API Nutrient Analysis – TN/TP, NO_3, NH_4, SRP

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Purpose: This procedure describes the steps to measure nutrients in water samples. The units of TN/TP, NO_3, NH_4, and SRP for this analysis are micrograms per liter.

Sample Holding Time:

- TNTP: ≤ 1 year, room temperature, ~100 mL preserved with 1mL concentrated HCl acid
- NO3, NH4, SRP: ≤ 1 year, frozen

Materials Required:

• Astoria-Pacific Astoria2 Analyzer • 15mL glass vials • Black test tube caps • 15mL plastic vials • 1-5mL pipette • 5mL pipette tips • Amber repipettor • WypAlls • Kimwipes	• Reagents: *Reagent prep varies among different tests. See* Preparing Reagents *for test-specific reagents/standards.* • Standards: *Standard prep varies among different tests. See* Preparing Standards/ICVs *for test-specific standards.*

Glassware Preparation: Glassware used in reagent prep and standard bottles must be acid washed before use. (Refer to "Acid Washing" SOP)

Personal Protective Equipment / Waste Disposal: Nitrile gloves and eye protection should be worn during this procedure. Always use chemical resistant gloves (not latex), safety glasses, lab coat, and a fume hood while using concentrated acids to prepare reagents. This is not only for your protection, but also to prevent contamination of samples. Proper personal protective equipment is always required for safety and contamination prevention.

Quality Assurance/Quality Control: The validity of the data collected by the instrument is checked with the following quality control samples:

- Reagent blank, plain Milli-Q water
- Initial Calibration Verification (ICV) sample(s) are prepared from a different stock material than the calibration curve.
- Every 10^{th} sample is duplicated.

Waste Disposal: Samples and washes can be disposed of down the drain. Throw glass vials in glass disposal boxes, and the black caps and plastic vials in the trash. Excess phenol reagent (NH4 assay) from the daily pour off must be disposed in the white carboy in the 101 lab.

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Sample and Standard Prep & Digestion for TNTP

1.0 Print labels from ChemLab 3.0

1.1 Username: lab, Password: Lovew@ter8!

1.2 *LTER → Search / View*

1.2.1 Select your event and test

1.2.2 Scroll to the bottle and select ***Print Labels*** and print.

(The print off will be formatted for the Avery 5160 labels located in the desk drawer - make sure you put the label sheets in the printer)

2.0 Stick labels to 15mL glass vials, duplicating every 10th sample.

2.1 Use excess empty labels to make labels for the duplicates

2.2 Label the duplicates the Sample ID + DUP with permanent marker

Ex) 123450 DUP

3.0 Stick a set of standard stickers onto tubes as well. A full set of standards will include C1-C10, C10(2), LOQ, ICV-L, ICV-M, ICV-H, and LRV. The C10(2) is a nitrite only standard of the same concentration as the nitrate C10 (2400ppb). The C10(2) is used to calculate the reduction efficiency of the cadmium column with the following equation.

$$e = \frac{[N - NO_3^-]}{[N - NO_2^-]} * 100\%$$

If e>105%, run C10 replicates until it drops. This usually will occur with a brand- new column or freshly conditioned column. If e<85%, recondition cadmium column using protocol on page 22. If column reduction capacity is unresponsive to conditioning, condition a new column.

4.0 Grab the TNTP standards from the fridge outside the WSEL lab and locate the box with your samples in the cabinets outside of the WSEL lab.

5.0 Using a 5mL pipette, pipette 5mL of standard/sample into your vial, repeating until you have pipetted all of your samples.

6.0 Once all your vials have sample in them, put a lab coat on to get ready to add the digestion reagent.

NOTE: If you spill digestion reagent on your clothes, it will create holes in your clothes if put through the dryer!

7.0 Empty the repipettor (should have MilliQ water in it) and place the amber glass container upside down on a WypAll. Let dry for about 3 minutes.

8.0 Add your digestion reagent to the container.

9.0 Take the beaker on the drying rack that is labeled ***Digestion Reagent*** and pump into the beaker until no air bubbles are present.

10.0 The repipettor should be set to dispense 2.5mL of digestion reagent. If it is not, set it.

11.0 Pump the repipettor to dispense the reagent into the vial.

12.0 Cap the and invert the vial

13.0 Bring your rack of samples and standards to the River Ecology lab and use autoclave to digest your samples. *(See Autoclave SOP)*

Sample and Standards Analysis

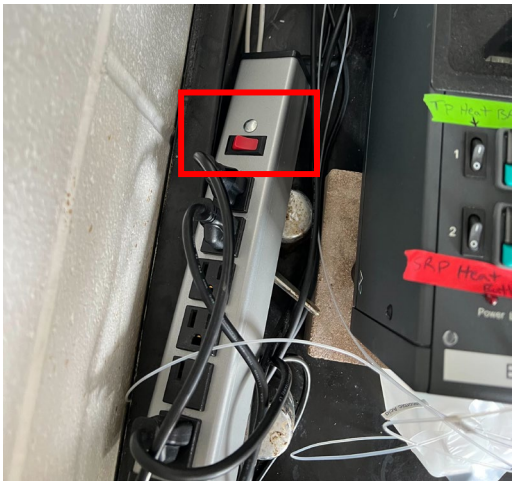
Start Up Procedure

1.0 Before operating, assure proper connections.

- **1.1** Make sure nothing is blocking the autosampler (above the blue sampler rack).
- **1.2** Sampler is connected to proper channels (T- intersect in tubing is going to correct modules on API).
- **1.3** Nitrogen pillow is connected to only in use channels.
- **1.4** Waste lines properly empty through underneath channel, into the trough.

