DETERMINATION OF NITRATE AND NITRITE IN WATER USING THE FIALYZER - 1000

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1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of nitrate (NO_3) and nitrite/nitrate (NO_2NO_3) in ground, surface, saline waters and wastes. Tests covered by this SOP are W-NO2NO3, W-NO2NO3-F, and W-NO3.

Calibration range	0 – 1.0 mg N/L
Minimum Detection Limit (MDL)	0.003 mg N/L
Practical Quantitation Limit (PQL)	0.010 mg N/L

The calibration range may be extended by dilution.

1.2. Nitrate concentration may be determined by calculation using the NO_2NO_3 concentration (analysis method described in this SOP) and the nitrite concentration (see DEP SOP NU-087). The MDL and PQL for calculated nitrate are 0.003 mg N/L and 0.010 mg N/L, respectively.

2. SUMMARY OF THE METHOD

2.1. This method is based on EPA method 353.2, Rev 2.0 (1993) and FIAlabs method NO3-W-2-1. Diethylenetriaminepentaacetic acid (DTPA) is used as complexing agent instead of ethylenediamine tetraacetic acid (EDTA).

2.2. A sample is passed through a column containing granular copper-cadmium catalyst, which reduces nitrate to nitrite. The nitrite originally present plus the reduced nitrate are determined by colorimetry. The nitrite is diazotized with sulfanilamide and coupled with N-(1-naphthyl)ethylenediamine dihydrochloride (NED) to form a reddish-purple azo dye, which is measured at a wavelength of 520 nm using a reference wavelength of 650 nm.

2.3. Interferences:

2.3.1. Build-up of suspended matter in the reduction column will restrict sample flow. Samples may be filtered prior to analysis.

2.3.2. Low results may be obtained for samples containing high concentrations of iron, copper and other metals. Diethylenetriaminepentaacetic acid (DTPA) is added to the buffer solution to eliminate these interferences.

2.3.3. Samples with background absorbance at the analytical wavelength may interfere.

2.3.4. Residual chlorine may reduce the efficiency of the nitrate conversion on the column. Samples may be checked and treated for residual chlorine prior to analysis (see Section 5.3.).

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3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation –

Instrumentation Unique ID	Component	Manufacturer	Model
FIA - A	System Unit	FIAlab Instruments	FIAlyzer - 1000
	XYZ Autosampler	FIAlab Instruments	ASX-560 Series
	Spectrometer/Detector	FIAlab Instruments	Tec5 spectrometer (310-1100nm, PN 64031)
	Light Source	Ocean Optics	HL-2000-LL (360-2000 nm, PN 17041)
	Flow Cell	FIAlab Instruments	SMA-Z-10-Cell (10 mm Optical Path, Polysulfone)
FIA - B	System Unit	FIAlab Instruments	FIAlyzer - 1000
	XYZ Autosampler	Lachat Instruments	ASX-5620 Series
	Spectrometer/Detector	FIAlab Instruments	Tec5 spectrometer (310-1100nm, PN 64031)
	Light Source	Ocean Optics	HL-2000-LL (360-2000 nm, PN 17041)
	Flow Cell	FIAlab Instruments	SMA-Z-10-Cell (10 mm Optical Path, Polysulfone)

3.2. Instrument Accessories –

Accessory	Manufacturer/Vendor	Model(s)/Catalog Number
3-Stop PVC Pump Tubing , 0.64 mm ID (*Orange/White*) (12 pk)	FIAlab Instruments	273064
3-Stop PVC Pump Tubing , 0.76 mm ID (*Black/Black*) (12 pk)	FIAlab Instruments	273076
3-Stop PVC Pump Tubing , 1.14 mm ID (*red/red*) (12 pk)	FIAlab Instruments	273114
3-Stop PVC Pump Tubing , 1.85 mm ID (*green/green*) (12 pk)	FIAlab Instruments	273185
Teflon Tubing 1/16 th" OD , 0.02" ID , 20 ft	FIAlab Instruments	270160
1/4 – 28 Flangeless Nuts for 1/16 th" OD tubing (10 pk)	FIAlab Instruments	240150
1/4 – 28 Flangeless Ferrules for 1/16 th" OD tubing (10 pk)	FIAlab Instruments	240070
Carbon Probe 0.8 mm ID for ASX-560	FIAlab Instruments	61010
Mixing Breadboard	FIAlab Instruments	792093
Mixing Coil	FIAlab Instruments	79209

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4-Way Manual Switching Valve	FIAlab Instruments	24055
Sample loop, 3 inches, 35 uL	FIAlab Instruments	792502
Female Luer Connector	FIAlab Instruments	240231
Or Equivalent		

