DETERMINATION OF NITRITE/NITRATE IN KCL EXTRACTED SEDIMENTS USING THE LACHAT QUIKCHEM 8500 AUTOANALYZER

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1. SCOPE AND APPLICATION

1.1. Sediments and soils are analyzed for extractable nitrite/nitrate, using a 2M KCl extraction technique.

1.2. The calibration range is from the method detection limit (MDL) 0.020 mg N/L to 20.0 mg N/L. The practical quantitation limit (PQL) is 0.060 mg N/L. The results are reported as mg/kg. The test covered by this SOP is S-NO2NO3.

2. SUMMARY OF THE METHOD

2.1. This method is based on Lachat method 10-107-04-1-B. Sediments are mixed with 2M KCl, shaken, and allowed to settle. The acidified KCl extract is filtered and is then ready for analysis. (See DEP SOP NU-052).

2.2. The KCl extract is analyzed for nitrite/nitrate using a flow injection analyzer equipped with an autosampler. A filtered sample is passed through a column containing granular copper-cadmium, which reduces nitrate to nitrite. The nitrite originally present plus the reduced nitrate can then be determined by colorimetry. The nitrite is diazotized with sulfanilamide and coupled with N-1-naphthylethylenediamine dihydrochloride to form a highly colored azo dye, which absorbs at a wavelength of 520 nm.

2.3. Interferences - Color and turbidity may interfere with the absorbance readings. Samples containing particulate matter must be filtered. Color correction (6.9.) is performed to correct for interference due to background absorbance.

3. EQUIPMENT AND SUPPLIES

3.1. Lachat QuickChem FIA+ 8500 Series Flow Injection Analyzer.

3.2. Pump Tubing:

- Carrier is 2 M KCL solution (4.2.1.) and the carrier line is orange.
- Sulfanilamide color reagent line is white.
- Buffer line is yellow and blue.
- Sample line is green.
- Wash line is green.

3.3. Glassware and pipettes:

- 250 mL Erlenmeyer Flasks.
- Various analytical pipettes for standard preparation.

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- 10 mL borosilicate test tubes.
- 15 mL centrifuge tubes used for spiking and diluting samples.
- 10 mL, 100 mL, 1 L and 2 L volumetric flasks (Class A) to make reagents and standards.

4. REAGENTS AND STANDARDS (DEP SOPS NU-041 and NU-042)

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water.

Note: Before preparing reagents or standards, or running samples, read the safety notes in the safety/hazardous waste section of this SOP and the MSDS's for the chemicals used in this analysis.

Note: All standard and reagent preparations should be documented in the NU-RGTSTD Reagent and Standard Logsheet located on the network at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\NO2NO3\S-NO2NO3 KCL. This sheet needs to be updated whenever a reagent or standard is newly prepared. All 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 used for the extraction and analysis are generated in the module and documented in the prep worksheet in LIMS or in the logsheet submitted with each analysis folder. The Certificate of Analysis for the SRM is kept in a binder in C307. If available, a Certificate of Analysis for each purchased reagent is also maintained in the REAGENT_PREP folder on the Nutrients drive. 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 altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals:

4.1.1. Granular copper-cadmium column

4.1.2. Hydrochloric acid (HCl)

4.1.3. Ammonium hydroxide (NH_4OH)

4.1.4. Disodium Ethylenediamine Tetraacetate (EDTA)

4.1.5. Phosphoric acid (H_3PO_4)

4.1.6. Sulfanilamide ($C_6H_8N_2O_2S$)

4.1.7. N-1-naphthylethylenediamine dihydrochloride (NED)

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4.1.8. Sulfuric acid, concentrated (H_2SO_4)

4.1.9. Sodium chloride (NaCl)

4.1.10. Potassium nitrate (KNO_3)

4.1.11. Chloroform ($CHCl_3$)

4.1.12. Potassium Chloride (KCl)

4.1.13. 1000 mg N/L nitrite standard purchased from RICCA Chemical Company.

4.1.14. A certified standard reference material (SRM) purchased from Environmental Resource Associates (Anions in soil, cat #543 - concentration varies with lot #).

4.2. Reagents:

4.2.1. 2M KCl Solution (carrier) - To a 1 L bottle or beaker, add 700 mL of DI water. Add 150 g of KCl (4.1.12.) and acidify with 2 mL H_2SO_4 (4.1.8.). Transfer to a 1 L volumetric flask and bring to volume. Stir/shake to dissolve and mix. Alternatively, you may acidify an aliquot of the extraction solution (DEP SOP NU-052, 4.2.1.), for use as carrier. The carrier is also used for un-extracted blanks i.e., continuing calibration blanks (CCBs), the MDL, and filter check blanks. Store at room temperature. Holding time, 90 days.

