NITRITE NITROGEN TEST KIT METHOD

Thermo Orion Method AC2046

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1.0 Scope and Applications

1.1 This method is a convenient packaging of the technology in "Standard Methods" 4500-NO_2^- B Colorimetric Method *(Ref 16.1*), and in EPA method 354.1 (*Ref 16.2.)* to determine nitrite ion in waters. Nitrite nitrogen (see *Definitions, Section 3.1*) is CAS Registry number 7727-37-9.

1.2 The method is applicable to drinking, surface and saline waters, domestic and industrial wastes in the range of 0.05-0.50 mg NO_2-N/L. Where sample results exceed the applicable range of the method, the sample must be diluted to within the applicable range and reanalyzed.

1.3 This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

1.4 The MDL for this method was determined to be 0.009 mg NO_2-N/L, using an AQUAfast II Colorimeter and a Thermo Orion AC2046 Test Kit. This value was derived from the analysis of nine aliquots with a concentration of 0.06 mg/L mg NO_2-N/L.

1.5 Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the quality control procedure in Section 9.2.

2.0 Summary of Method

This method uses one reagent tablet for the reaction. An intermediate compound is formed by first reacting nitrite ion with sulfanilamide at the appropriate pH to form a diazonium salt, and this salt is then coupled with N-(1-naphthyl)-ethylenediamine dihydochloride to produce a highly colored azo dye. The absorbance of the dye is measured at 528 nm.

3.0 Definitions

3.1 Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate, and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. It generally is more stable in neutral or alkaline solutions.

3.1.1 Unless noted otherwise, nitrite units are in nitrite as N.

3.2 Material Safety Data Sheet (MSDS)- Written information provided for each chemical reagent or standard about a chemical's toxicity, health hazards, physical properties, flammability, and reactivity. It also includes storage, spill, and handling precautions.

3.3 Method Detection Limit (MDL)-The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.4 Calibration Blank (CB)- A volume of reagent water fortified with the same matrix as the calibration standard, but without the analytes, internal standards or surrogate analytes.

3.5 Calibration Standard (CAL)- A solution prepared from the primary dilution standard or stock standards and the internal standards and surrogate analytes. Used to calibrate the instrument response with respect to analyte concentration.

3.6 Instrument Performance Check Solution (IPC)- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.7 Laboratory Fortified Blank (LFB)- An aliquot of reagent water or other blank matrices to which known quantities of the method analyte is added in the laboratory. The LFB is analyzed exactly like the sample and is used to determine whether the methodology is in control, and if the laboratory is capable of making accurate and precise measurement.

3.8 Laboratory Reagent Blank (LRB)- An aliquot of reagent water or other blank matrices that are treated as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. Use the LRB to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.9 Quality Control Sample (QCS)- A solution of method analytes of known concentration that is used to fortify an aliquot of LRB or sample matrix. Obtain the QCS from a source external to the laboratory that is different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.10 Matrix Spike (MS) An aliquot of an environmental sample, to which a known quantity of the method analyte is added in the laboratory. The MS is analyzed exactly like a environmental sample to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analyte in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations.

3.11 Matrix Spike Duplicate (MSD) An aliquot of the same sample used for the MS, to which the exact same known quantity of the method analyte has been added as for the MS, and which is analyzed separately with the identical procedure. Analysis of the MS and MSD give a measure of the precision associated with the laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.12 Calibration Range The range of concentration of analyte for which the use of the method has been approved and for which the instrument has been pre-programmed with internal calibration, as given in the Method Scope, Section 1.

4.0 Interferences

4.1 Chemical incompatibility makes it unlikely that nitrite, free chlorine, and nitrogen trichloride will coexist. NCl_3 imparts a false red color when color reagent is added. Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. The following ions can interfere under certain conditions by precipitation: antimony (III), iron (III), lead, mercury (I), silver, chloroplatinate, metavanadate.

4.2 Turbidity and sample color are normally compensated for during the “zeroing” step; if excessive turbidity is present, the instrument will indicate “+Err”. Suspended solids in excess of that which can be “blanked out” can be removed by filtering the solution.

5.0 Safety

5.1 Use good laboratory practices throughout the test procedure. Follow the test procedure carefully and observe all precautionary measures.