3.3. Equipment –

Equipment	Manufacturer/ Vendor[1]	Model(s)/Catalog
Analytical Balance	Mettler Toledo/Fisher	MS204TS
Stir Bar, 50.8mm/2 In.	Fisher Scientific (SR)	14-513-61
Adjustable Pipette, 10-100 µL	Eppendorf/Fisher	3123000047/13690029
Adjustable Pipette, 100 -1000 µL	Eppendorf/Fisher	3123000063/13690032
Adjustable Pipette, 1-10 mL	Eppendorf/Fisher	3123000080/13690034
Filtering Flask, 1 L	Pyrex/Fisher (SR)	10-180F
Glass Beaker, 1000mL	KIMAX/Fisher (SR)	02-555-20E
Glass Beaker, 600mL	KIMAX/Fisher (SR)	02-555-20C
Glass Beaker, 400mL	KIMAX/Fisher (SR)	02-555-20B
Glass Beaker, 250mL	KIMAX/Fisher (SR)	02-555-20A
Glass Beaker, 50mL	KIMAX/Fisher (SR)	02-539G
Glass Beaker, 20mL	KIMAX/Fisher (SR)	02-539-1
Volumetric Flask, Class-A, 2000 mL	KIMAX/Fisher (SR)	10-208M
Volumetric Flask, Class-A, 1000 mL	KIMAX/Fisher (SR)	10-212-5L
Volumetric Flask, Class-A, 500 mL	KIMAX/Fisher (SR)	10-212E
Volumetric Flask, Class-A, 250 mL	KIMAX/Fisher (SR)	10-205D
Volumetric Flask, Class-A, 100 mL	KIMAX/Fisher (SR)	10-212B
Magnetic Filter Funnel, 47 mm	Cytiva/Fisher	NC0444102
Graduated Cylinder, Class-A, 250 mL	KIMAX/Fisher (SR)	08-564A
Glass Funnel	KIMAX/Fisher	10-346-5B
Volumetric Pipette, Class A, 2mL	Fisher (SR)	13-650-2C
Volumetric Pipette, Class A, 10 mL	Fisher (SR)	13-650-3L
Volumetric Pipette, Class A, 20 mL	Fisher (SR)	13-650-2N
Bulb Pipet, Black	Fisherbrand/Fisher (SR)	13-681-50
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

3.4. Consumables and Supplies –

Equipment	Manufacturer/ Vendor[1]	Model(s)/Catalog No.
Sample Beakers, Polypropylene, 5 mL	Fisher (SR)	08-732-119
Sample Beakers, Polypropylene, 20 mL	Fisher (SR)	08-732-120
Sample Beakers, Polypropylene, 50 mL	Fisher (SR)	08-732-122
Pipette Tips, 200 µL	Eppendorf/Fisher (SR)	05-403-41
Pipette Tips, 1000 µL	Eppendorf/Fisher (SR)	05-403-43
Pipette Tips, 1-10 mL	Eppendorf/Fisher (SR)	05-403-116
Syringe, Polypropylene, 10 mL	Fisher (SR)	B303134

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Whatman Puradisc Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Cytiva/Fisher (SR)	05-713-404A
Tube Centrifuge 15 mL Polypropylene	Corning/Fisher	05-538-59A
Weigh Dish, Hexagonal 1 ½ X 1	Fisher (SR)	02-202-100
Weigh Dish, Hexagonal 4 X 3	Fisher (SR)	02-202-102
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Transfer Pipet, 5 mL	Fisher (SR)	13-711-5AM
Culture Tubes 13 X 100 mm	Fisher (SR)	14-961-27
Bottle, Polyethylene, 1000 mL	Fisher (receiving)	05-719-88
Bottle, Amber Glass, 1000 mL	Fisher (receiving)	05-719-91
Parafilm	Fisher (SR)	13-374-12
Nitrocellulose Membrane	Fisher brand/Fisher	SA1J792H5
PH Paper 0.00 – 13.0	Micro Essential Lab/Fisher (SR)	14-850-2
SenSafe Test Strip, Chlorine	Industrial Test System/Fisher (SR)	481026/NC9116053
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, please 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 must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the Reagent and Standard Logsheet NU-RGTSTD located on the network at: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Forms\. This sheet needs to be updated, printed, and submitted as part of the analysis folder. If available, a Certificate of Analysis for each purchased standard and reagent is maintained in the following folder \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\CertificatesofAnalysis\. 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 adjusted by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

Note: The following formula can be used to determine the correct dilution:

$$M_1V_1 = M_2V_2,$$

where M_1 = Concentration of stock solution used (4.1),

V_1 = Volume of stock required,

M_2 = Concentration of working standard and

V_2 = Volume being prepared.

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4.1. Chemicals –

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Storage Temperature
4.1.1.	Simple Nutrients certified standard	SIM NUT AMP	ERA	505	N/A	Room
4.1.2.	Potassium Nitrite	KNO_2	Fisher	P267-250	Certified ACS	Room
4.1.3.	Brij-L23 Solution 30 % (w/v) in H20	Brij	Sigma-Aldrich	B4184-1L	N/A	Room
4.1.4.	Ammonium Hydroxide	NH_4OH	Fisher (SR)	A669S-212	Certified ACS Plus	Room
4.1.5.	Hydrochloric Acid	HCl	Fisher (SR)	A144S-212	Certified ACS	Room
4.1.6.	N-(1-Naphthy)-Ethylenediamine	NED	Thermo Scientific (SR)	42399-0250	98+% ACS Reagent	Room
4.1.7.	o-Phosphoric Acid (85%)	H_3PO_4	Fisher (SR)	A242-212	Certified ACS	Room
4.1.8.	Sulfanilamide	$C_6H_8N_2O_2S$	Acros Organics(SR) (SR)	AC13285-5000	0.98%	Room
4.1.9.	Sulfuric Acid	H_2SO_4	Fisher (SR)	A300S-212	Certified ACS	Room
4.1.10.	Barium Chloride	$BaCl_2$	Fisher	B34-500	Crystalline/	Room
4.1.11.	Chloroform	$CHCl_3$	Fisher	C298-500	Certified ACS	Room
4.1.12.	Diethylenetriamine-	DTPA	Thermo Scientific	114322 500	≥ 98%	Room
4.1.13.	Potassium Nitrate	KNO_3	Fisher	P383-500	Certified	Room
4.1.14.	Sodium Thiosulfate	$Na_2S_2O_3 \cdot$	Fisher	SS370-1	Certified	Room
4.1.15.	Granular Copper-Cadmium Column	Cadmium Column	FIAlab	7101050237A	N/A	Room
4.1.16.	Deionized Water	H_2O	In-House	N/A	N/A	Room

Or Equivalent
[1]**Note:** (SR) designates supply 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.

