

**Standard Operating Procedure**  
**Nitrate/Nitrite Lachat operation**  
**Soil Environmental Chemistry Program, The Ohio State University**  
Version 5

## 1.0 Scope

Lachat may be used to determine the following ions in solution: Nitrite ( $\text{NO}_2^-$ ) and Nitrate ( $\text{NO}_3^-$ ).

### 1.1 The application ranges:

- 0.01-2.0 mg N/L as  $\text{NO}_2^-$  and  $\text{NO}_3^- + \text{NO}_2^-$  when using the Sample loop 40.5 cm
- 0.20-20 mg N/L as  $\text{NO}_3^- + \text{NO}_2^-$  when using the Microloop
- MDL:
  - Nitrate and Nitrite: 0.1 mg N/L when using the Microloop
  - Nitrate and Nitrite: 0.01 mg N/L when using the sample loop 40.5 cm
  - Nitrite 0.01 mg N/L

### 1.2 Throughput:

- 30 injections/hour

## 2.0 Definitions

2.1 Check standard: Standard that is independently made, that is used for Quality control.

2.2 Matrix: also known as the carrier, it matches the substance the samples are in.

## 3.0 Equipment and Supplies

3.1 Balance

3.2 Volumetric flask (v.f.)

3.3 Hamilton Autodiluter

3.4 Various graduated cylinders

3.5 Stir bars

3.6 Ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA)

3.7 ACS grade Ammonium Hydroxide

3.8 Sulfanilamide

3.9 N-1-naphthylethylenediamine dihydrochloride (NED)

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3.10 ACS grade Potassium Nitrate

3.11 ACS grade Potassium Nitrite

3.12 Hydrochloride acid (HCl)

**4.0 Reagents and Standards**

**\*Note strong bases and acids will be use, caution is advised.**

4.1 Reagent 1: Ammonium Chloride buffer

4.1.1 Mix 105 mL HCl, 95 mL ammonium hydroxide, and 1.0g EDTA in 700 mL DI in a 1000 mL v.f. Bring to volume

4.1.2 Store in HDPE screw top bottle, good for 2 months

4.2 Reagent 2: Sulfanilamide Color reagent

4.2.1 Mix 100ml of phosphoric acid, 40.0g sulfanilamide and 1.0g NED in 800 mL DI in a 1000 mL v.f. Bring to volume

4.2.2 Store in dark screw top bottle, discard when bright pink

4.3 Nitrate Standard : 200 mg N/L as  $\text{NO}_3^-$

4.3.1 Stock solution: Dissolve 1.444g Potassium Nitrate in 800 mL matrix in a 1000 mL v.f. Bring to volume

4.3.2 Repeat for check standard

4.4 Nitrite Standard : 200 mg N/L as  $\text{NO}_2^-$

4.4.1 Stock solution: Dissolve 1.214g Potassium Nitrite in 800 mL matrix in a 1000 mL v.f. Bring to volume

4.4.2 Repeat for check standard

4.5 Blank Standard: Matrix/ Carrier

4.6 Reagent 3: Copper Sulfate

4.6.1 Mix 2.5g of Copper Sulfate in 1000mL v.f. with 700 ml. Bring to volume.

4.6.2 Good for one week.

4.6.3 Add 5 drops/1000mL of Reagent 1 to reagent 1.

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**5.0 Sample Collection, Preservation and Storage**

- 5.1 Note if samples can't be run within 48 hours, samples should be adjusted to pH <2 with sulfuric acid and refrigerated to 4°C. Samples can be held in the refrigerator up to 28 days. Samples can also be frozen for longer period.
- 5.2 Samples should be filtered with a .45um Nylon filter that have been checked for contaminants and if possible new syringes to make sure all particles are free from the sample and to avoid contamination.
- 5.3 Sample Runlists
- 5.3.1 Sample runlists (ie. Lachat 16-1.xls) should be created and stored to Wdrive>SEC Lab>Lachat> >year (i.e 2016)>Runlist. A runlist template is in Wdrive>SEC Lab>Lachat.
- 5.3.2 Each time the method is started or stopped and restarted, the runlist must be updated immediately with the timestamp corresponding to the sample.

**6.0 Lachat Setup**

- 6.1 Turn on all instrument components and then start the OMNION software.
- 6.2 Clamp down tubing on the pump, Turn on pump by hitting manual start/stop, make sure the speed is 35.
- 6.3 Pump DI water through all the reagent lines and check for leaks and smooth flow. Switch to reagents one by one and wait for the system to equilibrate until a stable baseline is achieved for each one. Baseline should be around 0.5 and 0.6.
- 6.3.1 If baseline is running high or won't stabilize, i.e baseline wobbling. Do the following steps in this order until a solution is reached
- 6.3.1.1 Check the flow cell for any bubbles, if there are lightly flick the cell to dislodge the bubbles
- 6.3.1.2 Check the flow cell for leaks
- 6.3.1.3 Clean the flow cell area and interference plate with IPA
- 6.3.1.4 While running through the lines have one line run 0.1M HCl for about 30 minutes to help with staining.

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6.4 If running for Nitrate/Nitrite then turn on the Cadmium Column and wait for the system to equilibrate.

6.5 Templates are in Documents> OmnionData> OSU>Templates>NOx or NO2

**Commented [WU1]:** We need separate templates for NO2 and NO2+NO3. The analyte should be clearly indicated in the method.

