Hach Company TNTplus™ Nitrite – Spectrophotometric Measurement of Nitrite in Water and Wastewater

Hach Company TNTplus™ 839 Nitrite Method 10207

Revision 1.0
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Spectrophotometric Measurement of Nitrite in Water and Wastewater

1.0 Scope and Application

1.1 This method is applicable to the determination of nitrite in drinking water, surface water, and wastewater.

1.2 The method is applicable in the range from 0.01 to 0.60 mg NO_2-N /L.

2.0 Summary of Method

2.1 The diazonium compound formed by diazotization of sulfanilic acid by nitrite in water under acidic conditions is coupled with 8-amino-2-napthalene sulfonic acid to produce a reddish-purple color, which is read in a spectrophotometer at 515 nm.

3.0 Interferences

3.1 There are few known interferences at concentrations less than 1,000 times that of the nitrite; however, the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations. High alkalinity (>600 mg/L) will give low results due to a shift in pH.

4.0 Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. It is suggested that the laboratory perform personal hygiene monitoring of each analyst using this method and that the results of this monitoring be made available to the analyst.

4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.

4.3 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 any chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 15.4-15.5.

5.0 Equipment

Note: *Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

5.1 Sampling equipment

5.1.1 Sample collection bottles—Glass, approximately 1-L, with PTFE-lined screw cap.
Note: *In those instances necessitating collection of a smaller aliquot, a smaller sample container may be used.*

5.1.2 Cleaning

5.1.2.1 Bottles—Detergent water wash, tap water rinse, cap with aluminum foil, and bake at 110°C for 1 h minimum prior to use.

5.1.2.2 Liners for screw caps—Detergent water wash, tap water rinse, and bake at 110°C for 1 h minimum prior to use.

5.1.3 Bottles and liners must be lot-certified to be free of artifacts by running laboratory blanks according to this method (per Section 10). If blanks from bottles and/or liners without cleaning or with fewer cleaning steps than required above show no detectable materials, the bottle and liner cleaning steps may be omitted.

5.2 Equipment for glassware cleaning

5.2.1 Oven—Capable of maintaining a temperature within ± 5°C in the range of 100–250°C.

5.3 Equipment for sample analysis

5.3.1 Hach DR 5000, DR 3800, or DR 2800 spectrophotometer.

5.4 Equipment for calibration

5.4.1 Analytical balance – Capable of weighing 0.1 mg.

5.4.2 Volumetric flask – Glass, 1000-mL.

5.4.3 Volumetric flask – Glass, 50-mL.

5.4.4 Volumetric pipette – glass, assorted sizes.

6.0 Reagent and Standards

6.1 Reagent water – Water in which nitrite is not detected at or above the method level of this method. Bottled distilled water, or water prepared by passage of tap water through ion exchange and activated carbon have been shown to be acceptable sources of reagent water.

6.2 Hach Company TNTplus Nitrite Kit (TNT839, 0.01 – 0.60 mg/L NO_2-N).

6.3 Chloroform, distilled in glass.

6.4 Sodium nitrite, reagent grade

6.5 Quality control stock solution.

6.5.1 Dissolve 0.5007 g of dried anhydrous sodium nitrite (24 hours in desiccator) in reagent water and dilute to 1000 mL. Preserve with 2 mL chloroform per liter. 1.0 mL = 0.102 mg NO_2-N

6.6 Quality control standard spiking solution

6.6.1 Dilute 50.0 mL of the stock solution to 1000 mL. 1.0 mL = 0.005 mg NO_2-N.

6.7 Method detection limit solution

6.7.1 Prepare 7 or more replicate MDL solutions by diluting 0.10 mL of standard spiking solution to 50 mL. Final concentration = 0.01 mg/L NO_2-N.

6.8 Initial precision and recovery solution

6.8.1 TNT839: 0.01 – 0.60 mg/L NO_2-N.

6.8.1.1 Prepare 4 or more replicate IPR solutions by diluting 1.0 mL of standard spiking solution (Section 6.6.1) to 50 mL. Final concentration = 0.10 mg/L NO_2-N.

6.9 On-going precision and recovery

6.9.1 TNT839: 0.01 – 0.60 mg/L NO_2-N.

6.9.1.1 Prepare 1 or more solutions by diluting 1.0 mL of standard spiking solution (6.6.1) to 50 mL. Final concentration = 0.10 mg/L NO_2-N.

7.0 Sample Collection Preservation and Storage

7.1 Samples should be analyzed as soon as possible. They may be stored for 24 to 48 hours at 4^0C.

8.0 Quality Control

8.1 It is recommended that each laboratory that uses this method be required to operate a formal quality assurance program (Reference 15.1). The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analyses of laboratory prepared water standards as a test of continued performance to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery sample that the analysis system is in control. This procedure is described in Sections 8.3.