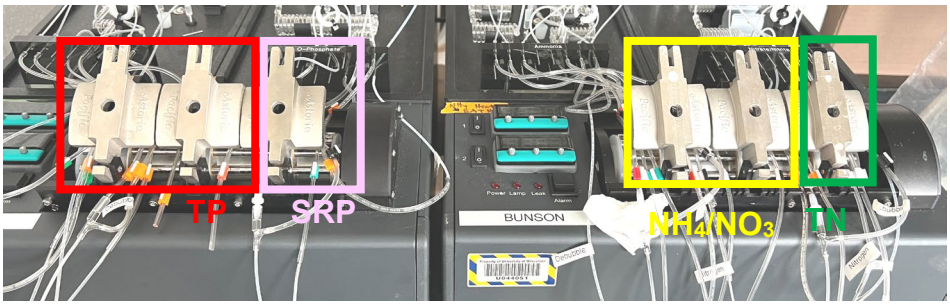
2.0 Take out any refrigerated/frozen reagents needed to thaw.

3.0 Toggle the ON switch on the power strip on the left side of the API, near the wall. The instrument should make a noise.



4.0 Latch the platens according to which test is being run:

- **4.1** TN requires the far-right platen on the right module.
- **4.2** TP requires the far-left and middle platen on the left module.
- **4.3** NH_4/NO_3 requires far-left and middle platen on the right module.
- **4.4** SRP requires far-right and middle platen on the left module.



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5.0 For NO_3 and NH_4, open the nitrogen pillow by twisting the metal nozzle to the left/counterclockwise (also indicated on the bag).

For start-up, ensure that the reagent lines are placed in proper wash bottles/start-up solutions. Turn on pumps by pressing the red buttons on the top back of the API. Information for prepping start-up solution can be found in *Preparing Reagents.*

- **TP/SRP:** Dowfax and MQ
- **TN/NO_3/DRSi:** TX10 and MQ
- **NH_4:** Brij-35 and MQ

Watch bubble pattern while reagent lines are in start-up, introducing a bubble by lifting the reagent lines out of solution is helpful to watch for bubble movement. Surging/stuttering bubbles may indicate that tubes need to be replaced.

		
(lots of small bubbles)	*(some small bubbles)*	*(bubbles are evenly spaced and the same size)*
Bad! Troubleshoot.	**Generally OK, may need more surfactant.**	**Good!**

6.0 Begin the start-up rinse cycle.

Remember to wipe reagent lines with a Kimwipe before placing in different solutions.

6.1 Start-up solution for 3 minutes

6.2 ChemWash for 5 minutes

6.3 Back to start-up solution for 20 minutes.

6.3.1 Within the last 5 minutes of this step, toggle on the heating baths located at the left side of the API.

6.4 *Use this time to prep reagents and to set up computer (step 8).*

6.4.1 Reagents to prep (see *Preparing Reagents)*:

- **TN:** Working NH_4Cl EDTA Buffer
- **TP:** Working Ascorbic Acid, Working Ammonium Molybdate Buffer
- **NH_4:** Working Complexing Reagent, Poured-off Alkaline Phenol, Working Sodium Hypochlorite, Poured-off Sodium Nitroferricyanide
- **NO_3:** Working NH_4Cl EDTA Buffer
- **SRP:** Working Color reagent

7.0 At the Computer:

7.1 Open the FASPac 2.4 application located in the center of the desktop screen. When prompted for the password, click ***OK*** (user: LTER, password: blank).

7.2 Select configuration based on test(s) from the tool bar drop down. Configurations with the latest date behind them will be most updated and should be used.

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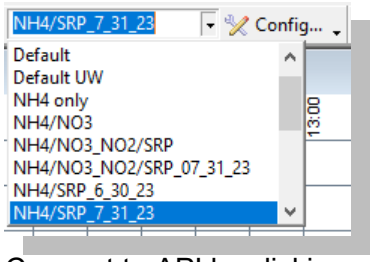


7.3 Connect to API by clicking on the green plug in the tool bar.
Confirmation of connection will be signified by a handshake icon that appears.

7.4 Name the run using your project, the test name, date (MM_DD_YYYY)
Ex) LTER_TNTP_07_24_2023

8.0 Monitor the baseline through FASPac by right clicking on the channel window and selecting ***Display Signal***.

8.1 Baseline can wobble, but major fluctuation suggest bubbles may be stuck in the flow cell. If the bubble does not exit by itself, quickly squeezing the tygon tubing near the waste port should work.

8.2 Correct the baseline by right clicking and selecting ***Zero Signal***.

9.0 Once 3-5-20 start-up procedure is finished, reagent lines can be placed in their respective reagents with parafilm covering the open bottles. *Move the wash pot line to the proper reservoir.*

9.1 Reagent lines are tagged with proper reagents needed:

- **NH_4:** Nitroferricyanide, hypochlorite, complexing reagent, phenolate
- **NO_3:** Buffer, color reagent
- **TP:** Ascorbic Acid, Mo/Sb (buffer), Diluent (same as Start-up)
- **DRSi:** Mo/Sb (buffer), Stannous Chloride, Tartaric Acid
- **SRP:** SRP color reagent

9.2 Wash pot guide:

- **NH_4/NO_3/SRP:** MQ
- **TNTP:** Sodium Bisulfate solution
- **DRSi:** 1% HCl (optima)

10.0 For NO_3 and TN: Condition Cd Column (column located in labeled drawer under API reagents shelf*).*

Addition of Cd column must occur after the NH_4Cl buffer signal displays on FASPac. If no clear buffer signal displays, 10 minutes should be sufficient time for buffer to run through system.

