DETERMINATION OF NITRATE AND NITRITE IN WATER USING THE LACHAT QUICKCHEM 8500 AUTOANALYZER

TABLE OF CONTENTS:

Page

1. SCOPE AND APPLICATION 2
2. SUMMARY OF THE METHOD 2
3. EQUIPMENT AND SUPPLIES 2
4. REAGENTS AND STANDARDS 4
5. SAMPLE PREPARATION 9
6. SAMPLE ANALYSIS AND QUANTITATION 10
7. DATA ARCHIVAL/VALIDATION 13
8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES 14
9. NITRATE DETERMINATION BY CALCULATION 15
10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION .. 16
11. REFERENCES/DEFINITIONS 16

APPENDIX OF SIGNIFICANT CHANGES 17

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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 – 0.80 mg N/L
Minimum Detection Limit (MDL)	0.004 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 0.004 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 Lachat method 10-107-04-1-C. 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 can then are determined by colorimetry. The nitrite is diazotized with sulfanilamide and coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to form a highly colored azo dye, which is measured at a wavelength of 520 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. Color correction is used to eliminate this interference on a per sample basis.

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 5.4).

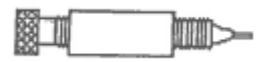
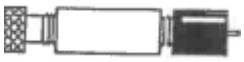
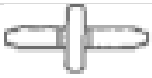
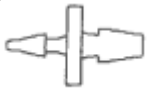
3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation –

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Instrumentation Unique ID	Component	Manufacturer	Model
Lachat 2	System Unit (Detector, Manifold)	Lachat Instruments	Quickchem 8500 Series 2
	XYZ Autosampler	Lachat Instruments	ASX-520 Series
	Reagent Pump	Lachat Instruments	RP-150 Series

3.2. Instrument Accessories –

Accessory	Manufacturer/Vendor	Model(s)/Catalog Number
 Pump Tube Adapter	Lachat Instruments	50905
 Pump Tube Adapter	Lachat Instruments	50907
 Connecting Nipple	Lachat Instruments	50015A
 Connecting Nipple	Lachat Instruments	50015
 Connecting Nipple, Reducing	Lachat Instruments	50014
Tygon Tubing (various)	Cole-Parmer/Fisher	NC0603997, 14-171-216, 14-171-208
Pump Tubing 07 Black-Black 0.030 i.d.	Lachat Instruments	54407L
Pump Tubing 08 Orange-Orange 0.035 i.d.	Lachat Instruments	54408L
Pump Tubing 14 Green-Green .073 i.d.	Lachat Instruments	54414L
Pump Tubing 15 Purple – Purple 0.081 i.d.	Lachat Instruments	54415L
Pump Tubing 19 Yellow – Blue 0.060 i.d.	Lachat Instruments	54419L
MR16 Precise Lamp (UV Control)	Lachat Instruments	31069
Or Equivalent		

3.3. Equipment –

Equipment	Manufacturer/ Vendor[1]	Model(s)/Catalog Number
Analytical Balance	Mettler Toledo/Fisher	MS204TS
Stirrer	Fisher Scientific	11-100-49S (Discontinued)
Stir Bars, Assorted	Fisherbrand/Fisher	14-513-61; 14-513-58; S68069
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)	10180F
Glass Beaker, Assorted	Pyrex/Fisher (SR)	02-540-K; 02-540L; 02-540P;
Volumetric Flask, Class- A, Assorted	Pyrex/Fisher (SR)	10-210-5C; 10-210-5E; 10-210-5F; 10-210-5G; 10-210-5H
Magnetic Filter Funnel, 47 mm	Andwin Scientific/Fisher	NC0006472
Graduated Cylinder, assorted	KIMAX/Fisher (SR)	08-548D; 08-564A
Glass Funnel	Fisher (SR)	10-346-5B
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.4. Consumables and Supplies –

Equipment	Manufacturer/ Vendor[1]	Model(s)/Catalog Number
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
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
Reagent Bottles, Amber, 1 L	Fisher (SR)	03-313-8F
Parafilm	Fisher (SR)	13-374-12
Nitrocellulose Membrane	Fisherbrand/Fisher	SA1J792H5
PH Paper 0.00 – 13.0	Micro Essential Lab/Fisher	14-850-2
SenSafe Test Strip, Chlorine	Industrial Test System/Fisher	481026/NC9116053
Bulb Pipet, Black	Fisherbrand/Fisher	13-681-50
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_W-NO2NO3 located on the network at: \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\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\Lachat\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.