4.2. Reagents:

4.2.1. Buffer Solution –

Hydrochloric Acid (4.1.5.)	210 mL
Ammonium Hydroxide (4.1.4.)	190 mL
Diethylenetriamine-pentaacetic Acid (4.1.12.)	2.0 g
DI Water (4.1.16.)	to 2000 mL

Fill a 2L volumetric flask with approx. 1 L of DI water. Place it on a stirrer in a laboratory hood. Slowly add 210 mL of concentrated hydrochloric acid while stirring. Slowly add 190 mL ammonium

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hydroxide and 2.0 g of DTPA. Stir for five minutes and allow to cool. Dilute to the mark.

Note: This acid base reaction is exothermic and generates enough heat to cause burns through the flask. Use caution. Use a hood to prepare the solution to minimize exposure to fumes from the reagents. This solution should be prepared in a room that is separate from Ammonia and TKN analysis due to the possibility of contamination.

After cooling is complete check the pH of the solution using pH paper. The pH should be ≈ 8.5. Buffer solution should be made as needed.

Holding time, 30 days.

4.2.2. Sulfanilamide Color Reagent (COLOR REAGENT) –

Phosphoric Acid (4.1.7.)	200 mL
Sulfanilamide (4.1.8.)	80 g
NED (4.1.6.)	4.0 g
DI Water (4.1.16.)	to 2000 mL

To a 2 L volumetric flask containing approx. 1.2 L of DI water, add 200 mL of 85% phosphoric acid. Mix and add 80 g of sulfanilamide and 4.0 g of N-(1-naphthyl)ethylenediamine dihydrochloride (NED).

Stir for twenty minutes and dilute to the mark. Filter reagent using an aspirator with a 0.45 μm filter. Transfer solution to a dark container.

Holding time, 30 days.

4.2.3. Carrier Solution (CAR, 0.2% H_2SO_4) –

This solution is used for the instruments carrier stream, MBLKs, Continuing Calibration Blanks (CCBs), as the diluent for preparation of calibrants (4.3.2. and 4.3.3.), and ALTQC working solution (4.3.4.2), as well as for sample dilutions when necessary.

Sulfuric Acid (4.1.9.)	4 mL
DI Water (4.1.16.)	to 2000 mL

To a 2 L volumetric flask containing approx. 200 mL of DI water, add 4 mL of conc. sulfuric acid. Dilute to mark and invert to mix.

Prepare fresh daily.

4.2.4. Granular Copper-Cadmium Column (4.1.15.) –

The supplier for the granular copper-cadmium columns gives an expiration date. The holding time of the columns can be extended an additional 180 days past the expiration date, based on a passing column efficiency check (CATCHCK Standard, 4.3.7.) and a passing ALTQC standard (4.3.4.2.). The column will be reentered in LIMS and a new Reagent ID will be generated with an expiration date, 180 days after the manufacture's expiration.

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4.2.5. Probe Wash Stock (Brij Stock) –

Brij-L23 Solution (4.1.3.)	3.3 mL
DI Water (4.1.16.)	to 1000 mL

To a 1000 mL volumetric flask containing aprox. 800 mL of DI water, add 3.3 mL of Brij-L23 solution. Dilute to mark with DI water and mix well.

Holding time, 180 days.

4.2.6. Probe Wash (Brij Wash) –

Probe Wash Stock (4.2.5.)	200 mL
DI Water (4.1.16.)	to 1000 mL

To a 1000 mL Volumetric flask containing aprox. 600 mL of DI water, add 200 mL of the probe wash stock. Fill to mark with DI water and mix well.

Holding time, 180 days.

4.3. Standards and Spiking Solutions:

4.3.1. Nitrate Stock Standard (1000 mg N/L as NO_3) (1000 PPM NO3) –

Potassium Nitrate (4.1.13.)	7.218 g
Chloroform (4.1.11.)	2 mL
DI Water (4.1.16.)	to 1000 mL

In a 1 L volumetric flask containing approx. 800 mL DI water, dissolve 7.218 g potassium nitrate (KNO_3). Add 2 mL of chloroform and dilute to the mark with DI water. Invert flask 12 times to mix thoroughly.

Store in the refrigerator. Holding time, 180 days.

Note: Only use glass when handling chloroform.

Note: Molecular Weight Factor – 0.139

4.3.2. Nitrate Intermediate/Spiking Standard (20 mg N/L as NO_3) (20 PPM NO3 / N1 / N2) –

1000 mg N/L Nitrate Stock (4.3.1.)	20 mL
Carrier Solution (4.2.3.)	to 1000 mL

To a 1 L volumetric flask containing approx. 500 mL of carrier solution, add 20 mL of 1000 mg N/L nitrate stock standard. Dilute to the mark with carrier solution and invert 12 times. This working standard is used to prepare the calibration standards including PQCK1 and CHCK standards, LFBs, and the matrix spikes.