10.1 Fill all Cd column syringes first (labeled, four syringes total).

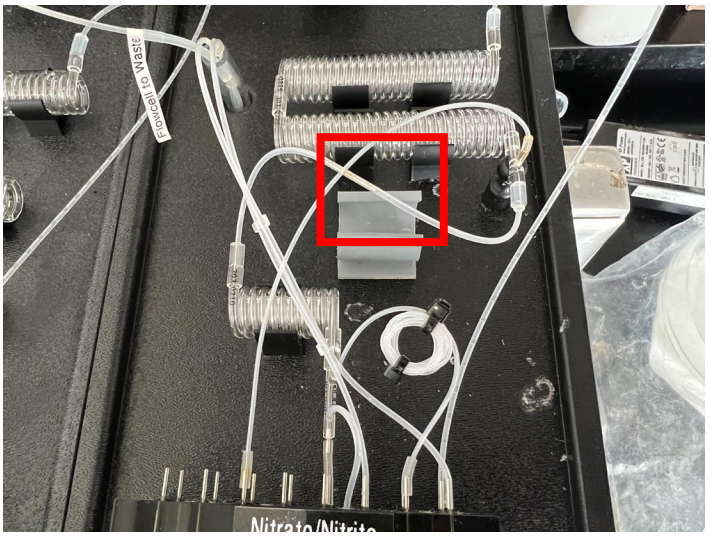
10.2 Open Column by sliding one side off of the metal connecter with a fingernail. **Avoid pulling on tubes to separate, as this causes tubes to stretch (decreases efficacy).**

10.3 Follow the order of conditioning indicated on the bottles, flush solutions through the side with the dated tag which has the metal connector piece (inlet).

NOTE: Do not expend all the syringe contents into the Cd column. Air/bubbles that may be in the syringe isn't good for Cd column longevity.

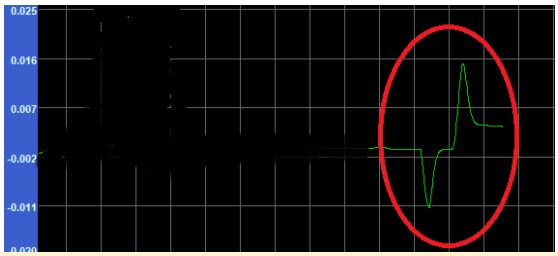
1. MQ (refill immediately after use)
2. HCl (be ready to flush with MQ after)
3. MQ (refill immediately after use)
4. $CuSO_4$ (push one syringe through until copper flakes are observed, typically ¾ of one syringe)
5. MQ (keep flushing until no more copper flakes occur)
6. NH_4Cl buffer (no TX-10)

10.4 Attach column to nitrate channel (middle of module)



10.4.1 Attach inlet first to avoid introduction of air/bubbles

10.4.2 Cd Column signal will display:



NOTE: Bubble pattern after attaching column may be uneven, stability can be determined from baseline monetarization.

11.0 Double check that TP/DRSi/NH_4 heater is on and up to temp, and make sure reagents that came out of the fridge are back in the fridge.

12.0 Modify sample table. When FASPac opens with the correct configuration, the sample table will appear at left. Add SIDs to the cell after standards

12.1 Standards will autofill, note that SYNC (first spot in sample rack) is the highest standard prepped (ex. C10 for TNTP). The following three slots will be washes (the same as the wash pot)

12.2 Slots in the sample rack are dictated by $\#_a$:$\#_b$ where $\#_a$ indicates which individual tray (up to 4), and $\#_b$ indicates the specific spot in the tray (numbered on the base of the tray)

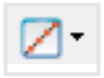
13.0 Once the baseline is steady, standards and samples can be poured off and placed into their assigned spots in the sampler trays via step 13. Make sure to check periodically that samples are going into the correct spot.

14.0 Begin the run by clicking the green arrow located in the tool bar.

14.1 SYNC signal will display within 3-4 minutes. Record this initial reading in the run log binder

14.2 For nitrate, an indicator that sampling is occurring can be observed by a pink/magenta color in the coils

15.0 As the standards are being ran, monitor the calibration curve by clicking the calibration drop down from the tool bar **(linear chart icon)**.



15.1 Typically a R^2 value of 0.999 or higher is considered acceptable

15.2 Manipulation of up to two points on the calibration curve can be accomplished by clicking on individual points to exclude them.

15.3 DO NOT click on the QC Chart button (scatter plot). You will anger the API.

16.0 Enter ICVs in QC Check spreadsheet.

17.0 Monitor the sampling throughout the entire run (timing of run depends on amount of samples, one sample ~1-1.5 min of run time)

Shut Down (plan about 45 minutes)

1.0 When the run is finished, the software will automatically end the run which can be observed by the tool bar graying out.

2.0 **Before run is saved/exported, look over data for anomalies.** Eyeball similarity of duplicates. Duplicates that are vastly different may indicate a rerun is needed.

3.0 Save and export your run

3.1 To save in FASPac: File → Save → OK

3.2 To export: File → Export → Run File (FPX) AND File → Export → Results → Comma Delimited file (*.csv).

3.3 Change location of export if necessary. *For LTER: O:Drive → LTER Water Chem Lab → RAW DATA → (Test ran) → (Year)*

4.0 For NH_4 and TP: Turn off the heat bath.

5.0 Shut valve on nitrogen pillow (twist metal piece to the right as indicated).

6.0 Remove Cd Column:

6.1 **TURN OFF PUMP FIRST**

6.2 Detach outlet first and then inlet.

6.3 Flush column with one syringe of MQ (some copper particles may expel).

6.4 Push one syringe of NH_4Cl buffer (no TX-10).

6.5 Close, and return to drawer.

7.0 Begin the shut-down rinse cycle.

Remember to wipe reagent lines with Kimwipe before placing them into a new solution.

7.1 MQ + Soap (same as start-up) for 5 minutes

7.2 ChemWash for 10 minutes

7.3 MQ + Soap for 15 minutes

8.0 While 5-10-15 rinse cycle is going, dispose of reagents and samples. Rinse reagent bottles three times with MQ water before placing on drying rack.

9.0 When rinse cycle is done, unclamp the platens and toggle off the power (left of API).

9.1 Ensure that wash pot line is moved back to MQ for storage

9.2 Ensure that any open containers are either capped, or covered with parafilm

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Preparing Reagents

Total Nitrogen and Total Phosphorus (TN/TP)

Sampler Wash

Safety Overview:	
Sodium Bisulfate Monohydrate ($NaHSO_4H_2O$)	Causes burns by all exposure routes.
Sodium Chloride (NaCl)	May cause skin/eye/respiratory irritation.

