DETERMINATION OF NITRITE IN WATER USING THE AQ2 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. This SOP is applicable to the determination of nitrite in surface, ground and saline water, and in domestic and industrial wastes. The applicable range for this method is from the method detection limit (MDL) of 0.002 mg N/L to 0.20 mg N/L NO_2 as N. Samples containing levels higher than 0.20 mg N/L should be diluted to be within the calibration range. The practical quantitation level (PQL) is 0.005 mg N/L.

1.2. Tests covered by this SOP are W-NO_2 and W-NO_2-F.

2. SUMMARY OF THE METHOD

2.1. This method is based upon EPA method 353.2, Rev. 2.0 (1993) and Seal Analytical AQ2 method EPA-137-A Rev.1.

2.2. The diazonium compound, formed by diazotation of sulfanilamide by nitrite in water under acid conditions, is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to produce a reddish-purple azo dye, which is measured colorimetrically at a wavelength of 520 nm.

2.3. Interferences: Background absorbance (color) at the analytical wavelength may interfere. Residual chlorine in the sample may cause low spike recoveries – this can be removed by adding sodium thiosulfate to the sample aliquot prior to spiking (see section 5.4 in DEP SOP NU-066 for chlorine test and treatment procedures). Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation: Seal AQ2 Discrete Analyzers, AQ2 Instrument 1, AQ2 Instrument 2 and AQ2 Instrument 3.

3.2. Glassware and pipettes (all volumetric glassware used is class A):

3.2.1. 1000, 100, and 10 µL pipettes

3.2.2. 15 mL centrifuge tubes

3.2.3. 4 x 250 mL volumetric flasks

3.2.4. 500 mL volumetric flask

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3.2.5. 3 x 1 L volumetric flasks

3.2.6. 600 mL glass beaker

3.2.7. 2 mL sample cups for AQ2 57-position sample tray (Seal Analytical catalog #6015)

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

Note: Prior to using the chemicals in this analysis, please read their MSDS and the safety notes found in the safety/hazardous waste section of this SOP.

Note: 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 (NU-RGTSTD). This excel sheet needs to be updated and submitted as part of the analysis report folder. If available, a Certificate of Analysis for each purchased standard or reagent is maintained in the \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\NO2 AQ2\ folder. . 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. Sodium Hydroxide, NaOH

4.1.2. Ethylenediamine Tetraacetic Acid (EDTA) disodium salt hydrate

4.1.3. Phosphoric Acid, Concentrated, H_3PO_4

4.1.4. Ammonium Chloride, NH_4Cl

4.1.5. Sulfanilamide, $C_6H_8N_2O_2S$

4.1.6. N--(1-naphtyl)-Ethylenediamine Dihydrochloride, NED

4.1.7. Potassium Nitrite, KNO_2

4.1.8. Chloroform, $CHCl_3$

4.1.9. Sodium Chloride, NaCl

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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. NED Color Reagent - To a 600 mL beaker containing about 250 mL of deionized (DI) water add 2.0 g sodium hydroxide pellets and 1.0 g ethylenediamine tetraacetic acid disodium salt hydrate (EDTA). Stir to dissolve completely. Add 50 mL of concentrated phosphoric acid. Caution, the beaker will become warm! While stirring add 50 g ammonium chloride, 12.5 g sulfanilamide and additional DI water to bring the volume to about 450 mL. Dissolve completely. Add 0.625 g N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) and stir to dissolve completely. Dilute to 500 mL. Filter the solution using a 0.45 micron filter and transfer to a dark container. Store this reagent in the refrigerator. Holding time, 30 days. Discard if it develops a significant pink color.

4.2.2. Alkaline EDTA Rinse (Cuvette Wash) - Add approximately 250 mL DI water to a 500 mL volumetric flask. Add 5 g sodium EDTA (4.1.2.) and 10 g NaOH (4.1.1.) to the flask and swirl until the NaOH dissolves. Dilute to volume with DI water and mix well. Holding time, 60 days.

4.3. Standards:

4.3.1. ALTQC (QCNI and CCV), 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) in a glass beaker and place it in an oven for 24 hours at 105 °C. 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 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 Stock Standard, 0.20 mg N/L - Add 2.0 mL of the 20.0 mg N/L working stock standard (4.3.3.) to a 200 mL volumetric flask containing approximately 150 mL of DI water. Bring to volume with DI water and invert 12 times. Store in refrigerator. Holding time, 30 days.