Store the standard in the refrigerator. Holding time, 90 days.

Note: It is suggested to prepare using a volumetric pipette.

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4.3.3. Calibration Standard Preparation –

20 mg/L Standard (4.3.2.)	See Table
Carrier Solution (4.2.3.)	See Table

The method uses an eight points calibration, including a zero point. The calibration standard levels are shown in the table below. To prepare, partially fill a volumetric flask with carrier. Add the specified volume of the nitrate intermediate standard and mix. Fill to the mark with carrier and mix well. The eight cups with standards are loaded in the standards rack.

Holding time, 30 days.

Concentration (mg N/L)	Vol of 20 mg N/L Standard (4.3.2.) added (mL)	Final Volume (mL) (made up using carrier, 4.2.3.)
1.00 (CHCK2)	0.500 (1000 ppm Stock 4.3.1.)	500
0.500	0.250 (1000 ppm Stock 4.3.1.)	500
0.200 (CHCK)	10	1000
0.050	1.25	500
0.020	0.500	500
0.010 (PQCK1)	0.500	1000
0.005 (MDLSPK)	0.250	1000
0	n/a	Carrier as needed

4.3.4. ALTQC standard –

This standard is purchased from a different manufacturer than the calibration stock standard or at least has a different lot number. If available, a certified standard reference material is used (such as can be obtained from Environmental Resource Associates; Simple Nutrients Ampule (4.1.1.)). This quality control standard is prepared from the ampule according to the manufacturer's instructions. For the concentration and expiration date of the ampule, see the manufacturer's certificate.

4.3.4.1. ALTQC Stock (NOX ERA STOCK) –

Simple Nutrients Ampule (4.1.1.)	see ERA's instructions
Sulfuric Acid (4.1.9.)	to 0.2%
DI Water (4.1.16.)	see ERA's instructions

This standard is called based on its source, e.g. NOX ERA STOCK# on the run, where '#' indicates the stock standard increment.

Store refrigerated. Holding time, 180 days.

4.3.4.2. Working ALTQC(ALTQCNOX) –

Stock ALTQC (4.3.4.1.)	refer to certified value
Carrier Solution (4.2.3.)	to 1000 mL

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In a 1 L volumetric flask containing approx. 800 mL of carrier solution, add enough of the stock ALTQC standard to dilute it so the concentration falls within the calibration range. This standard is called based on its source, e.g. ALTQCNOX# on the run, where '#' indicates the stock standard increment.

Holding time, 30 days.

Note: Do not dilute with carrier solution or acidify the nitrite standard. The presence of acid will oxidize the nitrite to nitrate.

4.3.5. Nitrite Stock Standard (2000 mg N/L as NO2) (2000 PPM KNO2)

KNO2 (4.1.2.)	12.14 g
Chloroform (4.1.11.)	2 mL
DI Water (4.1.16.)	to 1000 mL

Pour the potassium nitrite (KNO_2) into a glass beaker or metal weigh boat and place it in an oven for 24 hours at 105 ºC. Dissolve 12.14 g of the dried potassium nitrite in approximately 800 mL of DI water contained in a 1 L volumetric flask. Add 2 mL of chloroform. Bring to mark with DI water and mix well.

Store in refrigerator. Holding time , 180 days.

Note: Only use glass when handling chloroform.

Note: Molecular Weight Factor – 0.1646

4.3.6. Nitrite Intermediate Stock Standard (20.0 mg N/L as NO2)(20 PPM KNO2)

Nitrite Stock Standard (4.3.5.)	10.0 mL
DI Water (4.1.16.)	to 1000 mL

Into a 1L flask containing approximately 500 mL DI water, pipette 10.0 mL of 2000 mg N/L Nitrite stock standard. Bring to volume with DI water and mix well.

Store in refrigerator. Holding time , 90 days.

Note: It is suggested to prepare using a volumetric pipette.

4.3.7. CATCHCK Standard Solution (CATCHCK)–

Nitrite Intermediate Stock Solution (4.3.6)	1 mL
DI Water (4.1.16.)	to 100mL

Add 1 mL of the 20 mg N/L KNO_2 standard to a 100 mL volumetric flask partially filled with DI water. Bring to volume with DI water and mix well.

Holding time is 2 days.

5. SAMPLE PREPARATION (SEE DEP SOP NU-033)

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5.1. Samples are preserved at the time of collection with sulfuric acid to a concentration of 0.2% to achieve a pH of less than 2 units. They are stored at ≤ 6°C (not frozen) in the Nutrients refrigerator located in receiving room C301. The samples must be analyzed within 28-days of the collection date to be reported unqualified.

Note: Samples are occasionally preserved incorrectly with nitric acid rather than sulfuric acid. These will give a very high NO2NO3 value (typically > 200 PPM). A $BaCl_2$ test can be used to check for the presence of sulfuric acid. If sulfuric acid is present the $BaCl_2$ will form a precipitate and a white, cloudy solution. If the sample was not preserved with sulfuric acid, the precipitation will not form. This test may not be as easy to determine in high salinity samples.

