Parameter: Nitrite Nitrogen  
Method: SM 4500-NO₂⁻ B-2000 (Aqueous)  
Manual Spectrophotometric

AUDITOR GUIDE

EQUIPMENT:
- Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer. **Model:**
- Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm. **Model:**
- Filtration apparatus, for use with 0.45-µm-pore-diam membrane filters.

ANALYSIS REAGENTS:
- Nitrite-free water
- Color reagent
- Sodium oxalate, 0.025M (0.05N) [Na₂C₂O₄]
- Hydrochloric acid, 1N
- Ferrous Ammonium Sulfate, 0.05M (0.05N)
- Stock Nitrite Solution, 1.00 ml = 250 µg N
- Standard Potassium Permanganate titrant, 0.05N
- Ammonium hydroxide, 1N

PLEASE COMPLETE CHECKLIST IN INDELIBLE INK
Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

<table>
<thead>
<tr>
<th>GENERAL</th>
<th>LAB</th>
<th>SOP</th>
<th>EXPLANATION</th>
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</table>
| 1 | What is the most recent review/revision date of the SOP? [15A NCAC 2H .0805 (a) (7)] | | Date:  
Verify proper method reference. During review notate deviations from the approved method and SOP. Recommend an annual review. Update SOPs any time changes are made to procedure and make a list or highlight any changes that were made to methodology. |
| 2 | Is there North Carolina data available for review? | | If not, review PT data. |
| PRESERVATION and STORAGE | LAB | SOP | EXPLANATION |
| 3 | Are samples iced to above freezing but ≤ 6 ºC during shipment? [40 CFR 136.3 Table II and footnote 18] | | 40 CFR footnote 2 allows 15 minutes for sample preservation, including thermal. This means that if a sample is received in the lab within 15 minutes it is not required to be on ice. Document temperature downward trend for short transport samples. |
| 4 | Are samples refrigerated above freezing to 6°C during storage? [40 CFR 136.3 Table II and footnote 18] | | |
| 5 | Are samples analyzed within 48 hours of collection? [40 CFR 136.3 Table II] | | |
| PROCEDURE –Calibration | LAB | SOP | EXPLANATION |
| 6 | Is a standard curve constructed by plotting absorbance of standards against NO₂⁻ -N concentration? [SM 4500- NO₂⁻ B-2000 (5)] | | Prepare a standard curve by plotting absorbance of standards against NO₂⁻ -N concentration. Compute sample concentration directly from curve. |
| 7 | List the values of standards used for the calibration: [15A NCAC 2H .0805 (a) (7) (I)] | | For analytical procedures requiring analysis of a series of standards, the concentrations of these standards must bracket the concentration of the samples analyzed. One of the standards must have a concentration equal |
Is a minimum correlation coefficient of 0.995 achieved for calibration curves? [NC WW/GW LC Policy]

When linear regression is used, use the minimum correlation coefficient specified in the method. If the minimum correlation coefficient is not specified, then a minimum value of 0.995 (or a coefficient of determination, r², of 0.99) is required.

### PROCEDURE – Sample Preparation

<table>
<thead>
<tr>
<th>LAB</th>
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<tbody>
<tr>
<td>9</td>
<td>Are samples filtered to remove suspended solids? [SM 4500-NO₂⁻ B-2000 (1) (b) and (4) (a)]</td>
<td>Remove suspended solids by filtration. If sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter. NOTE: If this is required, a filtered blank and laboratory fortified blank must also be analyzed.</td>
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<tr>
<td>10</td>
<td>Is sample pH adjusted to between 5 and 9, when outside this range? [SM 4500- NO₂⁻ B-2000 (4) (b)]</td>
<td>If sample pH is not between 5 and 9, adjust to that range with 1N HCl or NH₄OH as required.</td>
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### PROCEDURE – Sample Analysis

<table>
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<tr>
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<tbody>
<tr>
<td>11</td>
<td>What volume of sample is analyzed? [SM 4500- NO₂⁻ B-2000 (4) (b)]</td>
<td>To 50.0 ml sample, or to a portion diluted to 50.0 ml, add 2 ml color reagent and mix.</td>
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<tr>
<td>12</td>
<td>Are 2 ml color reagent added to each sample and quality control standard? [SM 4500- NO₂⁻ B-2000 (4) (b)]</td>
<td>To 50.0 ml sample, or to a portion diluted to 50.0 ml, add 2 ml color reagent and mix.</td>
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<tr>
<td>13</td>
<td>Is absorbance of each standard and quality control standard measured at 543 nm between 10 min and 2 hours after adding the color reagent? [SM 4500- NO₂⁻ B-2000 (4) (c)]</td>
<td>Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm.</td>
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### QUALITY ASSURANCE