5.2 An, MSDS, information and assistance may be obtained by calling 1-800-225-1480 or 1-978-232-6000. Alternately, visit the website at www.thermo.com/waterapps. Search on “AC2046”.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of all chemicals. Additional information on laboratory safety can be found in *Ref. 16.3*

6.0 Equipment and Supplies

6.1 Thermo Orion Cat. No. AC2046, Nitrite Test Kit, or equivalent. This kit contains the necessary reagents.

6.2 Photometer, Thermo Orion AQ2046, or equivalent.

6.3 Balance, capable of weighing to the nearest milligram, for preparing standards.

6.4 Volumetric flasks, 1 L, 100 mL.

6.5 Pipettes, 1 and 10 mL.

7.0 Reagents and Standards

7.1 Thermo Orion Cat. No. 954606 0.1 M $NaNO_2$, not included in kit.

8.0 Sample Preservation, Collection and Storage

8.1 Nitrite is not stable in acid solutions, and acid preservation should never be used. Make the determination promptly on fresh samples to prevent bacterial conversion of nitrite to NO_3^- or ammonia. Samples to be reported for wastewater or drinking water compliance monitoring under the Clean Water Act or the Safe Drinking Water Act must be preserved by cooling to 4^o C and analyzed within 48 hours.

8.2 Details on sampling techniques from conduits may be found in *Reference 16.4.*

9.0 Quality Control

9.1 Each laboratory using this method for compliance reporting is required to operate a formal quality control (QC) program *(see Reference 16.5).* The minimum requirements of this program are initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks, fortified samples, duplicates and other laboratory solutions as a continuing performance check. The laboratory must maintain performance records that define the quality of the data that are generated. See Section 17.1 for QC Performance Criteria.

9.2 Initial Demonstration Of Performance

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Calibration Range Verification - The calibration range must be verified at least every six months or whenever a significant change in instrument response is observed. The verification must use a minimum of a blank and three standards that cover the entire calibration range. Any verification data must verify that the instrument is within ±10% of calibration for each concentration used. If any verification data exceeds ±10% of calibration, recalibrate the instrument and repeat the procedure. If the problem is not resolved, call the kit manufacturer for assistance.

9.2.3 Quality Control Sample (QCS)- Obtain an independent quality control standard for nitrite available from commercial sources (preferably verified against American Association for Laboratory Accreditation or National Institute of Standards and Technology reference materials, if available). If needed, dilute the standard according to the directions supplied with the standard to obtain standard concentrations within the calibration range.

When beginning the use of this method, on a quarterly basis, or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within 15% of the stated values, performance is unacceptable. Identify and correct the source of

the problem before proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL)- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values (40 CFR, Part 136, Appendix B), take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times S$$

Where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom]. S is the standard deviation of the replicate analyses. [t= 3.14 for seven replicates]

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB)- The laboratory must analyze at least one LRB with each batch of samples. Perform a reagent blank determination according to Section 11.0, *Procedure,* substituting DI water for the sample. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination may be present and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB)- The laboratory must analyze at least one LFB fortified with 100 μg/L of nitrite with each batch of samples. Calculate accuracy as percent recovery (see Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is out of control. Identify and resolve the problem before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data are available (at least 20-30 analyses), the analyst may develop optional control limits from the

percent mean recovery (x) and the single analyst standard deviation (S) of the mean recovery. Use these data to establish the upper and lower control limits as follows:

Upper Control Limit = x + 3S Lower Control Limit = x - 3S

The optional control limits must be equal to or better than the required control limits of 85-115%.

After each 5-10 new recovery measurements, calculate new control limits using only the most recent 20-30 data points. Also, use the standard deviation (S) data to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC)

For all determinations the laboratory must analyze the IPC (a midrange check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required), and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within + or – 10% .

If the calibration is not within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms the calibration is outside the limits, halt sample analysis, and determine the cause. In the case of drift, recalibrate the instrument. Reanalyze all samples following the last acceptable IPC solution. If the problem is not resolved, call the kit manufacturer for assistance. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 Assessing Analyte Recovery And Data Quality

9.4.1 Matrix Spike and Matrix Spike Duplicate (MS and MSD). The laboratory must add a known amount of analyte to an aliquot of at least 10% of the routine samples. The MS and MSD aliquots must be duplicates of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample level and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated MS/MSD recovery range of 85-115%. In regulated monitoring, the percent relative range (RR) must be calculated using the following equation:

$$RR = 100 \frac{(|C1-C2|)}{(C1+C2)/2}$$

where:
C1 = Concentration of the analyte in the MS sample, and
C2 = Concentration of the analyte in the MSD sample.

The result obtained should be compared with the precision requirement listed in Section 17.1, “QC Performance Criteria”.

9.4.3 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (see Section 9.3), the problem encountered with the LFM is matrix or solution related, not system related.

9.4.4 If reference materials are available, analyze them to provide additional performance data. Analyzing reference samples is valuable for demonstrating the ability to perform the method acceptably.

10.0 Calibration

10.1 General Precautions

10.1.1 Thoroughly clean vials, caps and stir rod after each analysis in order to prevent carry-over errors. Even minute reagent residues lead to incorrect measurements. Use the brush supplied with the kit for cleaning.

10.1.2 Ensure that the outer walls of the vials are dry and clean before performing the analysis. Fingerprints or water droplets on the light entry surfaces of the vials lead to incorrect measurements.