- 15.244g Sodium Bisulfate Monohydrate ($NaHSO_4H_2O$)
- 13.8g Sodium Chloride (NaCl)
- Milli-Q
- 2L volumetric flask

Add 15.244g sodium bisulfate monohydrate to 1.5L Milli-Q in a 2L volumetric flask. Mix to dissolve. Add 13.8g sodium chloride and dissolve. Dilute to 2L with Milli-Q.

Total Nitrogen (TN)

Safety Overview:	
Ammonium Chloride (NH_4Cl)	Harmful if swallowed. Irritating to eyes.
Ammonium Hydroxide (NH_4OH)	Harmful if swallowed. *Use in fume hood.*
Disodium Ethylenediamine Tetraacetate (disodium EDTA, $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$)	Slightly hazardous in case of skin/eye contact or ingestion.
Concentrated Phosphoric Acid (H_3PO_4)	Causes burns by all exposure routes. *Use in fume hood.*
N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2$ 2HCl)	Harmful if swallowed, inhaled or absorbed through skin. Causes irritation to skin, eyes, and respiratory tract.
Sulfanilamide ($C_6H_8N_2O_2S$)	Irritating to eyes, skin, and respiratory system.

TN start-up solution

- ~500mL Milli-Q
- 1-2mL TX-10
- 500mL rectangular bottle

Fill 500mL rectangular bottle to the shoulder with Milli-Q. Use 1mL disposable pipette to add ~1mL of TX-10. Turn the pump on and watch the bubble pattern. If the bubbles are breaking up, add more TX-10.

STOCK Ammonium Chloride EDTA Buffer:

- 85g Ammonium Chloride (NH_4Cl)
- 0.10g Disodium Ethylenediamine Tetraacetate (disodium EDTA, $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$)
- 6.5mL of Ammonium Hydroxide (NH_4OH)
- 1000mL volumetric flask
- 1000mL Nalgene bottle

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Dissolve ammonium chloride and EDTA in 900mL of Milli-Q water. Add ammonium hydroxide. Check pH (aiming for 8.5). Dilute to 1000mL with Milli-Q. *Store in 1000mL Nalgene on API reagent shelf.*

WORKING Ammonium Chloride EDTA Buffer:

- 250mL stock reagent
- ~0.5mL TX-10
- 250mL working reagent Nalgene bottle (labeled)

Pour off stock into 250mL working reagent bottle to the shoulder, add ~4 drops of TX-10. *Prepare fresh daily.*

Color Reagent:

- 20g Sulfanilamide ($C_6H_8N_2O_2S$)
- 50mL Concentrated Phosphoric Acid (H_3PO_4)
- 1g N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2$ 2HCl)
- 500mL volumetric flask
- 500mL glass amber bottle

Add sulfanilamide and concentrated phosphoric acid to 400mL of Milli-Q water. Dissolve completely (heat and stir on a hot plate if needed). Add N-1-Naphthylethylenediamine Dihydrochloride and dissolve. Dilute to 500mL. *Store in dark bottle at 4ºC.*

Total Phosphorus (TP)

Safety Overview:	
Acetone (CH_3COCH_3)	Extremely flammable liquid/vapor. Eye/respiratory irritant. Breathing may cause drowsiness/dizziness.
Ammonium Molybdate ($(NH_4)Mo_7O_{24}{\cdot}4H_2$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Antimony Potassium Tartrate [$(K(SbO)C_4H_4O_6{\cdot}½H_2O$]	Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Ascorbic Acid ($C_6H_8O_9$)	Air/light sensitive. Fire/explosion risk when in contact with oxidizing agents.
Concentrated Sulfuric Acid (H_2SO_4)	Causes severe burns by all exposure routes. May be fatal if inhaled. Reacts violently with water. Contact with combustible material may cause fire. *Use in fume hood.*

TP Start-up Solution/Diluent:

- ~500mL Milli-Q
- 1-2mL Dowfax
- 500mL rectangular bottle

Fill 500mL rectangular bottle to the shoulder with Milli-Q. Use 1mL disposable pipette to add ~1mL of Dowfax. Turn the pump on and watch the bubble pattern. If the bubbles are breaking up, add more Dowfax.

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STOCK Ascorbic Acid:

- 6.0g Ascorbic Acid ($C_6H_8O_9$)
- 200mL Acetone (CH_3COCH_3)
- 200mL Milli-Q
- 500mL Nalgene bottle

Dissolve 6.0g ascorbic acid in a mixture of 200mL acetone and 200mL Milli-Q. *Prepare fresh monthly or as needed. Store in 500mL Nalgene bottle at 4ºC (in WSEL LTER fridge).*

Working Ascorbic Acid:

- 10mL Stock Ascorbic Acid
- 50mL Milli-Q
- 100mL working reagent Nalgene bottle (labeled

Pipette 10mL stock ascorbic acid into the labeled bottle. Add 50mL Milli-Q. *Prepare fresh daily.*

Antimony Potassium Tartrate:

- 1.5g Antimony Potassium Tartrate [$(K(SbO)C_4H_4O_6 \cdot ½H_2O$]
- Milli-Q
- 500mL Nalgene bottle

Dissolve antimony potassium tartrate in 300mL of Milli-Q in a 500mL volumetric flask. Dilute to 500mL. *Store in 500mL Nalgene bottle at 4ºC (in WSEL LTER fridge).*

STOCK Molybdate/Antimony Reagent:

- 70mL Concentrated Sulfuric Acid (H_2SO_4)
- 6.0g Ammonium Molybdate ($(NH_4)Mo_7O_{24} \cdot 4H_2$)
- 50mL Stock Antimony Potassium Tartrate
- Milli-Q