8.1.2 Accompanying QC for the determination of nitrite is required per analytical batch. An analytical batch is a set of samples processed during a contiguous 8-hour period. Each analytical batch must be accompanied by an ongoing precision and recovery sample, matrix spike sample, and matrix spike duplicate sample resulting in a minimum of four analyses (1 OPR, 1 sample, MS, and MSD).

8.2 Initial demonstration of laboratory capability.

8.2.1 To establish the ability to detect nitrite the analyst shall determine the MDL and ML per the procedure in 40 CFR 136, Appendix B (Reference 15.3) using the apparatus, reagents, and standards that will be used in the practice of this method. An achieved MDL and ML less than or equal to the MDL in Section 12.0 is recommended prior to the practice of this method.

8.2.2 Prepare and measure seven replicates of the MDL standard according to the procedure beginning in Section 6.7.

8.2.3 Initial precision and recovery (IPR) - To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

8.2.3.1 Prepare and measure four samples of the IPR standard according to the procedure beginning in Section 6.8.

8.2.3.2 Using the results of the set of four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (*s*) for nitrite. Use the following equation for calculation of the standard deviation of the percent recovery:

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

where:

n = Number of samples
x = % recovery in each sample

8.2.3.3 Compare *s* and x with the corresponding limits for initial precision and recovery in Table 1. If *s* and x meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, *s* exceeds the precision limit or x falls outside the range for recovery, system performance is unacceptable. In this event correct the problem, and repeat the test.

8.3 Ongoing precision and recovery - To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

8.3.1 Prepare a precision and recovery standard with each analytical batch according to the procedure beginning in Section 6.9.

8.3.2 At the end of each analytical batch of samples, analyze a precision and recovery standard and compare the concentration recovery with the limits for ongoing precision and recovery in Table 3. If the recovery is in the range specified, measurement process is in control and analysis of samples may proceed. If, however, the recovery is not in the specified range, the analytical process is not in control. In this event, correct the problem, re-analyze analytical batch, repeating the ongoing precision and recovery test.

8.3.3 The laboratory should add results that pass the specification in Section 12.0 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of the percent recovery (sr). Express the accuracy as a recovery interval from R - 2sr to R + 2sr. For example, if R = 95% and sr = 5%, the accuracy is 85% to 105%.

8.4 Depending upon specific program requirements, field replicates may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9.0 Calibration and Standardization

9.1 The Hach Company DR series spectrophotometers have a built-in calibration that is automatically used when the TNTplus 839 Nitrite sample vial is placed in the cell holder of the instrument. No further initial calibration is required. However, the instruments have the capability of developing a user-calibration. See manufacturer's manual for instructions.

10.0 Procedure

10.1 Instrument Setup – follow the instrument manufacturer's instructions for instrument setup.

10.2 Sample Preparation

10.2.1 Adjust sample pH to 3 – 10.

10.2.2 Sample temperature should be 15 – 25^{0}C.

10.2.3 Remove the protective foil lid from the DosiCap^{TM} *Zip*. Unscrew the cap from the vial.

10.2.4 Carefully pipet 2.0 mL of sample into the vial.

10.2.5 Flip the DosiCap *Zip* over so that the reagent side faces the vial. Screw the cap tightly onto the vial.

10.2.6 Shake the capped vial until reagent in DosiCap *Zip* has dissolved.

10.2.7 Allow the reagents and sample to react for 10 minutes.

10.2.8 Place test vial into spectrophotometer and read result.

11.0 Data Analysis and Calculations

11.1 Nitrite concentration is calculated automatically against internal instrument calibration.

12.0 Method Performance

Acceptance Criterion	Section	Limit
Method Detection Limit	8.2.1	0.004 mg/L NO_2-N
Method Limit	8.2.1	0.01 mg/L NO_2-N
Initial Accuracy	8.2.3	101%
Initial Precision	8.2.3	0.9%
On-going Accuracy	8.3.2	98%

13.0 Pollution Prevention

13.1 Follow guidelines in Section 14.

14.0 Waste Management

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

14.2 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 Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 References

15.1 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-CI, Cincinnati, OH 45268, EPA-600-4-79-019, March 1979.

15.2 Standard Methods for the Examination of Water and Wastewater, 20th Edition, (1998).

15.3 40 CFR 136, Appendix A, B.

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

15.5 "Safety in Academic Chemistry Laboratories," American Chemical Society, Committee on Chemical Safety, 3rd Edition, 1979.

15.6 "Water Analysis Handbook", 5th Edition, Hach Company (2008).

16.0 Tables

16.1 Acceptance Criteria for Performance tests – The QC performance criteria for this method was performed with a Hach Company DR 5000 spectrophotometer using Hach Company TNT839 Nitrite Kit.