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4.3.5. PQNI (PQL level standard), 0.005 mg N/L - Add 50 µL of the 20 mg N/L working stock standard (4.3.3.) to a 200 mL volumetric flask containing approximately 150 mL of DI water. Bring to volume with DI water and invert 12 times. Store in refrigerator. Holding time, 30 days.

4.3.6. 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.

5. SAMPLE HANDLING AND PRESERVATION

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

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

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

Note: Samples containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer.

Note: Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook.

6.1. Initial Preparation:

Remove standards and reagents from the refrigerator and check the expiration dates. Discard expired standards and/or reagents and reprepare if necessary.

6.2. Calibration:

6.2.1. The nitrite calibration is prepared from the 0.20 mg N/L working stock standard (4.3.4.).

6.2.2. The diluent for nitrite analysis is DI water.

6.3. Reagents, Run Controls, and Stock Standard Loading:

6.3.1. Label one AQ2 reagent vessel and fill it with the NED color reagent (4.2.1.).

6.3.2. Reagent Location - The reagent tray is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the

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assigned locations. Place the NED color reagent (4.2.1.) in position 7.

6.3.3. Controls (CCV/check blank CCB) - Fill one reagent vessel with check standard (QCNI/CCV, 4.3.1.) and place it in position 8. Fill another vessel with DI water (CCB) and place it in position 9.

6.4. Batch QC:

6.4.1. A laboratory fortified blank (LFB) and a sample either ran 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 (4.3.6.) 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. The spike code in QC Manager is LO.

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

6.5. Analysis Setup/Sequence Setup:

6.5.1. Turn on the computer and start the software by double-clicking on the AQ2 icon on the screen. The username is '**Instrument 1, 2 or 3' depending on the instrument used** and the password is 'password'. Next turn on the AQ2 Analyzer (there are two switches, turn on the large switch on the right side behind the instrument - the small switch right next to it is for the chiller and is not used for this test).

6.5.2. Clean and refill the DI water reservoir and the waste containers. Replace all used reaction segments.

6.5.3. In the software: Go to the 'Settings' menu and select maintenance. Zero the reaction segment counter.

6.5.4. Go back to the 'settings' menu and select the daily start-up option.

6.5.5. Setup of the analytical run: Click on the scheduling icon on the left side of the screen. Select a free tray and **reagent set 2**. Name the run as follows:

Instrument 1: NOyymmddK1(use 2 for the 2nd run that day, etc.)
Instrument 2: NOyymmddQ(1)
Instrument 3: NOyymmddR(1)

Now the sequence screen appears. Leave lines one and two empty. Type the sample sequence in the 'Sample ID' column. Highlight the typed lines and select the NO_2 test. The test should appear in the requested test column to the right of the sample ID's. Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

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An example run sequence (see Section 9 for use of the Alkaline EDTA rinse (4.2.2) in case of carryover) includes:

CCV (4.3.1.)
CCB (6.3.3.) DI water, also used as the MDL
MBLK (6.4.2.) DI water
PQNI (4.3.5.)
QCNI (ALTQC, 4.3.1.)
LFB (6.4.1.)
Batched samples, with duplicate spikes (6.4.1.) for each batch
CCV
CCB

6.5.6. Pouring the analytical run - Cup positions #1 and #2 are reserved for the calibration standards. For NO_2 the calibration standard (4.3.4.) is placed in position #2 (position #1 on the sample tray is unused). Now, start pouring the samples according to the cup position on the bench sheet.

6.5.7. Double-click on the 'Run' icon on the left side of the screen. A dialog box appears. Select the tray that contains the sequence to be analyzed. Click 'ok'. Another dialog box appears. Check the auto-standardize box and click 'ok' (if the analysis will use the previous calibration do not check the auto-standardize option.). Select 'ok' on the following dialog box.

6.5.8. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start. If not, the run will stop and display the reagent which has insufficient volume. Fill up the reagent and re-start the run according to 6.5.7. While the run is in progress, the method should be printed to be included in the analysis file. Go to the 'settings' menu and select the option 'Test Parameters'. Select the Test 'EPA353_2 Inst1, 2 or 3' and print.

6.6. Post-Run Procedure:

6.6.1. After the run has finished click on the 'Reporting' icon on the left side of the screen. Select the appropriate reporting format and print the run report in the chronological reporting format.. It contains the calibration and all the data.

