DETERMINATION OF NITRITE IN WATER USING THE LACHAT QUICKCHEM 8500 AUTOANALYZER

1. SCOPE AND APPLICATION

This method is applicable to the determination of nitrite (NO_2) in drinking, surface waters, and wastes. The calibration range for nitrite is from the method detection limit (MDL) of 0.003 mg N/L, to 0.20 mg N/L. The range may be extended by dilution. Tests covered by this SOP are W-NO_2 and W-NO_2-F. The practical quantitation limit (PQL) is 0.005 mg N/L

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. The complexing agent Ethylenediaminetetraacetic acid (EDTA) is replaced by Diethylenetriaminepentaacetic acid (DTPA) in the buffer.

2.2. The nitrite present in the sample is 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 flow analyzer or injector needle can cause interference. 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.

3. EQUIPMENT AND SUPPLIES

3.1. Lachat QuickChem Series 2 FIA+ 8500 Series Flow Injection Analyzer; SN# 08120001071 (Instrument 2)
Omnion 3.0.222 Software.
Lachat RP-150 Series Reagent Pump; SN# A82000-2090
Lachat ASX-520 Series XYZ Autosampler. SN# 08120001798

3.2. Pump Tubing:

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Orange = Carrier (water)
Black = Sulfanilamide Color Reagent (4.2.2.)
Blue/Yellow = Buffer Solution (4.2.1.)
Green = Sample Intake
Purple = Rinse

3.3. Glassware and pipettes - Class A type volumetric glassware and analytical pipettes.

Variable Eppendorf pipettes
15 mL Centrifuge Tubes
50 mL, 250 mL, 1 L and 2 L volumetric flasks
2 L Beaker

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: All reagent and standard preparations must be documented in the Reagent and Standard Logsheet for NO_2 analysis located on the network at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\NO2 Lachat\. This excel sheet needs to be updated and submitted as part of the analysis report folder.
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 are generated in the module and documented in the Reagent and Standard Logsheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard or reagent is also maintained in the REAGENT_PREP folder. The Certificate of Analysis for the standard reference material is kept in a binder in C307. 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. Hydrochloric acid, concentrated (HCl)

4.1.2. Ammonium hydroxide (NH_4OH)

4.1.3. Diethylenetriaminepentaacetic acid (DTPA)

4.1.4. Phosphoric acid, concentrated (H_3PO_4)

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4.1.5. Sulfanilamide

4.1.6. N-(1-naphthyl)ethylenediamine dihydrochloride (NED)

4.1.7. Sodium chloride, reagent grade (NaCl)

4.1.8. Potassium nitrite (KNO_2)

4.1.9. Chloroform ($CHCl_3$)

4.1.10. 1000 mg N/L nitrite stock standard reference material solution from RICCA Chemical Company, purchased from Fisher (or equivalent).

4.2. Reagents:

4.2.1. Buffer solution:

4.2.1.1. In a laboratory hood, slowly add 210 mL of concentrated hydrochloric acid (HCl, 4.1.1.) to 1 L deionized (DI) water in a 2 L volumetric flask while stirring. Slowly add 190 mL ammonium hydroxide (4.1.2.) and 2.0 g of DTPA (4.1.3.). Stir for five minutes and allow to cool. Dilute to the mark.

Note: Use caution - this acid base reaction is exothermic and generates enough heat to cause burns through the flask. 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.

4.2.1.2. After cooling is complete check the pH of the solution is ≈ 8.5 using pH paper. Solution should be made as needed. Record each new batch in the reagent log. Holding time, 30 days.

4.2.2. Sulfanilamide color reagent:

4.2.2.1. Add 100 mL of 85 % phosphoric acid (4.1.4.) to 600 mL of DI water in a 1 L volumetric flask. Mix and add 40 g of sulfanilamide (4.1.5.) and 2.0 g of N-(1-naphthyl)ethylenediamine dihydrochloride (NED, 4.1.6.).