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<tr>
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<tbody>
<tr>
<td>14</td>
<td>Is each new analyst required to perform an initial demonstration of capability prior to analyzing samples? [SM 4020 B. (1) (a) - 2011]</td>
<td>Before new analysts run any samples, verify their capability with the method. Run a laboratory-fortified blank (LFB) (4020B.2e) at least four times and compare to the limits listed in the method.</td>
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<tr>
<td>15</td>
<td>Is the minimum reporting level (MRL) verified initially and at least quarterly (preferably daily) by analyzing a QC sample (subjected to all sample-preparation steps) spiked at a level 1 to 2 times the MRL? [SM 4020 B. (1) (c) - 2011]</td>
<td>Verify quantitation at the MRL initially and at least quarterly (preferably daily) by analyzing a QC sample (subjected to all sample-preparation steps) spiked at a level 1 to 2 times the MRL. A successful verification meets the method’s or laboratory’s accuracy requirements at the MRL. Laboratories must define acceptance criteria for the operational range — including the MRL — in their QA documentation.</td>
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<tr>
<td>16</td>
<td>Is each calibration point back-calculated and evaluated against established criteria? [SM 4020 B. (2) (a) - 2011]</td>
<td>Back calculate the concentration of each calibration point. The back-calculated and true concentration should agree within ±10%, unless different criteria are specified in an individual method. At the lower limit of the operations a range, acceptance criteria are usually wider. Such criteria must be defined in the laboratory’s QA plan.</td>
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<td></td>
<td>Question</td>
<td>Answer</td>
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<td>17</td>
<td>Is at least one method blank analyzed with each batch of 20 or fewer samples? [SM 4020 B. (2) (d) - 2011]</td>
<td>The reagent/method blank is treated exactly like standards and samples. Include at least one MB daily or with each batch of 20 or fewer samples, whichever is more frequent.</td>
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<td>18</td>
<td>Is the method blank concentration less than or equal to ½ the reporting limit? [NC WW/GW LC Policy]</td>
<td>For analyses requiring a calibration curve, the concentration of reagent/method and calibration blanks must not exceed 50% of the reporting limit, unless otherwise specified by the reference method.</td>
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<td>19</td>
<td>What corrective action is taken if the method blank is not acceptable? [15A NCAC 2H .0805 (a) (7) (F)]</td>
<td>Our Rule requires corrective action any time quality control results indicate a problem. SM states: If any MB measurements are at or above the reporting level, take immediate corrective action as outlined in Section 1020 B.5. This may include re-analyzing the sample batch.</td>
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<tr>
<td>20</td>
<td>Is a calibration verification standard (mid-range) analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift? [NC WW/GW LC Policy]</td>
<td>The calibration blank and calibration verification standard (mid-range) must be analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift. If either fall outside established quality control acceptance criteria, corrective action must be taken (e.g., repeating sample determinations since the last acceptable calibration verification, repeating the initial calibration, etc.). SM states: Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically, after each batch of ten samples and at the end of the run. The calibration verification standard's analyte concentration should be varied over the calibration range to determine detector response.</td>
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<td>21</td>
<td>What is the acceptance criterion for the calibration verification standard? [SM 4020 B. (2) (b) - 2011]</td>
<td>Results must not exceed ±10% of its true value and calibration blank results must not be &gt; one-half the reporting level (unless the method specifies otherwise).</td>
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<tr>
<td>22</td>
<td>Does the laboratory take appropriate corrective action if the calibration verification standard result exceeds ±10% of the true value? [SM 4020 B. (2) (b) - 2011]</td>
<td>SM states: If the calibration verification fails, immediately cease analyzing samples and initiate corrective action. Then, re-analyze the calibration verification. If the calibration verification passes continue the analysis. Otherwise, repeat initial calibration and reanalyze samples run since the last acceptable calibration verification.</td>
</tr>
<tr>
<td>23</td>
<td>Is a calibration blank analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift? [NC WW/GW LC Policy]</td>
<td>The calibration blank and calibration verification standard (mid-range) must be analyzed initially (i.e., prior to sample analysis), after every tenth sample and at the end of each sample group to check for carry over and calibration drift. If either fall outside established quality control acceptance criteria, corrective action must be taken (e.g., repeating sample determinations since the last acceptable calibration verification, repeating the initial calibration, etc.).</td>
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<tr>
<td>24</td>
<td>What is the acceptance criterion for the calibration blank? [SM 4020 B. (2) (b) - 2011]</td>
<td>Calibration blank results must not be &gt; one-half the reporting level (unless the method specifies otherwise).</td>
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</tbody>
</table>
|25 | Does the laboratory take appropriate corrective action if the calibration blank results are greater than one-half the reporting level? [SM 4020 B. (2) (b) - 2011] | SM states: If the calibration verification fails, immediately cease analyzing samples and initiate corrective action. Then, re-analyze the calibration verification. If the calibration verification passes continue the analysis. Otherwise, repeat initial calibration and reanalyze samples run since the last acceptable calibration verification.
Does the laboratory analyze a laboratory-fortified blank (LFB) at least daily or per batch of 20 or fewer samples? [SM 4020 B. (2) (e) - 2011]