4.1. Chemicals –

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Simple Nutrients certified standard	SIM NUT AMP	ERA	505	N/A	See CoA	Room
4.1.2.	Potassium Nitrite	KNO_2	Fisher	P267-500	Certified ACS	None	Room
4.1.3.	Nitrite Nitrogen standard (not in use)	NO2 ALT STOCK	Ricca	R5444-900-120C		See CoA	See CoA
4.1.4.	Ammonium Hydroxide	NH_4OH	Fisher (SR)	A669S-212	Certified ACS Plus	3 years	Room
4.1.5.	Ethylenediamine Tetraacetic Acid	EDTA	Fisher	S311-500	Crystalline/Certified ACS	None	Room
4.1.6.	Hydrochloric Acid	HCl	Fisher (SR)	A144S-212	Certified ACS Plus	None	Room
4.1.7.	N-(1-Naphthy)-Ethylenediamine	NED	Acros Organics	AC42399-0250	ACS Reagent	None	Room
4.1.8.	o-Phosphoric Acid (85%)	H_3PO_4	Fisher (SR)	A242-212	Certified ACS	None	Room
4.1.9.	Sodium Chloride	NACl/ Salt	Fisher (SR)	S271-500	Crystalline/ Certified ACS	None	Room
4.1.10.	Sulfanilamide	$C_6H_8N_2O_2S$	Acros Organics (SR)	AC132855000	0.98%	None	Room
4.1.11.	Sulfuric Acid	H_2SO_4	Fisher (SR)	A300S-212	Certified ACS Plus	None	Room
4.1.12.	Barium Chloride	$BaCl_2$	Fisher	B34-500	Crystalline/ Certified ACS	None	Room
4.1.13.	Chloroform	$CHCl_3$	Fisher	C298-500	Certified ACS	None	Room
4.1.14.	Diethylenetriamine-pentaacetic Acid	DTPA	Acros Organics	114322 500	≥ 98%	None	Room
4.1.15.	Potassium Nitrate	KNO_3	Fisher	P383-500	Certified	None	Room
4.1.16.	Sodium Thiosulfate Pentahydrate	$Na_2S_2O_3 \cdot 5H_2O$	Fisher	SS370-1	Certified	2 years	Room
4.1.17.	Granular Copper-Cadmium Column	Cadmium Column	Hach Instruments	50237A	N/A	6 months or more (4.2.5)	Room
4.1.18.	Deionized Water	H_2O	In-House	N/A	N/A	None	Room

Or Equivalent

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

Note: Routinely used expiration ranges are listed in the table. Follow CoA if more stringent.

4.2. Reagents:

4.2.1. Buffer solution –

Hydrochloric Acid (4.1.6.)	210 mL
Ammonium Hydroxide (4.1.4.)	190 mL
Diethylenetriamine-pentaacetic Acid (4.1.14.)	2.0 g
DI Water (4.1.18.)	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 hydroxide and 2.0 g of DTPA. Stir for five minutes and allow to cool. Dilute to the mark.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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 (4.1.8.)	200 mL
Sulfanilamide (4.1.10.)	80 g
NED (4.1.7.)	4.0 g
DI Water (4.1.18.)	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 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), and for sample dilutions when necessary.

4.2.3.1. For freshwater runs (<5,000 µmhos/cm or <3 PPTh Salinity) –

Sulfuric Acid (4.1.11.)	4 mL
DI Water (4.1.18.)	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.

4.2.3.2. For high conductivity runs (≥ 5000 µmhos/cm or salinity > 3 PPTh) –

Sodium Chloride (4.1.9.)	70 g
Sulfuric Acid (4.1.11.)	4 mL
DI Water (4.1.18.)	to 2000 mL

High conductivity samples are analyzed separately from freshwater samples. For high conductivity runs, prepare a saline carrier solution by adding 70 g reagent grade sodium chloride and 4 mL of conc. sulfuric acid to approximately 1 L of DI water

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

in a 2 L volumetric flask. Dilute to the mark with DI water and mix well.

Prepare fresh daily.

4.2.4. Color Correction Reagent (COLOR CORR RGT) –

Phosphoric Acid (4.1.8.)	100 mL
NED (4.1.7.)	2.0 g
DI Water (4.1.18.)	to 1000 mL

To a 1 L volumetric flask containing approx. 600 mL of DI water, add 100 mL of 85% phosphoric acid. Mix well and add 2.0 g of N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Dilute to the mark with DI water and invert to mix. Filter reagent using an aspirator with a 0.45 µm filter. Transfer solution to a dark container.