4.2.2. Buffer:

4.2.2.1. To a 2 L beaker containing approx. 1 L of DI water slowly add 210 mL of HCl (4.1.2.) with stirring. Add 190 mL of NH_4OH (4.1.3.) and 2.0 g of EDTA (4.1.4.). Stir for 5 min and transfer to a 2 L volumetric flask; dilute to volume with DI water.

4.2.2.2. Let the solution cool to room temperature and check the pH using pH paper. The pH should be around 8.5. Adjust pH if necessary with NaOH or HCl. The solution should be prepared in a room that is separate from ammonia analysis due to the possibility of contamination. Holding time, 30 days.

4.2.3. Sulfanilamide color reagent:

4.2.3.1. Add 100 mL of 85 % H_3PO_4 (4.1.5.) to 600 mL of DI water in a 1 L volumetric flask. Add 40.0 g of sulfanilamide (4.1.6.) and 1.0 g of NED (4.1.7.).

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4.2.3.2. Stir the solution for 20 min then dilute to volume. Filter reagent with 0.45 µm Teflon filter prior to using. Holding time, 30 days.

4.3. Standards:

4.3.1. Nitrate stock standard and high-level spike solution (1000 mg N/L) - To a 1 L flask containing approx. 800 mL of DI water, add 7.194 g of dried KNO_3 (4.1.10.). Shake/stir to dissolve. Add 2 mL of $CHCl_3$ (4.1.11.). Acidify with 2 mL H_2SO_4 (4.1.8.). Bring to volume with DI water, invert 12 times, and refrigerate. This solution is used for the high level spike at 10 mg N/L (spike code S2). Holding time, 180 days.

4.3.2. Nitrate working standard and low-level spike solution (100 mg N/L) - To a 100 mL flask containing about 50 mL DI water, add 10 mL (using a volumetric pipette) of 1000 mg N/L nitrate stock standard (4.3.1.). Acidify with 0.2 mL H_2SO_4 (4.1.8.). Bring to volume, mix well and refrigerate. This solution is used for the low level spike at 1 mg N/L (spike code S1) and the PQL standard (PQCK2) at 0.06 mg N/L. Holding time, 90 days.

4.3.3. Working calibration standards are prepared from the nitrate stock standards 1000 mg N/L (4.3.1.), 100 mg N/L (4.3.2.) and intermediate calibration standard solutions as described below. Holding time, 30 days.

STD	Conc. (mg N/L)	Prepared from Std (mg N/L)	Volume of Std Added	Final Volume
C1 (CHCK5)	20.0	1000	2.0 mL	100 mL
C2	10.0	1000	1.0 mL	100 mL
C3	2.00	100	2.0 mL	100 mL
C4 (CHCK8)	1.00	100	1.0 mL	100 mL
C5	0.500	100	0.50 mL	100 mL
C6	0.200	2.0	1.0 mL	10 mL
C7	0.100	100	0.10 mL	100 mL
C8	0.050	20	0.25 mL	100 mL
C9	0.025	0.05	5.0 mL	10 mL
C10 (BLK)	0.000			100 mL

Note: Standards are made in acidified 2M KCL carrier (4.2.1.) solution.

4.3.4. Nitrite catalyst check standard - The quality control standard CATCHK1 is prepared as a 1.0 mg N/L nitrite solution. Add 100 µL of the 1000 mg N/L nitrite standard (4.1.13.) to 50 mL 2M KCl extraction solution (see DEP SOP NU-052, section 4.2.1.) in a 100 mL volumetric flask and dilute to volume. **Do not dilute with carrier solution or acidify the nitrite**

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standard. The presence of acid will oxidize the nitrite to nitrate. Holding time, 48 hours.

4.3.5. Nitrate/Nitrite SRM standard (4.1.14.) - Prepared according to the manufacturer's instructions. The supplier (Environmental Resource Associates) differs from the supplier of the calibration standards. The standard may be diluted appropriately (by weighing out a smaller amount of soil standard) so that the concentrations falls within the calibration range. For the concentration and expiration date see the manufacturer's certificate.