List value(s) and acceptance criterion of standard used.

The LFB may be either a primary or secondary source standard so it may serve dual roles.

If the LFB is primary source, it may be equivalent to the CVS (refer to question #25). Analyze at least one daily or per batch of 20 or fewer samples. Use control charts to establish limits or default to the CVS acceptance criterion of ±10%.

If the LFB is secondary source, it may be equivalent to the second source standard (refer to question #12). Analyze one daily or per batch of 20 or fewer samples. Use control charts to establish limits.

**SM states:** LFBs (and LFM) do not have to be made from a second source (unless the method specifies otherwise) as long as each initial calibration solution is verified via a second source. Ideally, vary LFB concentrations to cover the range from the midpoint to the lower part of calibration curve, including the reporting limit. Include one LFB daily or per each batch of 20 or fewer samples. Calculate %recovery, plot control charts and determine control limits for the LFB unless otherwise specified in the method.

What corrective action is taken if the LFB recovery is outside established control limits? [15A NCAC 2H .0805 (a) (7) (F)]

Our Rule requires corrective action any time quality control results indicate a problem. **SM states:** Establish corrective actions to take if the LFB does not satisfy acceptance criteria.

**SM states:** Laboratory fortified matrix is the same as a matrix spike; that is, a spiked sample. **Note:** No option to perform an environmental sample duplicate and then spike separately – must perform MS/MSD for this method. **SM states:** Include at least one LFM/LFMD daily or with each batch of 20 or fewer samples.

**SM states:** To prepare an LFM, add a known concentration of analytes (ideally from a second source) to a randomly selected routine sample without increasing its volume by more than 5%. Ideally the new concentration should be at or below the midpoint of the calibration curve, and for maximum accuracy, the spike should approximately double the sample’s original concentration. If necessary, dilute the spiked sample to bring the measurement within the calibration curve. Also rotate the range of spike concentrations to verify performance at various levels. If the spike solution contribution to the fortified sample is kept to 1% or less, a spike dilution correction does not have to be calculated.
What is the acceptance criterion for LFM/LFMD recovery? [SM 4020 B. (2) (g) -2011]

Will have two % recovery calculations for accuracy from spike recoveries and one RPD calculation for precision from duplicate calculation (see question 41).

**SM states:** Calculate recovery limits and RPD, and plot control charts to determine acceptance criteria.

What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for accuracy? [15A NCAC 2H .0805 (a) (7) (F)]

**Our Rule requires corrective action any time quality control results indicate a problem. Compare to LFB result and other QC. Reanalyze LFM. If it still fails, qualify the spiked sample result.**

**SM states:** Establish corrective actions to be taken if the LFM does not satisfy acceptance criteria.

What is the acceptance criterion for LFM/LFMD for precision (i.e., relative percent difference)? [SM 4020 B. (2) (g) -2011]

**SM states:** Calculate percent recovery and relative percent difference, plot control charts (unless method specifies acceptance criteria) and determine control limits for spikes at different concentrations. Ensure the method’s performance criteria are satisfied.

**Bottom line:** We are not requiring control charts but will instead accept a system of trend analysis. That is, the lab’s monitoring of the trends in the data. 40 CFR part 136.7 (viii) states: Control charts (or other trend analysis of quality control results).

What corrective action does the laboratory take if the LFM/LFMD results are outside of established control limits for precision? [15A NCAC 2H .0805 (a) (7) (F)]

**Our Rule requires corrective action any time quality control results indicate a problem.**

**SM states:** Ensure the method’s performance criteria are satisfied.

Is the data qualified on the Discharge Monitoring Report (DMR) or client report if Quality Control (QC) requirements are not met? [NC WW/GW LC policy]

When quality control (QC) failures occur, the laboratory must determine the source of the problem and apply corrective action. Part of the corrective action is notification to the end user. If data qualifiers are used to qualify samples not meeting QC requirements, the data may not be useable for the intended purposes. It is the responsibility of the laboratory to provide the client or end-user of the data with sufficient information to determine the usability of the qualified data.