Holding time, 30 days.

4.2.5. Granular copper-cadmium column (4.1.17.) –

The supplier for the granular copper-cadmium columns gives an expiration date of 6 month from the manufacture 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, 4.3.5.) 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 180 days after the manufacture's expiration.

4.3. Standards and spiking solutions:

Note: For runs with high conductivity samples (≥ 5000 µmhos/cm), the calibration standards (4.3.3.) including the PQL-level check standard (PQCK1, 4.3.3.) and the Continuing Calibration Verification standard (CHCK, 4.3.3.), the laboratory fortified blanks (LFBs, 5.3.), and ALTQC (4.3.4.2.) solutions described below are prepared using the salt carrier solution (4.2.3.2.).

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

Potassium Nitrate (4.1.15.)	7.218 g
Chloroform (4.1.13.)	2 mL
DI Water (4.1.18.)	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.

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

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

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

carrier solution and invert 12 times. This working standard is used to prepare the calibration standards including the PQCK and the CHCK standards, LFBs, and the matrix spikes.

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

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 a six 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 six 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.*)
0.800 (CHCK2)	20	500
0.200 (CHCK)	5	500
0.050	1.25	500
0.020	0.500	500
0.010 (PQCK1)	0.250	500
0	n/a	Carrier as needed

* For runs containing samples with conductivities < 5,000 µmhos/cm, use carrier solution prepared as described in 4.2.3.1. For runs containing high conductivity samples (≥ 5000 µmhos/cm), use salt carrier solution prepared as described in 4.2.3.2.

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 –

Simple Nutrients Ampule (4.1.1.)	see ERA's instructions
Sulfuric Acid (4.1.11.)	to 0.2%
DI Water (4.1.18.)	see ERA's instructions

Store refrigerated. Holding time, 180 days.

4.3.4.2. Working ALTQC –

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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

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. ERAALTQCNOX# on the run, where '#' indicates the stock standard increment.

Holding time, 30 days.

4.3.5. CATCHCK standard solution – The CATCHCK is a 0.20 mg N/L KNO_2 solution that can be prepared from a stock standard solution, such as 20 mg/L working stock standard used for Nitrite analysis (SOP NU-087). 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.

For runs with high conductivity samples, dilute the stock standard solution using a solution of DI water with 35 g/L NaCl added. **Do not dilute with carrier solution or acidify the nitrite standard. The presence of acid will oxidize the nitrite to nitrate.** Holding time is 2 days.

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.

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

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 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.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

5.3. For each batch prepare 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). Also prepare a 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). 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. Prepare one LFB at 0.20 mg N/L following the same procedure substituting carrier solution (4.2.3.) for the sample.

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

5.4.1. Use chlorine test strips (3.4.) to determine if chlorine is present and its approximate concentration. Pour about 15 ml of 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 dechlorination

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.

5.4.2. Record the volume of sodium thiosulfate solution used to dechlorinate the sample in the Excel spreadsheet template NU-CHLORINE found at:\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\Forms\.

After adding the sodium thiosulfate, take a small aliquot of the sample and reanalyze with a 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 as YYMMDDN#CL (Lachat 2) in\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\Chlorine\.

6. SAMPLE ANALYSIS/QUANTITATION

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Note: High conductivity samples (≥ 5000 µmhos/cm) are run with a salt calibration (4.3.3.) and a salt carrier solution (4.2.3.2.) is used for sample dilutions.

6.1. Instrument Setup:

6.1.1. Turn on all four instrument components and computer.

6.1.2. Check the NO2NO3 manifold for the condition of the tubing and column. Make sure all connections are made as shown in the Lachat method manual.

6.1.3. Lock down all tubing in the peristaltic pump and flush with reagents for at least 15 minutes prior to start of run. Pump tubing set up is as follows:

Orange	Carrier (0.2% H_2SO_4) (4.2.3)
Black	Sulfanilamide Color Reagent (4.2.2)
Blue/Yellow	Buffer Solution (4.2.1)
Green	Sample Intake
Purple	Probe Rinse (DI H_2O)

6.1.4. While tubing is flushing select the Omnion icon on the computer desktop to open the instrument software.

6.1.5. Select *New* icon on the Omnion toolbar.

6.1.6. Modify the sample run sequence of the template to reflect the new run. To print the sequence worksheet select *Run → Export Worksheet Data.*