5. SAMPLE PREPARATION

5.1. For sample collection, preservation, shipment, storage, and preparation instructions details see DEP SOP NU-052.

5.2. Samples and extracts are stored at ≤ 6°C (not frozen) while awaiting analysis.

5.3. All samples must be extracted and analyzed within 28 days of collection.

5.4. Pre-filter the aliquot to be analyzed using a syringe filter with a 0.45 µm nominal pore size. Also, analyze a filtered blank and check standard with this type of syringe filter.

6. SAMPLE ANALYSIS (SEE DEP SOP NU-033)

6.1. Print backlog for S-NO2NO3 to determine which samples have been extracted and are ready for analysis.

6.2. The reagents should be removed from the refrigerator in nutrients lab to allow reagents, etc. to come to room temperature.

6.3. Please refer to DEP SOP NU-066 for instrument startup and operation procedures.

An example run sequence includes:

- **Calibration – ten calibrants including a blank (4.3.3.)**
- **CCB (Carrier, 4.2.1.) and CHCK8 (4.3.4.) – these cups are scheduled to bracket every 10 samples on the run. An additional carrier cup may be scheduled between the 10th sample and the bracketing CCB and CHCK.**
- **PQCK2 (4.3.2.)**
- **CATCHCK1 (4.3.4.)**
- **MDL (Carrier, 4.2.1.)**

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- **ERAQCNO3SOIL# (SRM, 4.3.5.)**
- **CHCK5 (4.3.4) – this cup is followed with a Carrier (4.2.1.) cup to check carryover**
- **CHCK8_F and MBLK_F – filtered blank (4.2.1.) and CHCK (4.3.4.), see section 5.4 above**
- **Batched samples with extracted MBLKs and LFBs, and including sample duplicates, duplicate low level spikes, and one high level spike – check prep worksheet for batching information and prep batch IDs (see DEP SOP NU-052 for batching information). A carrier cup is added after the high level spike.**
- **CCB and CHCK8 – final bracket**

6.4. Locate the manifolds for high level nitrite/nitrate analysis. Check the condition of the pump tubing. Check the condition of the cadmium column. Check the condition of the tubing on manifold.

6.5. Clamp down the pump tubing. Pump fresh DI water through all tubing. The manifold should be cleaned once a week with a 25 % HCL solution. The cadmium column should be removed before pumping the acid solution through the manifold. The manifold should be rinsed with DI water before replacing the column.

6.6. Check that the pump tubing is placed into the appropriate reagents. The waste line must be connected to valve port 5 of the manifold. The sample line must be connected to valve port 6.

6.7. Start flushing the instrument with DI water for approximately 10 min. Flush with reagents 5-10 min. Calibrate the instrument with the 10 working standards and blank (4.3.3.).

6.8. After an acceptable calibration is obtained (the calibration plot of absorbance vs. concentration is quadratic (1/x weighted) and must have a correlation coefficient of > 0.995), analysis may begin.

6.9. Color Correction - Samples with background absorbance at the analytical wavelength may interfere. Color correction is used to eliminate sample background interference due to color and turbidity.

6.9.1. Any sample at or above the MDL displaying color should be run for color correction. At the end of the run remove the color line (sulfanilamide) and put it in the carrier beaker. Wait approximately 5 min until the system equilibrates. Set up a new tray starting with 5 blanks, the samples to be color corrected, followed by 5 more blanks at the end. Start the run as usual.

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6.9.2. Color correction calculations – Open an Excel spreadsheet template found at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\color corrections\. Enter all blank concentrations from the color correction run (Sample ID: MBLK), sample ID of samples that required color correction, their respective raw value (A) and color correction value (B). An average blank concentration ($MBLK_{Avg}$) is calculated from the 10 blanks ran before and after the samples (6.9.1.) and the color correction is calculated as follows:

Color correction = $A – (B – MBLK_{Avg})$

Subtract only if the color correction value B is at or above the MDL. Color corrected results are manually entered into the QC Manager.

6.10. Post-run procedure:

6.10.1. Exporting Files:

6.10.1.1. Save file as SNOyymmddM# (Lachat 1 instrument) or SNOyymmddN # (Lachat 2 instrument).

6.10.1.2. Select Run Tab in the Run Properties window.

6.10.1.3. Select *Export Data to File* button.

6.10.1.4. In the QC Manager, select the Instrument: Nutrients: Lachat 1 or 2.

6.10.1.5. Convert the file and save as raw data.

6.10.2. Printing Data:

6.10.2.1. Select the *Tools* then *Custom Report*

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

6.10.2.3. Calibration info will print along with data.

7. QUANTITATION AND CALCULATIONS

7.1. The instrument will give the analysis results for nitrite/nitrate in mg N/L. The QC-manager converts the results to mg/kg. The concentration of analyte in mg/kg dry weight is calculated as follows:

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A = (filtrate analysis results (mg/L)) × (final vol (0.1 L)) × (Dilution Factor)

B = (wt. of sample extracted (kg)) × (% solids as decimal fraction)

Analyte concentration (mg/kg) = A / B

7.2. 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 the FDEP Quality Manual.