Note: If it is suspected that nitric acid was used to preserve the sample. If available, a corresponding unacidified aliquot can be analyzed by ion chromatography to check if the NO3 is inherent to the sample. A supervisor should be notified if a sample has been preserved with nitric acid, and an NCR should be created.

5.2. Remove the samples from the refrigerator following the proper protocols to preserve the samples' chain of custody. Allow the samples to come to room temperature before pouring them into the tubes for analysis.

5.3. All ground water samples that feed residential and commercial wells and samples from wastewater treatment facilities where the field sheets indicate the presence of residual chlorine content are tested for chlorine. If residual chlorine is detected it is removed according to the following procedure:

5.3.1. Use chlorine test strips (3.4.) to determine if chlorine is present and its approximate concentration. Pour about 15 ml of the sample in a small plastic cup and swirl the test strip in the sample for 30 seconds. Compare the color of the test strip against the color templates on the test strip container to determine total residual chlorine concentration in ppm. Record the result on the bench sheet and the run report.

If chlorine is detected, use the following formula to determine the amount of 0.025 N sodium thiosulfate solution to add to the sample:

$$X = [(A) \times (\text{total sample volume}) / 886.3],$$

where X = Volume (mL) of 0.025 N sodium thiosulfate solution to be added to the total sample volume (mL) for dichlorination.

A = Concentration (mg/L) of total residual chlorine in the sample.

Note: A 0.0250 N thiosulfate solution is equivalent to 886.3 µg Cl as Cl_2 /1.00 mL.

5.3.2. Record the volume of sodium thiosulfate solution used to dechlorinate the sample in the Excel spreadsheet template NU-CHLORINE-1.0 found at: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Forms\ After adding the sodium thiosulfate, take a small aliquot of the sample and reanalyze with a

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chlorine test strip (see 5.4.1.) to ensure that all the chlorine in the sample has been treated. This procedure is consistent with DEP SOP TA-09.18. Save the completed worksheet under the appropriate year folder as YYMMDDA#CL (FIA-A), or YYMMDDB#CL (FIA-B) in \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Chlorine\

6. SAMPLE ANALYSIS/QUANTITATION

6.1. Instrument Setup:

6.1.1. Turn on all instrument components (autosampler / instrument main power / light source) and computer.

6.1.2. Inspect instrument for the condition of the tubing/column (such as discoloration) and ensure the column valve is in the OFF position.

6.1.3. Lock down all tubing onto the peristaltic pump. Pump tubing set up is as follows:

Orange/White	Carrier (0.2% H_2SO_4) (4.2.3.)
Black	Sulfanilamide Color Reagent (4.2.2.)
Red	Buffer Solution (4.2.1.)
Green	Sample Intake
Clear	Probe Wash (4.2.6.)

6.1.4. Insert all lines into a container filled with DI water.

6.1.5. Open FIAlab software

6.1.6. Under the “Devices” tab (bottom left) and “All Devices” (top left), select “Connect All Devices”

6.1.7. Also, under the “Devices” tab, find the “FIAlab Analyzers” drop down to select “FIAlab1”. Select “ON” to start the instrument pump (35% speed).

6.1.8. Also, under the “Devices” tab, find “Auto Sampler”. Select the “Probe Down” command then click the “Send” button. This will rinse the autosampler wash pot.

Note: Instrument “A” has a separate (built in) autosampler pump that will automatically turn on when the probe down command is initiated. Instrument “B” has a slightly different autosampler that does not have a separate pump. The wash pot tubing is directed over the instrument pump. This means if the instrument pump is ON the wash line will be pulling.

6.1.9. Flush everything with DI water for approximately 5 minutes.

Note: While the instrument begins to flush, briefly removed one line at a time and ensure that each line is smoothly pulling liquid. If the motion is not smooth or is spitting out liquid rather than pulling, troubleshooting should be performed.

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6.1.10. Remove autosampler line from DI so that wash pot drains. (~ 5 minutes). Then give the "Probe Up" command (Devices → Autosampler→Probe Up→ Send).

6.1.11. Turn the instrument pump OFF (FIAlab Analyzers→FIAlab1→OFF) **then** switch the cadmium column ON via the 4-way valve located on the front of the instrument.

Note: To prevent valve failure, only turn the column on/off when the instrument pump is OFF. Turning the valve while the instrument pump is on can cause pressure buildup. The additional pressure can cause damage to the valve.

6.1.12. Place lines into their appropriate reagents and turn the pump back ON (FIAlab Analyzers→FIAlab1→ON).

6.1.13. Allow the reagents to flush for approximately 5 minutes.

6.1.14. Once the instrument has been conditioned with reagents and while the pump is still running, navigate to Devices →Spectrometer1 and acquire the "Single Acquisition" reading. The highest peak point should be between 45,000 and 55,000 counts. If needed, the "Integration Time" can be adjusted until this is achieved.

Note: The pump can be turned off at this point to save reagent volume while samples are prepared for analysis.

6.2 Run Setup

6.2.1. Samples are batched according to their conductivity or matrix. Samples with conductivities <5,000 µmhos/cm(or <3 PPTh salinity) are considered freshwater and are **batched separately** from those samples with conductivities >5,000 umhos/cm (or >3 PPTh salinity). If no conductivity or salinity is given, then batch according to matrix identification in LIMS. Waste and Effluents are also batched separately

6.2.2. For each batch of 10 samples, the following will be prepared:

6.2.2.1. Two low-level (0.20 mg N/L) matrix spikes by adding 100 µL of the Nitrate Working Intermediate/Spiking Standard (4.3.2.) into 10 mL of a selected sample (spike code N1).