Table 1. Initial Precision and Recovery Method Performance

IPR Concentration	Average Recovery (%)	Standard Deviation (%)
0.10 mg/L NO_2-N	101	0.9

Table 2. Method Detection Limit and Method Limit Performance

MDL Test Concentration	MDL	ML
0.01 mg/L NO_2-N	0.004 mg/L NO_2-N	0.01 mg/L NO_2-N

Table 3. On-going Recovery Performance

OPR Concentration	Average % Recovery
0.10 mg/L NO_2-N	98%

17.0 Glossary of Definitions and Purposes

The definitions and purposes are specified to this method but have been conformed to common usage as much as possible.

17.1 Units of weight and measure and their abbreviations

17.1.1 Symbols

°C degrees Celsius

17.1.2 Alphabetical characters

mg/L milligram per liter

17.2 Definitions, acronyms, and abbreviations

17.2.1 MDL: Method detection limit

17.2.2 ML: Method limit

17.2.3 IPR: Initial precision and recovery

17.2.4 OPR: On-going precision and recovery

17.2.5 MS: Matrix spike

17.2.6 MSD: Matrix spike duplicate

Side-by-side Method Comparison

	EPA 354.1/4500-NO_2-B	Hach TNTplus Nitrite (TNT839)
Scope and Application	0.01 –1.00 mg NO_2-N/L	0.01 – 0.60 mg NO_2-N/L
Summary of Method	The diazonium compound formed by diazotization of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-napthyl) ethylenediamine dihydrochloride to produce a reddish-purple color, which is read in a spectrophotometer.	The diazonium compound formed by diazotization of sulfanilic acid by nitrite in water under acidic conditions is coupled with 8-Amino-2-napthalene sulfonic acid to produce a reddish-purple color, which is read in a spectrophotometer.
Interference	There are few known interferences at concentrations less than 1000 times that of nitrite; however, the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations. High alkalinity (>600 mg/L) will give low results due to a pH shift.	There are few known interferences at concentrations less than 1000 times that of nitrite; however, the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations. High alkalinity (>600 mg/L) will give low results due to a pH shift.
Equipment	Spectrophotometer	Spectrophotometer
Sample Handling/ Preservation	Samples should be analyzed as soon as possible. They may be stored for 24 to 48 hours at 4°C.	Samples should be analyzed as soon as possible. They may be stored for 24 to 48 hours at 4°C.
Reagents and Standards	Hydrochloric acid (source of acidity) Sulfanilamide (diazotium reagent) N-(1-napthyl) ethylenediamine dihydrochloride (chromophore) Sodium acetate (buffer)	Citric acid (source of acidity) Sulfanilic acid (diazonium reagent) di-Sodium hydrogen phosphate (buffer) 8-Amino-2-napthalene sulfonic acid (chromophore) Chlorhexidine diacetate (preservative)
Method Performance	**Precision and accuracy** EPA Method 354.1 - Not Provided Standard Method 4500-NO_2-B Single laboratory wastewater – standard deviations of four wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg NO_3^- + NO_2^- - N/L were ± 0.005, 0.004, 0.005, and 0.01, respectively. Single laboratory wastewater – recoveries of three wastewater samples at concentrations of 0.24, 0.55, and 1.05 mg NO_3^- + NO_2^- - N/L were 100%, 102%, and 100% respectively. **Method Detection Limit** Not provided	**Precision and accuracy** IPR Mean Recovery (0.10 mg NO_2-N/L spike) = 100% (0% bias) IPR Stdev. – 1.5% IPR RSD – 1.5% Effluent #1 (Loveland, CO) Average Matrix Spike Recovery (0.10 mg NO_2-N /L spike) = 104% % Stdev. – 0.76 Effluent #2 (Boston, MA) Average Matrix Spike Recovery (0.10 mg NO_2-N /L spike) = 106% (+6% bias) % Stdev. – 1.26 Effluent #3 (Ventura, CA) Average Matrix Spike Recovery (0.10 mg NO_2-N /L spike) = 103 % Stdev. – 1.51 **Method Detection Limit** MDL – 0.004 mg NO_2-N/L (0.01 mg NO_2-N/L spike) ML – 0.01 mg NO_2-N/L

Final Ratios of Reagents to Sample mg:mL	HCl – 0.3 **mmoles** of acidity Sulfanilamide – 0.04 N-(1-napthyl)ethylenediamine dihydrochloride – 0.04 Sodium acetate – 10.9 mg	Citric acid – 0.4 **mmoles** of acidity Sulfanilic acid – 0.65 8-Amino-2napthylene sulfonic acid – 1.1 di-sodium hydrogen phosphate – 7