In the fume hood, slowly add sulfuric acid to 600mL of Milli-Q while stirring in an ice bath. Add ammonium molybdate and stir until dissolved. Add stock antimony potassium tartrate and mix well. Allow to cool and dilute to 1000mL with Milli-Q. *Store in 1000mL Nalgene bottle on API reagent shelf.*

WORKING Molybdate/Antimony Reagent:

- 100mL stock reagent
- ~1mL TX-10
- 100mL working reagent Nalgene bottle (labeled)

Pour off stock into 100mL working reagent bottle to the shoulder, add ~8 drops of TX-10. *Prepare fresh daily.*

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Dissolved Nutrients

Nitrate (NO_3)

Safety Overview:	
Ammonium Chloride (NH_4Cl)	Harmful if swallowed. Irritating to eyes.
Ammonium Hydroxide (NH_4OH)	Harmful if swallowed. *Use in fume hood.*
Disodium Ethylenediamine Tetraacetate (disodium EDTA, $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$)	Slightly hazardous in case of skin/eye contact or ingestion.
Concentrated Phosphoric Acid (H_3PO_4)	Causes burns by all exposure routes. *Use in fume hood.*
N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2$ 2HCl)	Harmful if swallowed, inhaled or absorbed through skin. Causes irritation to skin, eyes, and respiratory tract.
Sulfanilamide ($C_6H_8N_2O_2S$)	Irritating to eyes, skin, and respiratory system.

STOCK Ammonium Chloride EDTA Buffer: *(Same as TN)*

- 85g Ammonium Chloride (NH_4Cl)
- 0.10g Disodium Ethylenediamine Tetraacetate (disodium EDTA, $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$)
- 6.5mL of Ammonium Hydroxide (NH_4OH)
- 1000mL volumetric flask
- 1000mL Nalgene bottle

Dissolve ammonium chloride and EDTA in 900mL of Milli-Q water. Add ammonium hydroxide. Check pH (aiming for 8.5). Dilute to 1000mL with Milli-Q. *Store in 1000mL Nalgene on API reagent shelf.*

WORKING Ammonium Chloride EDTA Buffer: *(Same as TN)*

- 250mL stock reagent
- ~0.5mL TX-10
- 250mL working reagent Nalgene bottle (labeled)

Pour off stock into 250mL working reagent bottle to the shoulder, add ~4 drops of TX-10. *Prepare fresh daily.*

Color Reagent:

- 20g Sulfanilamide ($C_6H_8N_2O_2S$)
- 50mL Concentrated Phosphoric Acid (H_3PO_4)
- 1g N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2$ 2HCl)
- 500mL volumetric flask
- 500mL glass amber bottle

Add sulfanilamide and concentrated phosphoric acid to 400mL of Milli-Q water. Dissolve completely (heat and stir on hot plate if needed). Add N-1-Naphthylethylenediamine Dihydrochloride and dissolve. Dilute to 500mL. *Store in dark bottle at 4ºC.*

Dissolved Nutrients

Ammonium (NH_4)

Safety Overview:	
Phenol, liquefied (C_6H_5OH)	Very hazardous in case of skin/eye contact or ingestion. Skin contact will produce burns. Corrosive, sensitizer, and permeator. *Use in fume hood.*
Sodium Citrate [$HOC(COONa)(CH_2COONa)_2 \cdot H_2O$]	May cause skin, eye, and respiratory irritation.
Sodium Hydroxide (NaOH)	Causes severe burns by all exposure routes. Water reactive. *Use in fume hood.*
Sodium Nitroferricyanide ($C_5H_4FeN_6Na_2O_3$)	Very hazardous in case of ingestion. Slightly hazardous in case of skin contact (permeator).
Sodium Potassium Tartrate ($KnaC_4H_4O_6 \cdot 4H_2O$)	May cause skin, eye, and respiratory irritation.
Concentrated Sulfuric Acid (H_2SO_4)	Causes severe burns by all exposure routes. May be fatal if inhaled. Reacts violently with water. Contact with combustible material may cause fire. *Use in fume hood.*

STOCK Complexing Reagent:

- 33g Sodium Potassium Tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)
- 24g Sodium Citrate [$HOC(COONa)(CH_2COONa)_2 \cdot H_2O$]
- 2mL Sulfuric Acid (H_2SO_4)
- Milli-Q water
- 1L volumetric flask
- 1L Nalgene bottle

Completely dissolve sodium potassium tartrate in 900mL of Milli-Q in volumetric flask. Add and dissolve sodium citrate. Add sulfuric acid. Dilute to 1L with Milli-Q. Use pH electrode to ensure pH is ~5.0. *Store in 1L Nalgene bottle on API reagent shelf.*

WORKING Complexing Reagent:

- 50 mL Stock Complexing Reagent
- 50mL Milli-Q
- ~0.5mL Brij-35
- 250mL working reagent Nalgene bottle (labeled)

Dilute stock 1:1 with Milli-Q in working bottle. Add 4 drops of Brij-35. *Prepare fresh daily.*

10N Sodium Hydroxide:

- 400g Sodium Hydroxide
- Milli-Q
- 1L volumetric flask
- 1L plastic Nalgene bottle

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In the fume hood, slowly add sulfuric acid to 600mL of Milli-Q while stirring in an ice bath. Once completely dissolved and cooled, dilute to 1L. *Store in 1L Nalgene bottle on API reagent shelf.*

Alkaline Phenol:

- 12mL Phenol, liquefied (C_6H_5OH)
- 85mL Sodium Hydroxide (NaOH)
- Milli-Q
- 1L volumetric flask
- 1L brown Nalgene bottle/amber glass bottle

In the fume hood, slowly add sodium hydroxide to 700mL of Milli-Q water in volumetric flask while stirring in an ice bath. Once solution is cooled, add phenol in small quantities, cooling completely between each addition. Dilute to one liter with Milli-Q. *Store in 1L brown Nalgene/glass amber bottle at 4ºC (in WSEL LTER fridge).*

STOCK Sodium Hypochlorite: Any good commercially available household bleach having 6.00% chlorine may be used.