Note: A sample duplicate and one (N1) spike can be prepared rather than two spikes to obtain a relative percent difference (RPD).

6.2.2.2. One single high-level (0.40 mg N/L) matrix spike by adding 200 µL of the Nitrate Working Intermediate/Spiking Standard (4.3.2.) into 10 mL of the selected sample (spike code N2).

Note: This high-level spike allows for reporting a spike recovery in case the low-level matrix spikes are buried due to high background levels of nitrate in the sample.

6.2.2.3. One LFB at 0.20 mg N/L following the same procedure as the low-level spike (6.2.2.1.), substituting carrier solution (4.2.3.) for the sample.

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6.2.2.4. One method blank (MBLK) prepared from carrier solution (4.2.3). The MBLK should have the same preparation process as a sample. For example, if most samples in a batch are filtered, the MBLK should also be filtered. Filtration is denoted by _F on the analytical run.

6.2.3. Navigate to the "Sample Table" tab (bottom left) to load templates located at \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Forms\ :

6.2.3.1. Select "Sample Table" select "Load Template" and import the "Generic_Table_Template". This is the run sequence.

6.2.3.2. Select "Standard Table" then "Load Template" and import "Standard_Table_PPB". This converts the data into PPB.

6.2.3.3. Select "Dataset Notes", then "Load Template" and import "Dataset_Notes_Templete". This adds the report header.

Note: The software configuration can be set to automatically pull the templates.

6.2.4. Modify the sample table sequence of the template to reflect the new run.

An example run sequence includes:

Initial QC
- **CalStd A-G** (Calibration Standards from 4.3.3. and a blank (4.2.3.))
- **MDLSPK** (4.3.3)
- **PQCK1** (4.3.3.)
- **CATCHCK** (4.3.7.)
- **ALTQCNOX#** (ALTQC, 4.3.4.2.)
- **CHCK2** (1.0 mg N/L standard (4.3.3.), to check for carryover)
- **CAR** (Carrier solution, 4.2.3.)
- **CHCK_F** (4.3.3., to check for filter effects on analyte)
- **CCB** (Carrier solution, 4.2.3.)

Batch QC
- **MBLK_F** (6.2.2.4)
- **LFB** (6.2.2.3.)
- **Sample 1 / Sample 1 dup-A**
- **Sample 1 dup-B or Sample 1 Low Level Spike-A - N1** (6.2.2.1.)
- **Sample 1 Low Level Spike-B - N1** (6.2.2.1.)
- **Sample 1 High Level Spike - N2** (6.2.2.2.; optional)
- **Sample(s)** (Samples 2 through 10) (6.1.15.)
- **CAR**(Carrier solution, 4.2.3.)
- **CHCK** (4.3.3.)
- **CCB**(Carrier solution, 4.2.3.)

(the notation "**_F**" indicates the sample was filtered)

Note: While building the sequence be mindful of the "Rack Position", and "Sample Type". A position such as RS1 indicates the use of the larger standard tubes (50 mL) that are along the back of the autosampler. RA1, RB1 or RC1 indicates a location on the samples racks.

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"A" being the far-left rack. If the "Sample Type" is a "Standard" it must correspond to the appropriate calibration "Level". The calibration levels and their corresponding concentrations can be found under the "Sample Table" tab then under "Standard Table". Unknowns will have a "Level" of "00"

6.2.5. Prepare the bench sheet. Manually type or copy and paste the sequence from the software to the word document then print. Save as NOYYMMDD**A#**_Bench for FIA-A or NOYYMMDD**B#**_Bench for FIA-B (where YY is the year, MM is the month, DD is for the day, and # is the 1st, 2nd, etc run of the day) into yearly folder located at :

\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\AnalysisFolders\Nitrate-Nitrite\Bench

6.2.6. Prepare and load samples following the bench sheet.

6.2.7. To start the run sequence, go to "Method Operation" tab and choose "Start Method".

6.2.8. To monitor the run, select the "Data Analysis" tab. Under "Plots" select "View by run"

6.2.9. To view the calibration, find the "calibration" drop down (under "Data Analysis" tab), followed by "Main view".

6.2.10. Once the run is complete, to save the sequence, go to "Save Template", navigate to the respective folder, and save under the following format: YYMMDD**A#** for FIA-A or YYMMDD**B#** for FIA-B (where YY is the year, MM is the month, DD is for the day, and # is the 1st, 2nd, etc run of the day).

6.3. Quantitation/Calculations:

6.3.1. The instrument will give the analysis results for NO_2NO_3 in ug N/L (PPB). The results will be converted in LIMS to mg N/L (PPM).

6.3.2. 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 (11.3).

6.3.3. The individual calibration standards are prepared according to section 4.3.3. The weighted linear calibration is calculated by the instrument software and is presented in Table 1 and Figure 1 of the analysis report. To evaluate the linear fit, the software includes the correlation coefficient and percent error (see section 8.6.).