An example run sequence includes:

CalStd A-F (Calibration Standards from 4.3.3. and a blank (4.2.3.))
PQCK1 (4.3.3.)
CATCHCK (4.3.5.)
ERAALTQCNOX# (ALTQC, 4.3.4.2.)
CHCK2 (0.800 mg N/L standard (4.3.3.), to check for carryover)
CAR (Carrier solution, 4.2.3.)
CHCK_F (4.3.3.)
CCB (4.2.3.)
Batched samples, with an **MBLK_F, LFB,** and **spikes** (5.3.) for each batch (the notation "**_F**" indicates the sample was filtered)
CAR
CHCK
CCB

6.1.7. Load samples.

6.1.8. Select *Start* button on the tool bar to begin analysis. If the analysis needs to be paused or stopped select the *Stop* button on the tool bar. If you want to resume analysis select *Resume* or select *Stop Now* to end current sequence.

6.1.9. After completing the run save the data by selecting Run → *Save As* → *FileName.* Use the notation YYMMDD**N**# for files run on Lachat 2

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

(where YY is for the year, MM is for the month, DD is for the day, and # is for the 1st, 2nd, etc run of the day).

6.2. Color Correction:

6.2.1. Manual color correction – Any sample with a concentration at or above the MDL and a visually noticeable color should be run for color correction. At the end of the run remove the intake line from the sulfanilamide (color reagent) and put it in another beaker containing Color Correction Reagent (4.2.4.). Wait approximately 5 minutes until system equilibrates. Meanwhile set up the new sequence beginning with five blanks followed by the samples to be corrected followed by another five blanks at the end.

6.2.2. Repeat steps 6.1.6. – 6.1.9.

6.2.3. Color correction calculations – In the Excel spreadsheet template NU-COLORCORR), found at:\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\Forms\, enter all blank concentrations from the color correction run (Sample ID: MBLK), sample ID of samples that required color correction, their respective raw result (A) and color correction value (B). An average blank concentration (Avg. Blank) is calculated from the 10 blanks run before and after the samples (6.2.1.) and the color correction is calculated as follows:

$$\text{Color correction} = A - (B - \text{Avg. Blank})$$

6.2.4. Save the worksheet as YYMMDDN# (Lachat 2) in \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\ColorCorrection\.

6.3. Quantitation/Calculations:

6.3.1. The instrument will give the analysis results for NO_2NO_3 in mg N/L.

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 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 concentrations of the calibrants which are used to manually calculate the percent recoveries (see section 8.6.).

6.4. Post-Run Procedure:

6.4.1. Printing Data:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.4.1.1. Select the *Tools -> Custom Report*

6.4.1.2. Select *Print* button. (Font size may be changed.)

6.4.1.3. Calibration info will print along with data.

6.5. Exporting Files:

6.5.1. Select Run tab in the Run Properties window.

6.5.2. Select *Export Data to File* button.

6.5.3. Save raw Omnion data file to the Nutrients drive under the appropriate instrument in the current year's subfolder by clicking *Open* in the Omnion Software→ right click on the current day's saved file →select copy →paste the data file in: \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers\Lachat-2\and selecting the appropriate subfolder for the current year.

6.5.4. Convert file in QC Manager, perform calculations and select sample results for upload.

6.5.5. Complete the data folder and submit it for further review by supervisor.

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 NOYYMMDDN# (Lachat 2)), 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 – the run report(s), the color correction calculations spreadsheet (if color correction was necessary), bench sheet(s), reagents and standard logsheet, the backlog containing the batching documentation, the thiosulfate log sheet (see section 5.4.2.) if applicable, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. If nitrate by calculation was performed a copy of the nitrate by calculation excel sheet (see section 9) should be included. If sample dilutions were prepared, the dilutions recipes and the pipettes used are documented on the NU-PIPETTE form.

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. Lachat instrument data excel 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\Lachat-2\

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.5. Lachat raw instrument files are stored in electronic format in the current year's subfolder located in: \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\FlowInjectionAnalyzers\Lachat-2\RawOmnionData\

7.6. Current worksheet templates are located in a subfolder on the Nutrients drive. \\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\Forms\.

7.7. 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\Lachat\NO2NO3\.

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 (4.3.4.2.) 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 PQCK standard are 70 – 130%. Acceptance limits for recovery for the ALTQC are 90 – 110%.