4.2.2.2. 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 - DI Water. Also used for MBLKs, MDL, CCBs (continuing calibration blanks), and dilutions on the run.

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4.3. Standards and spiking solutions:

4.3.1. ALTQC (QCNI and CHNI), 0.10 mg N/L - Into a 1 L flask containing approximately 500 mL DI water pipette 100 µL of the 1000 mg N/L nitrite stock standard (4.1.10). Bring to volume with DI water and invert 12 times. Store in refrigerator. Holding time, 2 days.

4.3.2. Stock Nitrite Standard, 2000 mg N/L - Pour the potassium nitrite (KNO_2) into a glass beaker and place it in an oven for 24 hours at 105 °C. Dissolve 12.14 g of dried potassium nitrite in approximately 800 mL of DI water contained in a 1 L volumetric flask. Add 2 mL of chloroform. Bring the volume to 1 L with DI water, invert 12 times and refrigerate. Holding time, 180 days.

4.3.3. Working Stock Standard, 20.0 mg N/L - Into a 1 L flask containing approximately 500 mL DI water, pipette 10.0 mL of the 2000 mg N/L nitrite stock standard (4.3.2.). Bring to volume with DI water and invert 12 times. Store in refrigerator. Holding time, 90 days.

4.3.4. Working Nitrite Spike Standard, 4.0 mg N/L - Add 500 µL of the 2000 mg N/L nitrite standard (4.3.2.) to a 250 mL volumetric flask containing approx. 100 mL of DI water. Dilute to volume, invert 12 times and refrigerate. Holding time, 90 days.

4.3.5. Calibration Standard Preparation - The method uses a five point calibration. The calibration standard levels are shown in the table below. Six cups are loaded in the standards rack, the 6th cup having carrier (4.2.3.) for the blank. Holding time, 30 days.

Concentration (mg N/L)	Volume added (mL) (20 mg/L Stock Standard, 4.3.3.)	Final Volume (mL) (using carrier, 4.2.3.)
0.200 (CHNI2)	2.50	250
0.050	0.625	250
0.020	0.250	250
0.010	0.125	250
0.005 (PQNI)	0.0625	250

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

5.1. Samples are collected and stored on ice or in refrigerator at ≤ 6°C (not frozen).

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5.2. Samples must be analyzed within 48 hours of collection time.

5.3. A laboratory fortified blank (LFB), a sample either analyzed in duplicate and/or spiked in duplicate must be analyzed for every batch of 10 samples. Use the 4.0 mg N/L stock solution (4.3.4.) as the spiking solution. Add 150 µL to 10 mL of sample (or DI water in the case of an LFB) for a 0.060 mg N/L spike (spike code, LO).

5.4. One method blank (MBLK) must be analyzed for every batch of 10 samples. DI water is used for the blank.

6. SAMPLE ANALYSIS/QUANTITATION

6.1. Instrument Setup:

6.1.1. Turn on all instrument components and computer.

6.1.2. Locate manifold, and check the condition of the tubing and column. Make sure all connections are made as shown in the Lachat Method 10-107-04-1-C. Lock down all tubing in the peristaltic pump and flush with water for at least 15 minutes.

6.1.3. While tubing is flushing select the Omnion 3.0 icon on the computer desktop to open the instrument software.

6.1.4. Select *Run → Open → NO2TEMPA.omn → OK*

6.1.5. 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 (4.3.5.) plus a blank (4.2.3.))
PQNI (4.3.5.)
MDL (4.2.3.)
QCNI (ALTQC, 4.3.1.)
CHNI2 (4.3.5.)
CCB (4.2.3.)
Batched samples, with an **MBLK, LFB,** and **spikes** (5.3.) for each batch
CAR
CHNI
CCB

6.1.6. Remove intake lines from water bath, place in appropriate reagent reservoirs and set pump to 35 rpm. Allow to flush while continuing with set-up. Preview the baseline before starting the run.