6.4. Post-Run Procedure:

6.4.1. Saving and Exporting Data:

6.4.1.1. Save raw data (.DAT) file. Once the run is complete, go to the " Data Analysis" tab and save the data under the appropriate instruments Raw Data folder (FIAsoftRawData) in the current year located at :

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\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers

6.4.1.2. Export the CSV file for LIMS and print a physical Graphical report.

Data Analysis → report (far left)→ report (drop down at the top) →Type→ CSV (for CSV) or Graphical (for print out)

Check boxes for:

- ✓ Header
- ✓ Calibrations
- ✓ Custom CSV (name,pos,conc1,resp1,datetime)
- ✓ Peaks
- ✓ Compact
- ✓ Auto scale
- ✓ Data Processing Parameters

Now, "Create report" then "export report" and save

6.4.1.2. Save report in the current year located at :

\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\AnalysisFolders\Nitrate-Nitrite\FIA-A\Graphical (or \FIA-B\Graphical)

or

\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers\FIA-A\CSV (or \FIA-B\CSV)

6.4.1.3. To print, open the "Chrome" graphical file, click the three dots in the top right corner of the web page, and select *Print*. (Calibration information will be printed along with data.)

6.5. Instrument Shut Down:

6.5.1. Once the run has completed. Turn Cadmium Column valve to OFF position. (The pump is automatically turned off at the end of the run sequence)

6.5.2. Place all lines in DI water, and turn on instrument pump

(FIAlab Analyzers→FIAlab1→ON)

6.5.3. Put the probe down (Devices → Autosampler→Probe Down→ Send)

6.5.4. Flush for approximately 5 minutes.

6.5.5. Remove all lines from DI water and air dry for 5-10 minutes.

6.5.6. After drying, put probe in the UP position
(Devices → Autosampler→Probe Up→ Send)

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6.5.7. Go to *FIAlab Analyzers* → *FIAlab 1* → and turn pump off.
6.5.8. Go to *All Devices* → *Disconnect all Devices* and then proceed to close the program.

6.5.9. Turn off all the components of the instrument.

6.5.10. Unclamp tubing from the peristaltic pump.

Note: If tubing is accidentally left clamped overnight, it is suggested that the tubing be replaced.

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-NO2NO3), filename/s (NOYYMMDDA# (FIA-A), or NOYYMMDDB# (FIA-B)), and initials of the analyst. If nitrate by calculation was performed as well the test W-NO3 is added to the label.

7.2. Each report folder should contain the following items:

- Bench sheet(s)
- The graphical run report with calibration and raw data
- Reagents and Standard logsheet
- NU-PIPETTE form
- The backlog containing the batching documentation
- If nitrate by calculation was performed a copy of the nitrate by calculation excel sheet (see section 9) should be included.
- the thiosulfate log sheet (see section 5.4.2.) if applicable
- A copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.3. The report folders are on file with the nutrients 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. FIAlab instrument data CVS transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers\FIA-A\CSV (or \FIA-B\CSV)

7.5. FIAlab raw instrument files (.DAT) are stored in electronic format in the current year's subfolder located in: \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers\FIA-A\FIAsoftRawData (or \FIA-B\FIAsoftRawData)

7.6. FIAlab graphical report are located in the current years subfolder located in:

\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\AnalysisFolders\Nitrate-Nitrite\FIA-A\Graphical (or \FIA-B\Graphical)

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7.7. Current bench sheet templates are located in a subfolder on the Nutrients drive. \\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Forms\

7.8. Method validation data is on file with the supervisor. Digital copy is also stored on the network:

\\floridadep\data\labs\Lab5\Chemistry\Inorganic\Nutrient\Validations\FIA

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

8.1. For method performance and acceptance criteria for QC measures and contingencies for handling out-of-control or unacceptable data refer to the DEP SOPs NU-038 and NU-043.

8.2. One practical quantitation limit level standard, PQCK1 (4.3.3.) and one ALTQC nitrate standard (4.3.4.) are analyzed on every analytical run. The ALTQC standard serves as the initial calibration verification standard (ICV) and is prepared from a stock of a different lot or manufacturer than the calibration standards. Acceptance limits for recovery for the PQCK1 standard are 70 – 130%. Acceptance limits for recovery for the working ALTQC are 90 – 110%.

8.3. CATCHCK, a nitrite standard used as a column efficiency check standard, (4.3.7.), is analyzed on every analytical run. If the recovery is greater than 115% the run is invalid, and the column needs to be replaced. If the recovery falls between 110 and 115% the column will need to be replaced as soon as possible. Recovery less than 85% indicates an analytical error and the analyst should discontinue analysis and report the failure to a supervisor.

8.4. A CHCK standard (4.3.3.) and CCB (4.2.3.) are run before samples are analyzed, every 10 samples and at the end of the run. The highest calibration level standard followed by a carrier is analyzed in the beginning of the run to check for carryover. Acceptance limits for recovery of the CHCK and CHCK2 standard are 90 – 110%. Acceptance limits for the MBLKs, and CCBs are < 0.003 and > -0.003 mg N/L.

8.5. An MBLK (4.2.3.), an LFB (6.2.2.3.), a matrix spike and a sample duplicated are required for each batch of ten samples. For practical reasons, two low-level duplicate matrix spikes, and one high-level matrix spike (6.2.2.1. & 6.2.2.2.) are usually prepared. Acceptance limits for the LFB and matrix spike recoveries are 90 – 110%. The sample/matrix spike reproducibility should be less than 20% RPD for results above the practical quantitation limit (PQL).