8.3. CATCHCK, a nitrite standard used as a column efficiency check standard, (4.3.5.), 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. A 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 < 0.004 and > -0.004 mg N/L.

8.5. An MBLK (4.2.3.), an LFB (5.3.), a matrix spike and a spike or 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 (5.3.) 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

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

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. The color correction runs use the calibration performed the same day for NO_2NO_3 analysis.

Five calibration standards and a blank are used to prepare a calibration curve. Un-weighted linear fit is applied. To verify the linear calibration range (LCR) the percent recovery for each calibration standard above the PQL must be within 90 – 110% and 70 – 130% for the PQL-level standard. The acceptance limits for the blank calibration point are below the MDL and above the negative value of the MDL (-MDL< blank < MDL). The analyst is responsible for calculating the percent recoveries for each of the calibrants at and above the PQL level. The correlation coefficient of the linear calibration must be equal to or larger than 0.995. Calibration that does not satisfy the above criteria should not be used for analysis. The cause of failing calibration should be evaluated and corrected.

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 PQCK1 (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-066,
$[NO_2]_{measured}$ = 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:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Nitrate-Nitrite\Lachat\Forms\.

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 lab packed, labeled, and stored in the hazardous waste storage room until disposal.

10.5. All excess acid used for preparing reagents should be neutralized using the acid neutralizing station located in the lab. 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. Lachat QuickChem Method 10-107-04-1-B.

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 NU-087, Determination of Nitrite in Water Using the AQ2 Discrete Analyzer.

11.11. DEP SOP TA-09.18 Dechlorination Procedure Using Sodium Thiosulfate.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

11.12. Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 1995.

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

Appendix of Significant Changes

02/20/2008 Updated SOP to comply with latest revision of method: 353.2, Rev 2.0 (1993).

02/11/2008 Changes were made to sections 3.1 and 4.6 to reflect the use of a new instrument and auto-dilutor.

02/09/2010 Changes were made to sections 3 and 4 to reflect the use of two instruments, one equipped with an autodilutor and the other without.

02/02/2011 The autodilutor was removed from the setup of instrument 2 (4/21/10). The MDL for samples with a conductivity ≥ 5000 µmho/cm was elevated to 0.005 mg N/L.

03/14/2011 Amended the list of chemicals needed in section 4.

02/27/2012 A note was added to section 4.3 about the preparation of calibration and QC standards in salt carrier for high conductivity runs.

The preparation of the ALTQC standard was modified and described in section 4.3.4.

A section (6.2.4.) describing the use of the Excel spreadsheet template for color correction calculations was added.

A procedure for the determination of nitrate by calculation is described in Section 9.

05/16/2012 The MDL was changed to 0.004 mg N/L for samples with high conductivity (≥ 5000 □mho/cm).

Instrument 1 was upgraded and appropriate information changed in Section 3.1.

07/01/2013 Section 5.4 has been added detailing the detection and removal procedure for residual chlorine in samples, if necessary.

02/10/2014 Replaced ethylenediamine tetraacetic acid (EDTA) complexing agent with DTPA.

11/06/2014 Color correction reagent was modified to resemble the color reagent matrix as closely as possible by adding phosphoric acid and NED.

01/30/2015 The concentration for the CHCK and CATCHCK standards were changed to 0.20 mg N/L. The source for the CHCK standard was changed from the ALTQC to the working stock standard (see section 4.3.2.). Data archival information was clarified in section 7.

01/21/2016 File paths were updated and Appendix A and B were added.

05/03/2017 Appendix A and B were removed. Location of worksheets and templates was updated. Assessment of the LCR was modified in section 8.

02/16/2018 Section 4.2.5. was added to address the cadmium columns expiration policy.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

02/25/2019	Reference to the method validation data was updated to include the file path under data archival. A note was added to section 8.6 about checking % residuals for calibrants. A section was added under quality control detailing ongoing MDL verifications. Section 10 was updated to include neutralizing excess acid.
02/19/2020	Section 6.3. and 8.6. were updated to include manually calculating the percent recoveries of the calibration standards. Replaced notes regarding % residuals with percent recoveries. Added control limit for the calibration blank.
6/03/2020	Removed references to the “MDL” standard; new ongoing MDL verifications procedures were introduced 02/25/2019.
02/25/2021	Clarified Section 1. Revised Sections 3, 4 and 6 to add and organize data in tables.
07/21/2023	Updated file path links. Added note for barium chloride test to check for incorrect preservation.
09/17/2024	Updated file path links. Instrument Lachat I was removed.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.