

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

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

8.5.7. 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. Write down all maintenance, troubleshooting, and service visits in the instrument's maintenance logbook.

9.2. Remove the PTFE membrane from the dialyzer block if the system will not be in use for a long time. Otherwise, the membrane can stick to block and damage dialyzer channels causing leaks.

9.3. Weekly Cleaning Procedure –

The following protocol should be done at the **minimum once a week** after samples are analyzed during the instrument shut down procedure.

It should also be done more frequently when more difficult sample matrices are analyzed to prevent contamination from building up in the system. This will help prevent from having to do a full system deep clean more often.

During the instrument daily shut down, once all reagents have been flushed from the system (6.10.2.) and with the pump set to "fast," place all reagent tubing in:

- 1:10 HCl (4.2.10.) (incl. wash line and sample probe/line) – 10 minutes
- DI water – 10 minutes

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- 1 N NaOH (4.2.11.) – 10 minutes
- DI water – 5 minutes
- 10% Bleach (4.2.12.) (Only the wash line (NOT incl. sample probe or reagent lines)) – 15 minutes
- DI water – 30 minutes

With the probe raised, clean the autosampler wash receptable with 10% bleach and a cleaning swab. Rinse thoroughly with DI water.

Continue following the instrument shut down procedure (6.10.4.).

9.4. Monthly Cleaning Procedure –

The following protocol should be done at the **minimum once a month** as preventative maintenance.

It should also be done if instrument has contamination issues that were not resolved through the weekly cleaning procedure (9.3.).

9.4.1. With the pump set to "fast," place all reagent tubing in:

- 1:10 HCl (incl. wash line and sample probe/line) – 15 minutes
- DI water – 15 minutes
- 1 N NaOH – 15 minutes
- DI water – 15 minutes
- 10% Bleach (4.2.12.) (**NOT** incl. sample probe/line) – 15 minutes
- DI water (incl. sample probe/line) – 30+ minutes

Note: **Do not allow bleach to go through the sample probe!** Bleach will aggressively strip the probe leading to sample breaking and carryover issues. This will lead to the probe needing to be replaced.

9.4.2. With the probe raised, clean the autosampler wash receptable with 10% bleach and a cleaning swab. Rinse thoroughly with DI water.

9.4.3. Change pump tubing and airbar tubing.

9.4.4. Change the autosampler peristaltic pump tubing.

9.4.5. Replace the dialyzer membrane.

Note: SEAL recommends replacing the autosampler's peristaltic pump heads at least every 6 months. Due to the corrosive nature of the TKN analysis, the pump tubing should be inspected regularly and changed sooner if deterioration is observed.

9.5. Troubleshooting Tips –

9.5.1. Drifting or noisy baseline:

- Make sure waste lines from dialyzer, autosampler, and colorimeter are not submerged. Submerged waste lines can cause a drifting baseline or air spikes. Replace dialyzer and colorimeter waste lines if necessary.
- Make sure bubble pattern is even and all bubbles are symmetrical. Be sure reagent lines are submerged.

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- When switching from wash solutions to reagents, the air introduced may get stuck before the glass injection joints and between the tubing joints. Remove these bubbles and check if baseline has improved.
- Baseline noise may also be caused by the air flow tubing (black-black). Check to be sure the flexible tubing is in the center of the air flow regulators and properly clamped on both sides.
- A noisy baseline with fresh reagents and diluent can indicate a contaminated autosampler wash receptacle or sampling probe. Clean the reagent tubing/line of the autosampler wash receptable with 10% bleach, the sampling probe with acid and base, and replace the sample line and sample probe if necessary.

9.5.2. An increase of air spikes in the run usually indicates pump tubing needs to be changed. Glass tubing joints may also need to be replaced if there is a white band and/or bubbles that are getting caught in the joint's gap.

9.5.3. If the tops of peaks are noisy or take a long time to come to a plateau, the membrane most likely needs to be changed.

9.5.4. Carryover should be below 2%. A higher carryover indicates the need for maintenance and/or troubleshooting.

9.5.5. Failing blanks can indicate contamination. There are multiple approaches to fixing this, such as:

- Check that standard and sample tubes are not filled above the autosampler wash receptable line. Ensure that the autosampler wash receptable is filling and emptying appropriately.
- Acid soak with diluted HCl all glassware used to make reagents and standards overnight and replace all bottles. Remake reagents and standards. Acid soak the standards rack glass tubes overnight.
- Clean the system, replace the pump tubing, and change the membrane (see 9.4). Replace the probe sample line.
- For deeper contamination issues, it may be necessary to acid soak the reagent tubing, replace the tubing connecting glass joints, change the sample probe, replace the Kel-F line from 10-turn coil to detector, and/or replace the reagent and autosampler wash tubing lines (connected to the manifold, autosampler, and reagent tubing).
- If the problem persists, the Buffer Reagent may have become contaminated and should be re-made with a new bottle of potassium tartrate (4.1.2.). If the stock bottle becomes contaminated, see "SEAL AQ2 Technical Tips Removal of Ammonia Contamination from Sodium Potassium Tartrate" for guidance.

9.5.6. If the primer peak drops significantly, the dialyzer block is likely to be contaminated and should be soaked in the 1:10 HCl cleaning solution

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(4.2.10.) overnight, followed by rinsing with DI water sufficiently to remove all traces of hydrochloric acid. Significant drop in primer peak can also be caused by uneven pressure on the tubing where the platen may needs to be adjusted. Please notify your supervisor before making any adjustment on the platen.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

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.

10.4. The AA500 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.

10.7. Excess DIC (4.1.1.) and DIC Reagent (4.2.9.) should be disposed of down the

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sink and should be flushed with copious amounts of tap water.

10.8 The 1:1 HCl solution for cleaning is disposed of down the sink and should be flushed with copious amounts of tap water during the rinse procedures.

11. REFERENCES/DEFINITIONS

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. DEP SOP NU-026, Manual Determination of pH

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

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

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

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

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

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

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

11.10. Quality Manual, Bureau of Laboratories, FDEP.

11.11. Laboratory Health and Safety Plan.

11.12. SEAL AutoAnalyzer 500 Method MK5-0154-23 Rev. 0

11.13. SEAL Analyzer AA500 Operation Manual System

11.14. SEAL AutoSampler all types Operation Manual

Appendix of Significant Changes

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