8.6. Calibration Range – See FDEP Quality Manual Appendix B, Section 7.0, ‘Protocol for Determining the Test Method Range of Applicability’. The calibration standards (4.3.3.) are prepared from a nitrate stock. Every day samples are analyzed a new calibration is performed at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

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Seven calibration standards and a blank are used to prepare the calibration curve. A weighted linear fit is applied (1/sqrt(response)). To verify the linear calibration range (LCR) the percent error for each calibration standard above the PQL must be within ±10 %, ±30 %, for the PQL-level standard and -40% to 50% for the MDLSPK. The acceptance limits for the blank calibration point are below the MDL and above the negative value of the MDL (-MDL< blank < MDL). The correlation coefficient of the calibration must be equal to or larger than 0.995. Calibrations that do not satisfy the above criteria should not be used for analysis. The cause of failing calibration should be evaluated and corrected. Results reported based on a failing calibration must be J-qualified.

8.7. If the CAR solution analyzed after the CHCK2 standard in the analysis run sequence fails acceptance limits for a CCB (see section 8.4) this usually indicates carryover from the CHCK2 standard. If carryover is observed the analysis should be stopped and the instrument maintained. Additional CAR standards may be added throughout the run as carryover checks. They are assessed in the same way as CCBs.

8.8. If samples are filtered prior to analysis to avoid clogging the column, filtered MBLKs and a filtered CHCK standard (CHCK_F) are included with the run to serve as positive and negative controls for filtration.

8.9. Method blank (4.2.3.) and MDLSPK (4.3.3.) results are used for annual MDL and PQL verification (see NU-043 for details).

9. NITRATE DETERMINATION BY CALCULATION

9.1. In order to calculate the nitrate content of a sample an acidified aliquot is analyzed for NO2NO3 as described herein, and an unacidified aliquot is analyzed for nitrite (see DEP SOPs NU-087). The nitrate concentration is then calculated from the raw data as follows:

$$[NO_3]_{calculated} = ([NO_2NO_3]_{measured} \times D_{f1}) - ([NO_2]_{measured} \times D_{f2}),$$

Where, $[NO_3]_{calculated}$ = Calculated concentration of nitrate in mg N/L,
$[NO_2NO_3]_{measured}$ = Raw NO2NO3 concentration in mg N/L as determined by NU-107,
$[NO_2]_{measured}$ = Raw nitrite concentration in mg N/L as determined by NU-087,
D_{f1} and D_{f2}= Dilution factors used in NO_2NO_3 and NO_2 measurements.

9.2. The calculation is documented in the excel spreadsheet NU-NO3-CALC and the result is reported to the Laboratory Information Management System (LIMS) using the Results Editor module. The excel template is located on the nutrients drive:

\\floridadep\data\labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\FIAlyzer\Forms\

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10. MAINTENANCE

10.1. Yearly Preventative Maintenance (PM) will be scheduled with an authorized technician to maintain instrument.

10.2. A 10% bleach solution can be used followed by a thorough DI water flush to clean. It is important to note that the column should remain OFF during cleaning.

10.3. A 0.1 – 1 % Liquiniox solution may be done monthly or as needed to reduce staining caused by sulfanilamide. This is not regularly stocked in the laboratory but can be purchased if needed. The column should remain OFF during this cleaning.

10.4. Occasionally, the autosampler probe wash will need cleaning. If there is visible discoloration or contamination issues, the analyst should consider cleaning. To clean:

10.4.1. Remove from the instrument

10.4.2. Place into a container that will hold enough liquid to cover the entire part

10.4.2. Fill the container with 50% bleach/50% DI water

10.4.3. Allow to sit overnight

10.4.4. Thoroughly rinse and reinstall on the instrument

10.5. If there is a large amount of discoloration on areas of tubing, contamination issues, suspected clogs, or kinks, the analyst should consider replacing tubing. During replacement it is important to keep the diameters and lengths of tubing the same. (see section 3.2.)

10.6 If green-green waste line is not flowing properly (stopping or stuttering), their may be a clog in the sample probe. Use syringe and luer connector to push DI or carrier into W port and out of the probe. If it needs to be more thoroughly cleaned, a 10% bleach solution can be pushed followed by plenty of DI.

10.7. Pump tubing will be regularly changed based on instrument performance.

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 a lab coat should always be worn when handling samples and reagents.

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

10.4. Used/expired cadmium columns are packaged and returned to FIAlab for recycling.

10.5. Brij-L23 is considered a hazardous waste and should not be allowed to enter the drains. The waste lines should be separated so that Brij waste is directed to a separate container. Any waste containing brij should be collected for hazardous

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waste. The hazardous waste team will bring it to the collection drum labeled " Polyoxyethylene Lauryl Ether (Brij-L23) Solution w/ Water"

10.6. Waste NOT containing Brij-L23 can be carefully neutralized and rinsed into the sink. Acidic waste is neutralized with a base such as sodium bicarbonate. In the case that the waste is too basic, add acid such as hydrochloric or sulfuric acid. The pH should be tested and be between 6-8 before discarding contents down the lab sink.

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 353.2, Revision 2 (1993).

11.2. FIAlab Method NO3-W-2-1_v0.4

11.3. Quality Manual, Bureau of Laboratories, DEP.

11.4. Laboratory Health and Safety Plan.

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

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

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

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

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

11.10. DEP SOP TA-09.18 Dichlorination Procedure Using Sodium Thiosulfate.

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

Appendix of Significant Changes

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