8. DATA ARCHIVAL

8.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (S-NO2NO3), filename/s (SNOyymmddM# (Lachat 1 instrument) or SNOyymmddN # (Lachat 2 instrument)), and initials of the analyst.

8.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, Reagent and Standard Logsheet including the prep worksheet ID(s) associated with the extraction, color correction calculations spreadsheet (if color correction was required), and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

8.3. 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.

8.4. Lachat instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\FIA\sediments\S-NO2NO3\.

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

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

9.2. The calibration standards (4.3.3.) and SRM (4.1.14.) are nitrate only. The Ricca standard (CATCHK1, 4.1.13.) is nitrite only. Do not acidify the nitrite standards,

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which will cause the nitrite to oxidize to nitrate.

9.3. A nitrite standard (CATCHK1) must be analyzed at the beginning of every run in order to check the efficiency of the cadmium column. A nitrate check standard (4.3.3.) should be run a minimum of every 20 cups throughout the run.

9.4. Each tray of extracted samples contains a PQCK2 (0.06 mg N/L (4.3.2)) and an SRM (4.3.5.). Acceptance limits for the PQCK2 recovery are 70 to 130 % and for the SRM standard are specified in the Certificate of Analysis provided by the manufacturer.

9.5. A blank and a check standard (CHCK, 1.0 mg N/L (4.3.3.)) are run every 20 cups. Acceptance limits for the MBLKs, MDL, and CCBs are < 0.020 and > - 0.020 mg N/L. The acceptance limits for recovery of the check standard and CATCHK1 are 85 to 115 %.

9.6. For every batch of 20 samples, a method blank (MBLK), laboratory fortified blank (LFB, 1.0 mg N/L), sample in duplicate and two low-level spikes (1.0 mg N/L) and one high-level spike (10 mg N/L) are extracted and analyzed. The acceptance limit for the RPD of replicates is less than 20 % for results greater than the PQL. Acceptable limits for spike recoveries are 80 to 120 % for results above the PQL. One filtered blank and one filtered check standard must be analyzed per calibration, if any samples on that calibration are filtered.

Note: Blanks and LFBs are prepared and extracted in the same way as samples, using Ottawa sand as the matrix (see DEP SOP NU-052).

9.7. Calibration Range - See Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the quadratic calibration must be equal to or larger than 0.995. The acceptance limits for the % error calculated for each calibrant should not exceed 10 % for all calibrants above the PQL and 30 % at the PQL.

9.8. 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.

10. MAINTENANCE

10.1. Carry out routine maintenance as recommended by Lachat and document any maintenance performed in logbook.

10.2. The manifold is cleaned with 1 M NaOH as necessary.

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11. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

11.1. Read the MSDS information (in the MSDS notebook) for safe reagent and sample handling. Read the MSDS on cadmium. **Cadmium is a harmful substance**.

11.2. Proper safety equipment including safety glasses, gloves, and lab coat must be worn at all times during analysis and sample preparation.

11.3. Refer to the MSDS for information about chemicals used in this analysis.

11.4. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

12. REFERENCES

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.

12.1. EPA method 353.2.

12.2. Lachat QuikChem Method No. 10-107-04-1-B.

12.3. Quality Manual, Bureau of Laboratories, FDEP.

12.4. Laboratory Health and Safety Plan.

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

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

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

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

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

12.10. DEP SOP NU-052, KCL Extraction of Nitrate/Nitrite and Ammonia from Soils and Sediments.

12.11. DEP SOP NU-066, Determination of Nitrate and Nitrite in Water Using the

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Lachat QuickChem 8500 Autoanalyzer.

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

Appendix of Significant Changes

08/12/2010 Changes were made to sections 6.8 and 7.0 to clarify reporting procedures and calculations.

08/04/2011 Expanded the range of the calibration curve. More details were also added to section 9 (Quality Control) to describe QC requirements and acceptance criteria for each run and batch.

07/05/2012 The color correction calculation procedure was described in more detail.

07/09/2015 Data archival information was clarified in Section 8.

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