10.1.3 'Zero calibration' and 'Test' must be performed using the same vial, since different vials can possess slightly different tolerances.

10.1.4 For 'Zero calibration' and 'Test', ensure that the vial is always positioned in the sample chamber in such a way that the graduation with the white triangle points toward the marking on the housing.

10.1.5 Always perform 'Zero calibration' and 'Test' with capped vials.

10.1.6 Bubbles on the inside walls of the vial lead to incorrect measurements. To prevent this, cap the vial and remove the bubbles by swirling the vial before performing the test. This is especially important in this test, because of the ten-minute waiting time.

10.1.7 Prevent water from penetrating into the sample chamber. The entry of water into the housing of the photometer can destroy electronic components and lead to corrosion damage.

10.1.8 Soiling of the lens (LED and photo sensor) in the sample chamber leads to incorrect measurements.

10.1.8.1 Check - and if necessary clean - the light entry surfaces of the sample chamber at regular intervals. Clean using a moist cloth and cotton balls.

10.1.9 Always add the reagent tablets to the samples straight from the foil without touching them with fingers.

10.1.10 Major temperature differentials between the photometer and the environment can lead to incorrect measurements, due to the formation of condensate in the area of the lens or on the vial.

10.1.11 Use a 10.0 mL pipette to measure samples

10.2 Zero Calibration

10.2.1 Switch the unit on using the 'power' switch.

10.2.2 The display shows the method.

10.2.3 Fill a clean vial with the sample up to the 10 mL mark, screw the cap on, and place in the sample chamber with the ∇ vial mark aligned with the Δ housing mark.

10.2.4 Press the 'zero/test' key.

10.2.5 The method symbol flashes for approx. 3 seconds, then the message ‘0.0.0’appears. This confirms zero calibration. Remove the vial from the sample chamber.

11.0 Procedure

11.1 Add one NITRITE LR tablet straight from the foil to the 10 mL sample, and crush using a clean stir rod.

11.2 Allow to dissolve completely, cap the vial, and align the Δ and ∇ marks.

11.3 Wait for a color reaction time of *ten minutes.*

11.4 Press the 'zero/test' key. The method symbol flashes for approx. 3 seconds, then the result appears in the display.

12.0 Data Analysis and Calculations

12.1 The meter will read the results in mg/L nitrite as N. To convert to mg/L nitrite as NO_2^-, multiply by 3.3.

12.2 Report results to the precision shown on the meter (2 decimal places).

13.0 Method Performance

13.1 The method detection limit (MDL) study was performed by one analyst, and was determined to be 0.009 mg/L nitrite as N.

13.2 The minimum level (ML) for this method is 0.05 mg/L nitrite as N

13.3 In a single laboratory, a single analyst performed replicate spiked sample analyses on a drinking water sample. The mean recovery for a 0.112 mg/L addition of nitrite as N to a baseline drinking water sample containing 0.024 mg/L of nitrite as N was 93.8%, with a relative standard deviation of 2.8 % for n = 7 replicates.

14.0 Pollution Prevention

14.1 The small quantities of dye and buffers do not contribute significantly to pollution.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to

protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 For further information on waste management, consult "*The Waste Management Manual for Laboratory Personnel*", and "*Less is Better: Laboratory Chemical Management for Waste Reduction*", both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 "Standard Methods for the Examination of Water and Wastewater", Method 4500 NO_2- B, 20th Edition, American Public Health Association, 1015 Fifteenth Street, NW, Washington, D.C. 20005, (1998); pp. 4-112 to 4-114.

16.2 "Methods for Chemical Analysis of Water and Wastes", 3rd Edition, Environmental Protection Agency, Environmental Monitoring Systems Laboratory-Cincinnati (EMSL-Ci), Cincinnati, Ohio 45268, EPA-600/4-79-020, Method 354.1, Storet # Total 00615.

16.3 "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

16.4 "Standard Practices for Sampling Water," ASTM Annual Book of Standards, Part 31, D3370-76, American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

16.5 40 CFR part 136, Appendix A, Methods 1624 and 1625. See also, "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMS-CI, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

17.0 Tables and Validation Data

17.1 QC Performance Criteria

Type	Frequency	Acceptance Criteria
Laboratory Reagent Blank (LRB)	Daily	<0.03 mg/L nitrite as N
Precision (duplicates MS and MSD)	5-10%	RR equal to, or less than, 15% at 0.5 mg/L nitrite as N
Accuracy (LFB or MS/MSD)	5-10%	85-115 % recovery at 0.5 mg/L nitrite as N
Instrument Performance Check (IPC)	Immediately after any calibration; after every 10th sample and at the end of a sample run	90-110% of the initial calibration by the analyst at 0.5 mg/L nitrite as N
Independent Standard (QCS)	Initially, or quarterly, and as required to meet data quality needs	85-115% recovery at 0.5 mg/L nitrite as N