6.5.1 Open template

6.5.2 Modify the template if necessary and save the file in Document>Omnion Data>OSU> Data>(Current year)>(runlist #)

6.6 Templates are created by inputting the following data system parameters:

**In the timing Tab:**

**Run:**

Cycle period: 90 s

Min. probe in wash period: 40 s

Probe in sample period: 25 s

**Nitrate/ Nitrite :**

Peak Base Width: 35 s

Inject to peak start: 13 s

**Channel 3:**

Load Period: 17.9 sec

Inject period: 27.1

Time to valve: 27

**Under the Analyte Tab:**

Chemistry: Direct/ Bipolar

Calibration Rep: Average

Calibration fit type: 2nd order polynomial

Weighting method: 1/X

Force through zero: no

Concentration Unit: mg/L

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Name: Nitrate/Nitrite

Clear calibration: should be checked, unless otherwise

6.7 From the stock 200 mg N/L Nitrate or Nitrite standard, prepare 50mL solutions, using the matrix as diluent, of 2.0, 1.0, 0.20, 0.10, 0.05, and 0.01 mg N/L. This can be done manually or using the auto diluter on the Lachat.

6.7.1 Using the Autodiluter

6.7.1.1 Put standard in its position

6.7.1.2 In Omnion select where the standard is going to be taken from.

6.7.1.3 If it need diluted click the ADF check mark and impute the auto dilution factor in the ADF box

6.7.1.4 Then under sample in run properties, impute the known concentration of the standard you are achieving by dilution.

6.8 From the 200mg N/L Nitrate and Nitrite check standard, prepare 50 mL solution, using the matrix as diluent of 1.0 mg N/L for the check standard.

6.8 Using the standards run a calibration curve.

6.8.1 Calibration curve should have a straight line and  $r= 1\pm.0010$

6.9 Run the Check standard and blank after calibration is completed

6.9.1 When running the NO<sub>x</sub> testing, a Nitrate and Nitrite standards should be run to test for Cd column efficiency. See 11.0 on how to do the efficiency testing.

6.10 Before running samples create a Composite (Comp), comp spk, and comp x5 solution.

6.10.1 **Comp**- make a solution with a mixture that is a composite of all the samples going to be run. Make about 15 ml of solution.

6.10.2 **Comp spk**- Take 5 ml of comp solution and add 0.125 mL of 10 mg N/L of Nitrate and Nitrite solution. Or another

6.10.3 **Comp x5**- dilute Comp solution x5

6.10.4 Calculate the % recovery for each. Should be approx.  $\pm 10\%$

6.11 Run samples as unknowns and check standard and blank after every 10 samples.

## 7.0 Quality Control

7.1 Run the Check standards, 1.0 mg N/L as prepared in 6.7 and blank on the Lachat.

7.1.1 Results: should fall with in  $\pm 10\%$

**Commented [WU2]:** Do we need a low limit check standard, low calibration standard maybe?

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- 7.2 Check standards as prepared in 6.7 and blank should be run after ever 10 samples.
- 7.2.1 If check standards fail then recalibrate instrument and re-run the previous 10 samples and check standards and blank
  - 7.2.2 If it continues to fail then remake standards and recalibrate and test using check standards
  - 7.2.3 If check standards fail then remake check standards and re-run check standard
- 7.3 When equilibrating in 6.1, after each reagent is added, it should go back down to the baseline after equilibration if it does not, check lines and remake reagent if necessary.

### **8.0 Data Summary**

- 8.1 Data automatically goes to excel sheet in the Document>Omnion Data>OSU> Data>(Current year).
- 8.1.1 This can be found and/or modified in the Configuration tab>Options>Data export>Data Items.
- 8.2 Move all excel sheets corresponding to a specific run to Document>Omnion Data>OSU>Data>(Current year)>(current runlist folder)
- 8.3 See Lachat Summary SOP for Data Summary.

### **9.0 Lachat Power off procedure**

- 9.1 Turn off Cadmium Column
- 9.2 Rinse all lines with DI water for 10 minutes
- 9.3 Allow air to pump through lines for five minutes
- 9.4 Restart Auto-sampler so that the sampling wand ends out of rinse.
- 9.5 Turn off all devices and computer

### **10.0 Waste**

- 10.1 All Waste lines from the Lachat should be positioned into a carboy, not down the sink

### **11.0 Cadmium Column Efficiency Testing**

- 11.1 Cadmium Column can go inefficient over time, it is good to test it at the beginning of every run.
- 11.2 To test the Column:

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11.2.1 Start up the instrument as in 6.0, but making sure Column is open and using the NO<sub>3</sub> Standard as your calibration.

11.2.2 Run a check standard of your NO<sub>3</sub> and NO<sub>2</sub> of the same concentration.

11.2.3 Using the following formula:  $\frac{NO_3}{NO_2} * 100\% = E$

11.2.4 E should be greater than 90%.

## 12.0 References

12.1 Lachat Applications Group. Determination of Nitrate in 2 M KCl Soil Extracts by Flow Injection Analysis Colorimetry. QuickChem Method 12-107-04-1-F. 2015

12.2 O'Dell, J.W. Determination of Nitrate-Nitrite by Automated Colorimetry. Environmental Monitoring Systems Laboratory. United States Environmental Protection Agency. Method 353.2. 1993

12.3 United States Geological Survey. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Brook 5 Chapter A1. United States Department of the Interior. Method I-2601-78