6.6.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.6.3. Click on the 'Accept All' Icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the convention described in 6.5.5. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current

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year's subfolder at \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\Nitrite.

6.6.4. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 1', 'Nutrients: Seal AQ2 2' or 'GenChem: Seal AQ2 3'.

6.7. Quantitation/Calculations:

6.7.1. The instrument will give the analysis results for Nitrite in mg N/L.

6.7.2. The QC Manager calculates relative percent differences (RPDs) for duplicate samples as well as recoveries for QC standards. For general calculations including RPDs, see Section 5.9 of the FDEP Quality Manual. Once calculated the results are uploaded into LIMS.

6.7.3. The high calibration standard is prepared according to section 4.3.4. The other standards used in the linear calibration are auto-diluted by the instrument from the high standard. The calibration consists of five standards and a calibration blank. The correlation coefficient for the calibration and the % error of the standards are calculated by the instrument software.

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 (NOYYMMDDQ# (where Q is for AQ2 2 (see section 6.5.5. for other AQ2 instrument codes), and the # indicates the 1st, 2nd etc. run of the day), and the 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), bench sheet(s), reagents and standard logsheet, 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. AQ2 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\AQ2\Nitrite\ .

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations .

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 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.

8.2. The 0.1 mg N/L QCNI/CCV standard (4.3.1.) and CCB will be run every 10 samples and at the beginning and end of each run. The QCNI standard is prepared from a different source than the calibration standard. Acceptance limits for the recovery of the QCNI/CCV are 90-110 %. Acceptance limits for the MBLK and MDL are < 0.0020 and > - 0.0020 mg N/L.

8.3. The PQL level standard (PQNI, 4.3.5.) is run on every analytical run and serves as the ongoing MDL and PQL verification spike. Acceptance limits for the recovery of the PQNI are 70-130 %. The LOD is verified on every run based on the PQNI.

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

8.5. 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. Acceptance limits for the percent error of calibration standards above the PQL are ±10%. Acceptance limits for the percent error of the PQL level standard (0.048 mg N/L) are ±30%. The acceptance limits for the blank calibration point results are below the MDL and above the negative value of the MDL (-MDL < blank < MDL).

8.6. Every day samples are analyzed a new calibration is performed at the beginning of the analysis and the LCR must be verified. Consecutive runs on one day may use the same calibration.

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9. TROUBLESHOOTING

If the run quality deteriorates (unacceptable calibration and/or QC results, high blanks, sample carry-over, etc.), the system should be washed with the alkaline EDTA rinse solution (4.2.2.) to remove any reagent deposits in the aspiration tube and cuvette. If carryover is expected or observed during a run, the Alkaline EDTA Rinse (4.2.2.) can be scheduled after every sample cup in the method parameters. The solution is placed in a reagent vessel in position 1 of the reagent tray and is called Control 1 in the sample sequence.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

10.1. Review the Laboratory Health and Safety Plan available on the Laboratory Internet site. Choose the 'Operations' menu and select 'Health and Safety'.

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

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

10.4. Instrument and sample waste is flushed down the drain with plenty of water.

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 2.0, August 1993.

11.2. Seal Analytical AQ2 method EPA-137-A Rev.1.

11.3. AQ2 Operator Handbook and AQ Software guide.

11.4 Quality Manual, Bureau of Laboratories, DEP.

11.5. Laboratory Health and Safety Plan.

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

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

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

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

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

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

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

12. TECHNICAL SUPPORT CONTACTS

Seal Analytical, Inc.
Mequon Technology Center
10520-C Baehr Rd.
Mequon, WI 53092
Phone: 262-241-7900
Fax: 262-241-7970
www.seal-analytical.com

Appendix of Significant Changes

12/01/2010 Changed MDL level to 0.002 mg N/L and PQL to 0.005 mg N/L. Made changes to reflect the use of all three Seal AQ2 instruments.

11/29/2011 A section was added to list the chemicals used for the Nitrite analysis.

11/19/2012 A troubleshooting section was added to help restore/maintain run quality using an alkaline/EDTA wash procedure.

11/05/2015 Data archival information was clarified in Section 7.

12/27/2018 Section 4 is modified to include a reference to new storage location for certificates of analysis. In Section 8, added a reference to use of the PQL level standard for the ongoing MDL and PQL verification data collection. Section 10 waste disposal procedure is clarified.

02/13/2020 Added control limits for the blank calibration standard.

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