WORKING Sodium Hypochlorite:

- 2.2mL Stock Sodium Hypochlorite
- 97.8mL Milli-Q
- 250mL working reagent Nalgene bottle (labeled)

Dilute stock sodium hypochlorite to 100mL with Milli-Q in working bottle. *Prepare fresh daily.*

STOCK Sodium Nitroferricyanide:

- 0.5g Sodium Nitroferricyanide
- Milli-Q
- 1L volumetric flask
- 1L brown Nalgene bottle/amber glass bottle

Dissolve sodium Nitroferricyanide in 900mL of Milli-Q. Dilute to 1L with Milli-Q. *Store in 1L brown Nalgene/glass amber bottle on API reagent shelf*

WORKING Sodium Nitroferricyanide: Pour off stock solution into working bottle.

Nutrient Analysis – API Astoria2 Analyzer

Dissolved Nutrients

Orthophosphate (SRP)

Safety Overview:	
Ammonium Molybdate ($(NH_4)Mo_7O_{24}{\cdot}4H_2$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Antimony Potassium Tartrate [$(K(SbO)C_4H_4O_6{\cdot}½H_2O$]	Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Ascorbic Acid ($C_6H_8O_9$)	Air/light sensitive. Fire/explosion risk when in contact with oxidizing agents.
Concentrated Sulfuric Acid (H_2SO_4)	Causes severe burns by all exposure routes. May be fatal if inhaled. Reacts violently with water. Contact with combustible material may cause fire. *Use in fume hood.*

Working Color Reagent:

1. 50mL Sulfuric Acid, 5N
2. 5mL Antimony Potassium Tartrate
3. 15mL Ammonium Molybdate
4. 30mL Ascorbic Acid
5. 0.5mL Dowfax

Add reagents in order and mix after each addition. *Prepare fresh daily.*

Color Reagent Intermediates

5N Sulfuric Acid

- 140mL Concentrated Sulfuric Acid (H_2SO_4)
- Milli-Q
- 1L volumetric flask
- 1L plastic Nalgene bottle

In the fume hood, slowly add sulfuric acid to 600mL of Milli-Q while stirring in an ice bath. Once completely dissolved and cooled, dilute to 1L. *Store in 1L Nalgene bottle on API reagent shelf.*

Antimony Potassium Tartrate: *(Same as TP)*

- 1.5g Antimony Potassium Tartrate [$(K(SbO)C_4H_4O_6{\cdot}½H_2O$]
- Milli-Q
- 500mL volumetric flask
- 500mL Nalgene bottle

Dissolve antimony potassium tartrate in 300mL of Milli-Q in a 500mL volumetric flask. Dilute to 500mL. *Store in 500mL Nalgene bottle at 4ºC (in WSEL LTER fridge).*

Ammonium Molybdate: *(Different from TP reagent)*

- 8g Ammonium Molybdate ($(NH_4)Mo_7O_{24}{\cdot}4H_2$)
- Milli-Q
- 200mL volumetric flask

- 250mL plastic Nalgene bottle

Dissolve ammonium molybdate in 100mL of Milli-Q, dilute to 200mL. *Store in Nalgene at 4ºC*.

Ascorbic Acid: *(Different from TP reagent)*

- 4.5g Ascorbic Acid
- Milli-Q
- 250mL volumetric flask
- 250mL plastic Nalgene bottle

Dissolve ascorbic acid in 150mL of Milli-Q. Dilute to 250mL. *Store in Nalgene bottle at 4ºC*.

Cadmium (Cd) Column Reduction Activation

Safety Overview:	
Cupric Sulfate ($CuSO_4 \cdot 5H_2O$)	Harmful if swallowed. Irritated to eyes, respiratory system, and skin.
Hydrochloric Acid (HCl)	Causes burns by all exposure routes.

STOCK Ammonium Chloride/EDTA Buffer: *(Same as TN and NO_3)*

- 85g Ammonium Chloride (NH_4Cl)
- 0.10g Disodium Ethylenediamine Tetraacetate (disodium EDTA, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$)
- 6.5mL of Ammonium Hydroxide (NH_4OH)
- 1000mL volumetric flask
- 1000mL Nalgene bottle
- 100mL Cd column Nalgene bottle (labeled)

Dissolve ammonium chloride and EDTA in 900mL of Milli-Q water. Add ammonium hydroxide. Check pH (aiming for 8.5). Dilute to 1000mL with Milli-Q. Transfer to labeled Cd column bottle. *Store in 1000mL & 100mL Nalgenes on API reagent shelf.*

Cupric Sulfate Solution:

- 10g Cupric Sulfate ($CuSO_4 \cdot 5H_2O$)
- Milli-Q
- 500mL volumetric flask
- Parafilm
- 100mL Cd column Nalgene bottle (labeled)

Dissolve cupric sulfate in ~400mL of Milli-Q water. Dilute to 500mL. Transfer to labeled Cd column bottle. *Store in 100mL Nalgene on API reagent shelf. Store remaining in volumetric flask – parafilm the stopper.*

0.1N Hydrochloric Acid

- 2.1mL Concentrated Hydrochloric Acid (HCl) (Optima brand)
- Milli-Q

Add f concentrated HCl to ~200mL of Milli-Q water. Dilute to 250mL. *Store in 250mL Nalgene on API reagent shelf.*

1.0N Hydrochloric Acid

- 8.3mL Concentrated Hydrochloric Acid (HCl) (Optima brand)
- Milli-Q

Add concentrated HCl to ~80mL of Milli-Q water. Dilute to 100mL. *Store in 100mL Nalgene on API reagent shelf.*

Preparing Standards/ICVs

TNTP

1.0 Go to standard calc sheet folder in share drive. *LTER Water Chem Lab → SOPs and Project Descriptions → STANDARD CALC SHEETS → TN-TP*

2.0 Make a copy of the file titled ***TN_TP-Template-BLANK***

3.0 Rename the file with the date that you plan to make the standards.

3.1 *Ex) TN_TP_07_14_2023*

4.0 Put the file in the file titled with the appropriate year.

5.0 Open the sheet and fill out your name and the date of preparation in the top right corner of the sheet, as well as the stock solution information.