Nitrite-free water: If it is not known that the distilled or demineralized water is free from NO₂⁻, use either of the following procedures to prepare nitrite-free water.

1. Add to 1L distilled water one small crystal each of KMnO₄ and either Ba(OH)₂ or Ca(OH)₂. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 ml of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent indicates the presence of permanganate.

2. Add 1 ml conc. H₂SO₄ and 0.2 ml MnSO₄ solution (36.4 g MnSO₄·H₂O/100 ml distilled water) to each 1 L distilled water, and make pink with 1 to 3 ml KMnO₄ solution (400 mg KMnO₄/L distilled water). Redistill as described in the preceding paragraph.

Color reagent: To 800 ml water add 100 ml 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

Sodium oxalate, 0.025M (0.05N): Dissolve 3.350 g Na₂C₂O₄, primary standard grade, in water and dilute to 1000 ml.

Ferrous Ammonium Sulfate, 0.05M (0.05N): Dissolve 19.607 g Fe(NH₄)₂(SO₄)₂·6H₂O plus 20 ml conc H₂SO₄ in water and dilute to 1000 ml. Standardize.
Stock nitrite solution: Commercial reagent-grade NaNO$_2$ assays at less than 99%. Because NO$_2^-$ is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine NaNO$_2$ content, add a known excess of standard 0.05N KMnO$_4$ solution, discharge permanganate color with a known quantity of standard reductant such as 0.025M Na$_2$C$_2$O$_4$ or 0.05M Fe(NH$_4$)$_2$(SO$_4$)$_2$-6H$_2$O, and back-titrinate with standard permanganate solution.

1. Preparation of stock solution – Dissolve 1.232 g NaN$_O_2$ in water and dilute to 1000 ml; 1.00 ml = 250 µg N. Preserve with 1 ml CHCl$_3$ (chloroform).

2. Standardization of stock nitrite solution – Pipet, in order, 50.00 ml standard 0.05N KMnO$_4$, 5 ml conc H$_2$SO$_4$, and 50.00 ml stock NO$_2^-$ solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while adding stock NO$_2^-$ solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient 10-ml portions of standard 0.025M Na$_2$C$_2$O$_4$. Titrate excess Na$_2$C$_2$O$_4$ with 0.05N KMnO$_4$ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below. NOTE: If ferrous ammonium sulfate solution is substituted for Na$_2$C$_2$O$_4$, omit heating and extend reaction period between KMnO$_4$ and Fe$^{2+}$ to 5 min before making final KMnO$_4$ titration.

3. Calculate NO$_2^-$ -N content of stock solution by the following equation:

$$A = \left(\frac{(B \times C) - (D \times E)}{F}\right) \times 7$$

Where:
- $A$ = mg NO$_2^-$ -N/ml in stock NaNO$_2$ solution.
- $B$ = total ml standard KMnO$_4$ used.
- $C$ = normality of standard KMnO$_4$.
- $D$ = total mL standard reductant added.
- $E$ = normality of standard reductant, and
- $F$ = ml stock NaNO$_2$ solution taken for titration.

Each 1.00 ml 0.05N KMnO$_4$ consumed by the NaNO$_2$ solution corresponds to 1725 µg NaNO$_2$ or 350 µg NO$_2^-$ -N.

Intermediate nitrite solution: Calculate the volume, $G$ of stock NO$_2^-$ solution required for the intermediate NO$_2^-$ solution from $G = 12.5/A$. Dilute the volume $G$ (approximately 50 ml) to 250 ml with water; 1.00 ml = 50.0 µg N. Prepare daily.

Standard nitrite solution: Dilute 10.00 ml intermediate NO$_2^-$ solution to 1000 ml with water; 1.00 ml = 0.500 µg N. Prepare daily.

Standard potassium permanganate titrant, 0.05N: Dissolve 1.6 g KMnO$_4$ in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure:

Weigh to the nearest 0.1 mg several 100- to 200-µg samples of anhydrous Na$_2$C$_2$O$_4$ into 400-ml beakers. To each beaker, in turn, add 100 ml distilled water and stir to dissolve. Add 10 ml 1 + 1 H$_2$SO$_4$ and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 ml solution. Run a blank on distilled water and H$_2$SO$_4$.

$$\text{Normality of KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.067}$$

Where: $A$ = ml titrant for sample, and
- $B$ = ml titrant for blank.

Average the results of several titrations.

Revised 08/2016
Additional Comments:

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