
Hach Company TNTplus 835/836 Nitrate Method 10206

**Spectrophotometric Measurement of Nitrate in Water
and Wastewater**

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Revision 2.1
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Spectrophotometric Measurement of Nitrate in Water and Wastewater

1.0 Scope and Application

- 1.1 These procedures cover the determination of nitrate in surface water and domestic and industrial wastes.
- 1.2 The method is applicable in the range from 0.20 to 35.0 mg/L $\text{NO}_3^- \text{ N}$.
- 1.3 This method is equivalent or better in performance to SM 4500- $\text{NO}_3^- \text{ E}$, EPA 353.2, and EPA 300.0, and is EPA approved for Clean Water Act purposes for regulatory reporting of nitrate and nitrate-nitrite (*Federal Register/Vol.82, No. 165/Monday, August 28, 2017/Rules and Regulations*).

2.0 Summary of Method

- 2.1 The Hach TNTplus Nitrate chemistry follows classical electrophilic aromatic substitution in that nitrate in the presence of sulfuric acid yields a nitronium ion ($^+\text{NO}_2$) and HSO_4^- . Nitronium ions are electrophiles that attack the aromatic ring of the dimethylphenol reagent to form intermediate nitro-carbonium ions. The basic HSO_4^- ion then extracts a hydrogen ion from the nitro-carbonium intermediate to yield a stable substitution product (o, or p-nitro-dimethylphenol). The nitrodimethylphenol product is a highly colored (directly related to the nitro functional group), quantifiable by its visible absorption spectra. Test results are measured at 345 nm.

3.0 Interferences

- 3.1 The items listed in the *Interfering substances* table have been individually checked up to the given concentrations and do not cause interference. The cumulative effects and influence of other ions have not been determined. High loads of oxidizable organic substances cause the reagent to change color and to give high-bias results. The test can thus only be used for wastewater analyses if the chemical oxygen demand (COD) is less than 500 mg/L. Measurement results can be verified using sample dilutions or standard additions.
- 3.2 Nitrite concentrations of more than 2.0 mg/L interfere (high-bias results). Add 50 mg of sulfamic acid (amidosulfonic acid) to 5.0 mL of sample, dissolve and wait for 10 minutes. Analyze the prepared sample as described in the procedure above.

Interfering substance	Interference level (mg/L)	Interfering substance	Interference level (mg/L)
Ag^+	100	Cu^{2+}	50
Cl^-	500	Ca^{2+}	50
Fe^{3+}	50	NO_2^-	2
K^+	500	Cd^{2+}	50
Na^+	500	Sn^{2+}	50
Ni^{2+}	50	Cr^{6+}	5

Pb ²⁺	50	Fe ²⁺	10
Zn ²⁺	50	Co ²⁺	10

3.3 Residual chlorine does not cause an interference with this method.

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 Sections 16.3 and 16.4.

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 – Preferably use polyethylene bottles for collecting and storing samples for nitrate analysis. Glass bottles are satisfactory if previously they have not contained high-nitrate solutions.

5.1.2 Cleaning

5.1.2.1 All glassware used should be washed with hot 1:1 HCl and rinsed with distilled water. Preferably, this glassware should be used only for the determination of nitrate and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl is only occasionally required.

6.0 Equipment for sample analysis

6.1 Hach Company DR 5000, DR 3800, or DR 2800 spectrophotometer, or equivalent

6.2 Equipment for standard preparation

6.2.1 Volumetric flask – Glass, 1000-mL.

6.2.2 Volumetric pipette – Glass, assorted sizes.

7.0 Reagents and Standards

7.1 Reagent water – Water in which nitrate 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.

7.2 Hach Company TNTplus Nitrate Reagent, Cat. No. TNT835 or TNT836.

7.3 Hach Company Nitrate Standard Solutions: 100 mg/L as NO_3^- -N (Cat. No. 194749) and 1000 mg/L as NO_3^- -N (Cat. No.1279249).

7.4 Method detection limit (MDL) solution

7.4.1 Prepare 7 or more replicate MDL solutions by diluting 3.0 mL of the 100 mg/L standard spiking solution (Section 7.3) to 1000 mL. Final concentration = 0.3 mg NO_3^- -N /L.

7.5 Initial precision and recovery (IPR) solution

7.5.1 Prepare 4 or more replicate IPR solutions by diluting 5.0 mL of the 1000 mg/L standard spiking solution (Section 7.3) to 1000 mL. Final concentration = 5 mg NO_3^- -N /L.

8.0 Sample Collection, Preservation and Storage

8.1 Samples may be collected in clean glass or plastic bottles.

8.2 Analyze samples as soon as possible. If immediate analysis is not possible, store at $\leq 6^\circ$ C.. Samples to be used for measurement of nitrate only must not be preserved with acid and must be analyzed within 48 hours of collection.

8.3 If longer storage is required (up to 28 days), adjust sample pH to less than 2 with sulfuric acid (about 2 mL per liter). Sample refrigeration is still required. If sample is acid preserved, results must be reported as nitrate-nitrite, also known as total nitrate.

9.0 Quality Control

9.1 It is recommended that each laboratory that uses this method be required to operate a formal quality assurance program (16.1). 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.