6.0 Print a copy of the calculation sheet.

7.0 Obtain fifteen clean 250 mL and two 100mL plastic Nalgene bottles.Brand new bottles are ideal, but freshly acid washed bottles are also okay. (See acid washing SOP)

8.0 Label the bottles with label tape:

8.1 INT, ICV INT, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, LOQ, ICV-L, ICV-M, ICV-H, LRV

8.2 On the left side of the tape write “TN TP STD” and on the right side write the day of preparation and your initials

8.3 *Tip: color coding the calibration standards vs. ICVs may also be helpful. For example, all calibration standards (C1-10) are green and all ICVs (LOQ, LRV, ICV-L, M, H) are blue.*

9.0 Use the file titled ***TN_TP-Template*** (located in the Cl-SO4 standard calc sheet folder) to find the concentrations of the stock solution you must use to make the standard and ICV solutions. There should be a copy of it near the analytical scale in the River Ecology lab. If not, print one out to reference.

10.0 Use an analytical scale and the template to make the standards and ICVs:

10.1 For calibration standards, LOQ, and LRV: first make the INT solution, then use the INT solution to make C1-C10

10.2 For ICVs: first make ICV INT solution, then use ICV INT to make the ICVs.

NOTE: The amount of the stock solution and MQ water on the calculation sheet is cumulative.

Ex) For C10, add 16.00g of the INT solution, then add 184.00g of MQ water (so that the total weight is now 200.00g).

Nutrient Analysis – API Astoria2 Analyzer

TN / TP Standard Calculations DATE:

NO_3-N & PO_4-P stocks are ***1,000 ppm*** *(1,000,000 ppb)* PREPARED BY:

		NO_3- N	PO_4-P
Calibration Standards	Brand of Stock:		
	Lot #:		
ICV Standards	Brand of Stock:		
	Lot #:		

	NO_3-N Stock (g)	PO_4-P Stock (g)	1% HCl (g)	[TN] ppb	[TP] ppb
INT	3.000	4.000	100.000	30000.000	10000.000

C	INT (g)	1% HCl (g)	[TN] ppb	[TP] ppb	
10	16.000	200.000	2400.000	800.000	10
9	12.000	200.000	1800.000	600.000	9
8	10.000	200.000	1500.000	500.000	8
7	8.000	200.000	1200.000	400.000	7
6	6.000	200.000	900.000	300.000	6
5	4.000	200.000	600.000	200.000	5
4	2.000	200.000	300.000	100.000	4
3	1.000	200.000	150.000	50.000	3
2	0.500	200.000	75.000	25.000	2
1	0.000	200.000	0.000	0.000	1
LOQ	0.100	200.000	15.000	5.000	LOQ
LRV	20.000	200.000	3000.000	1000.000	LRV

	NO_3-N Stock (g)	PO_4-P Stock (g)	1% HCl (g)	[TN] ppb	[TP] ppb
ICV INT	3.000	3.400	100.000	30000.000	4000.000

ICV	ICV INT (g)	1% HCl (g)	[TN] ppb	[TP] ppb	
H	8.000	200.000	1200.000	160.000	H
M	2.000	200.000	300.000	40.000	M
L	0.500	200.000	75.000	10.000	L

10.3 Place a 250mL Nalgene bottle on the scale, close all the doors on the scale, and tare.

10.4 Pour the stock solution into a disposable 10mL beaker. This is to limit contamination of the stock solution and also helps you have more control over the amount you pour.

10.5 For low-end amounts of stock solution use the 1mL disposable pipette. One droplet is ~0.01g

10.6 For high-range amounts of stock you can pour the stock straight from the bottle, until you get close to the desired weight, then switch back to using the disposable pipettes and beakers.

10.7 Write down the exact amount of stock solution on the blank calculation sheet you printed out, making sure to close the doors of the analytical scale before recording your measurement.

10.8 To add MQ:

10.8.1 Take the cap off the MQ squirt bottle, take the standard/ICV bottle off the scale, and pour water to about 0.5cm below the shoulder of the standard/ICV bottle.

10.8.2 Then, put the cap back on the MQ squirt bottle, return the standard bottle to the scale and squirt water into the bottle until you reach 250mL.

11.0 Once you have made all standards, put them on a lunch tray and put in the refrigerator outside of the WSEL lab. Dump old standards if needed.

12.0 Enter in your stock solution amounts into your standard calculation sheet on a computer to find the concentration values.

13.0 Print out your newly filled out sheet. Add this to the ***Astoria Pacific SOP*** binder that sits next to the API computer

Dissolved Nutrients

1.0 Go to standard calc sheet folder in share drive. *LTER Water Chem Lab → SOPs and Project Descriptions → STANDARD CALC SHEETS → NH4-NO3-SRP*

2.0 Make a copy of the file titled ***NH4_NO3_SRP-Template-BLANK***

3.0 Rename the file with the date that you plan to make the standards.

3.1 Ex) *NH4_NO3_SRP_07_14_23*

3.2 Put the file in the folder titled with the appropriate year.

4.0 Open the sheet and fill out your name and the date of preparation in the top right corner of the sheet, as well as the stock solution information.

5.0 Print a copy of the calculation sheet.

6.0 Obtain seventeen clean 100 mL plastic Nalgene bottles. Brand new bottles are ideal, but freshly acid washed bottles are also okay. (See acid washing SOP)

7.0 Label the bottles with label tape:

7.1 H-INT, L-INT, LOQ-INT, ICV-INT, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, LOQ, ICV-L, ICV-M, ICV-H

7.2 *Tip: color coding the calibration standards vs. ICVs may also be helpful*
Ex) all calibration standards (A-J and Blk) are blue and all ICVs (LOQ, ICV-L, M, H) are pink.