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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. The instrument is now in pause mode for a maximum of five minutes, after which the analysis will automatically end. 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 notation NYYMMDD**N**# (Using YY for the year, MM for the month, DD for the day, N for Lachat 2, and # for the number of the run).

6.2. Color Correction:

6.2.2. Manual color correction – Any sample at or above the MDL with a noticeable color should be run for color correction. At the end of the run remove the intake line from the sulfanilamide (color reagent), and place it in a beaker with DI water to flush for a few minutes. Then move the line to a beaker with 10 % phosphoric acid (v/v). 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.

6.2.3. Repeat steps 6.1.4. – 6.1.9.

6.2.4. Color correction calculations – In 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 (MBLKAvg) is calculated from the 10 blanks ran before and after the samples (6.2.2.) and the color correction is calculated as follows, Color correction = A – (B – MBLKAvg).

6.3. Quantitation/Calculations:

6.3.1 The Instrument will give the analysis results for NO_2 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.

6.3.3 The individual calibration standards are prepared according to section 4.3.5. The linear calibration is calculated by the instrument software and

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is presented in Table 1 and Figure 1 of the analysis report. It includes the correlation coefficient and % residuals that are used to evaluate the linear fit.

6.4. Post-run Procedure:

6.4.1. Printing Data – Single Channel:

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.

6.4.2. Exporting Files:

6.4.2.1. Select Run tab in the Run Properties window.

6.4.2.2. Select Export Data to File button.

6.4.2.3. Convert file in QC Manager, and save as raw file.

6.4.2.4. Format data file, select uploadable samples, and place in folder for further review by supervisor.

7. DATA ARCHIVAL

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(s) (W-NO2, W-NO2-F), filename/s (NYYMMDDN# (where N is for Lachat 2, and the # indicates the 1st, 2nd etc run of the day), 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 logs, the backlog containing the batching documentation, 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 DEP SOP NU-066, section 9) should be included.

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.

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

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

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 the following DEP SOPs: NU-038 and NU-043.

8.1. The 0.1 mg N/L CHNI standard (ALTQC, 4.3.1.) and a CCB are run every 10 samples and at the beginning and end of each run. The QCNI/CHNI standard is prepared from a different source than the calibration standard. Acceptance limits for the QCNI/CHNI recovery are 90 - 110 %. Acceptance limits for the MBLK, CCB, and MDL are < 0.003 and > - 0.003 mg N/L.

8.2. The PQL level standard (PQNI, 4.3.5.) is run on every analytical run and also serves as the limit of detection verification (LOD). Acceptance limits for the PQNI recovery are 70 – 130 %.

8.3. An LFB, sample in duplicate and/or matrix spike in duplicate are run for every batch of 10 samples analyzed. Acceptance limits for the LFB and spike recoveries are 90 – 110 %. The sample/matrix spike reproducibility should be less than 20 % RPD for samples above the PQL.

8.4. Linear Calibration Range (LCR) - See FDEP Quality Manual Appendix B, Section 7.0., 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear calibration must be equal to or greater than 0.995. Recoveries for calibration standards above the PQL must be 90 -110 % and 70-130 % at the PQL level.

8.5. Every day samples are analyzed a new calibration has to be performed at the beginning of the analysis and the LCR must be verified as described in section 8.4. Consecutive runs on one day may use the same calibration.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

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

9.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

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

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10. 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. Method validation data is on file with the supervisor. The limit of detection (LOD) is verified on every run based on the QCNI.

10.1. EPA Method 353.2, Rev. 2 (1993).

10.2. Lachat QuickChem Method 10-107-04-1-C

10.3. Quality Manual, Bureau of Laboratories, DEP.

10.4. Laboratory Health and Safety Plan.

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

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

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

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

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

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

10.11. DEP SOP NU-066, Determination of Nitrate and Nitrite in Water Using the Lachat QuickChem 8500 Autoanalyzer

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

Appendix of Significant Changes

07/20/2015 Data archival information was clarified in Section 7.

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