8.0 Use the file titled ***Cl_SO4-TEMPLATE*** (located in the Cl-SO4 standard calc sheet folder) to find the concentrations of the stock solution you must use to make the standard and ICV solutions. There should be a copy of it near the analytical scale in the River Ecology lab. If not, print one out to reference.

9.0 Use an analytical scale and the template to make the standards and ICVs:

NOTE: The amount of the stock solution and MQ water on the calculation sheet is cumulative.

Ex) For H-INT, add 2.000g of NH_4 stock, then 2.000g of NO_3 stock (so that the total weight is now 4.000g) and then add 0.200g of P stock (so that the total weight is now 4.200g). Then add 45.800g of MQ water (so that the total weight is now 50.000g).

Nutrient Analysis – API Astoria2 Analyzer

NH4-N / NO3-N / P Standard Calculations						Date:	
*ALL stocks are **1,000 ppm** (1,000,000 ppb)*						Prepared By:	
					NH_4	NO_3	PO_4-P
	Calibration Standards		Brand of Stock				
			Lot #				
	ICV Standards		Brand of Stock				
			Lot #				
	NH4-N Stock (g)	NO3-N Stock (g)	P Stock (g)	MQ (g)	[NH4-N] ppb	[NO3-N] ppb	[P] ppb
H - INT	2.0000	4.0000	4.2000	50.0000	40000.0000	40000.0000	4000.0000
C	H - INT (g)	MQ (g)			[NH4-N] ppb	[NO3-N] ppb	[P] ppb
12	10.0000	100.0000		12	4000.000	4000.000	400.000
11	7.5000	100.0000		11	3000.000	3000.000	300.000
10	5.0000	100.0000		10	2000.000	2000.000	200.000
9	2.5000	100.0000		9	1000.000	1000.000	100.000
8	1.2500	100.0000		8	500.000	500.000	50.000
7	0.5000	100.0000		7	200.000	200.000	20.000
	H - INT (g)			MQ (g)	[NH4-N] ppb	[NO3-N] ppb	[P] ppb
L - INT	1.2500			50.0000	1000.0000	1000.0000	100.0000
	L - INT (g)	MQ (g)			[NH4] ppb	[NO3] ppb	[P] ppb
6	10.0000	100.0000		6	100.000	100.000	10.000
5	5.0000	100.0000		5	50.000	50.000	5.000
4	2.0000	100.0000		4	20.000	20.000	2.000
3	1.0000	100.0000		3	10.000	10.000	1.000
2	0.5000	100.0000		2	5.000	5.000	0.500
1	0.0000	100.0000		1	0.000	0.000	0.000
	NH4-N Stock (g)	NO3-N Stock (g)	P Stock (g)	MQ (g)	[NH4-N] ppb	[NO3-N] ppb	[P] ppb
LOQ - INT	1.0000	1.3000	1.4000	*100*	10000.0000	3000.0000	1000.0000
	LOQ - INT	MQ (g)			[NH4] ppb	[NO3] ppb	[P] ppb
LOQ	0.1000	100.0000		LOQ	10.000	3.000	1.000
	NH4-N Stock (g)	NO3-N Stock (g)	P Stock (g)	MQ (g)	[NH4-N] ppb	[NO3-N] ppb	[P] ppb
ICV - INT	0.5000	1.0000	1.0500	50.0000	10000.0000	10000.0000	1000.0000
ICV	ICV - INT (g)	MQ (g)			[NH4-N] ppb	[NO3-N] ppb	[P] ppb
H	15.0000	100.0000		H	1500.000	1500.000	150.000
M	1.5000	100.0000		M	150.000	150.000	15.000
L	0.5000	100.0000		L	50.000	50.000	5.000

NOTE: use ICV standard stock solution to make the LOQ-INT

9.1 Place the Nalgene bottle on the scale, close all the doors on the scale, and tare.

9.2 Pour the stock solution into a disposable 10mL beaker. This is to limit contamination of the stock solution and also helps you have more control over the amount you pour.

9.3 For low-end amounts of stock solution use the 1mL disposable pipette. One droplet is ~0.01g

9.4 For high-range amounts of stock you can pour the stock straight from the bottle, until you get close to the desired weight, then switch back to using the disposable pipettes and beakers.

9.5 Write down the exact amount of stock solution on the blank calculation sheet you printed out, making sure to close the doors of the analytical scale before recording your measurement.

9.6 To add MQ:

9.6.1 Take the cap off the MQ squirt bottle, take the standard/ICV bottle off the scale, and pour water to about 0.5cm below the shoulder of the standard/ICV bottle.

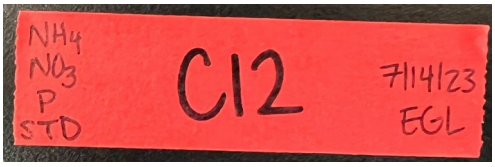
9.6.2 Then, put the cap back on the MQ squirt bottle, return the standard bottle to the scale and squirt water into the bottle until you reach desired weight.

Nutrient Analysis – API Astoria2 Analyzer

10.0 Once you have made all of your standards:

10.1 Pour standards into 20 mL Disposable Scintillation Vials.

10.2 Place label tape on each vial. On the left side of the tape write "NH_4 NO_3 P STD" and on the right side write the day of preparation and your initials. On the lid write what standard it is *(C1, ICV-H, etc.)*



10.3 Dump/toss old standards if needed.

11.0 Enter in your stock solution amounts into your standard calculation sheet on a computer to find the concentration values.

12.0 Print out your newly filled out sheet. Add this to ***Astoria Pacific SOP*** binder that sits next to the API computer