9.1.1 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 9.2. The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery sample that the analysis system is in control.

- 9.1.2 Accompanying QC for the determination of nitrate 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 a laboratory reagent blank (LRB, an ongoing precision and recovery sample (OPR), matrix spike sample (MS), and matrix spike duplicate sample (MSD) resulting in a minimum of five analyses (1 LRB, 1 OPR, 1 sample, MS, and MSD).
- 9.2 Initial demonstration of laboratory capability.
- 9.2.1 To establish the ability to detect nitrate the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (16.2) using the apparatus, reagents, and standards that will be used in the practice of this method. The analyst also shall calculate the Minimum Level (ML) of quantitation by multiplying the MDL by 3.18 and rounding to the number nearest to (1,2 or 5) x 10ⁿ, where n is a positive or negative integer. The calculated MDL should be less than or equal to the MDL in Section 13.0 prior to the practice of this method. Similarly, the calculated ML should be less than or equal to the ML in Section 13.0
- 9.2.2 Prepare and measure seven replicates of the MDL standard according to the procedure beginning in Section 7.4.1.
- 9.3 Initial precision and recovery (IPR) - To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:
- 9.3.1 Prepare and measure four samples of the IPR standard according to the procedure beginning in Section 7.5.
- 9.3.2 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 nitrate. Use the following equation for calculation of the standard deviation of the percent recovery:

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

where:

n = Number of samples

x_i = % recovery in sample i

- 9.3.2.1 Using the values of X and s calculated in the previous step, calculate the Relative Standard Deviation (RSD) using the equation below:

$$RSD = \frac{s}{X} * 100\%$$

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

9.4 Laboratory Reagent Blank (LRB) – The laboratory reagent blank (LRB) is an aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment and reagents that are used with other samples. The laboratory must analyze at least one LRB with each batch of samples. Data produced are to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective action should be taken before continuing the analysis.

9.5 Ongoing precision and recovery (OPR) - To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch. An analytical batch is a set of samples processed during a contiguous 8-hour period, not to exceed 20 samples. The analyst shall perform the following operations:

9.5.1 Prepare a precision and recovery standard with each analytical batch.

9.5.1.1 At the end of each analytical batch of samples, analyze a precision and recovery standard. If the recovery is within the acceptable range of 90 -110%, measurement process is in control and analysis of samples may proceed. If, however, the recovery is not in the acceptable 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.

9.5.1.2 The laboratory should add results that pass 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%.

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

9.6 Matrix Spike and Matrix Spike Duplicate Precision and Recovery (MS/MSD) – The laboratory must, on an ongoing basis, spike at least 5% of the samples from each analytical batch as defined in Section 9.4.

9.6.1. The concentration of the spike in the sample should be determined as follows:

9.6.1.1 If the concentration of a specific parameter in the sample is not being checked against a limit specific to that parameter, the spike should be at 5 mg/L or one to five times higher than the background concentration determined in Section 10, whichever concentration would be larger.

- 9.6.2 Analyze one sample aliquot to determine the background concentration (B).
- 9.6.3 Analyze the spiked MS and MSD aliquots following the procedure in Section 11.
- 9.6.5 Calculate each percent recovery (P) as $100(A-B)/T$, where A is the concentration of NO_3^- -N in the spiked samples and T is the known true value of the spike.
 - 9.6.5.1 Compare the percent recovery (P) NO_3^- -N with the corresponding QC acceptance criteria found in Section 17, Table 3.
- 9.6.6 Calculate the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = Concentration of analyte in the MS, D_2 = Concentration of analyte in the MSD.

- 9.6.6.1 Compare the calculated RPD with the corresponding QC acceptance criteria found in Section 17, Table 3.

10.0 Calibration and Standardization

- 10.1 The Hach DR series spectrophotometers have a built-in calibration that is automatically used when the TNTplus nitrate vial is inserted into 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.

10.2 Calibration Verification

- 10.2.1 To verify that the instrument is measuring nitrate properly, analyze a 0.3 mg/L and 10.0 mg/L nitrate nitrogen standard. Results should be within 15 percent of the actual value. Perform this calibration verification daily while instrument is in use.

11.0 Procedure

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

11.2 Preparation

- 11.2.1 For LR TNT 835: Pipet 1.0 mL of sample into the reagent vial.
For HR TNT836: Pipet 0.2 mL of sample into the reagent vial.

11.3 Reaction

- 11.3.1 For LR TNT835: Pipet 0.2 mL of Solution A into the vial.
For HR TNT836: Pipe 1.0 mL of Solution A into the vial.

11.3.2 Cap and invert the reaction tube 2-3 times until no more streaks can be seen in the reaction tube solution.

11.3.3 React for 15 minutes.

11.4 Analysis

11.4.1 Wipe the vial and insert the prepared vial into the spectrophotometer. The instrument reads the barcode, then selects and performs the correct test. No zero is required. Results are in mg/L NO₃⁻-N

12.0 Data Analysis and Calculations

12.1 Nitrate concentration is calculated automatically against internal instrument calibration.

13.0 Method Performance

Performance of the method was demonstrated in multi-lab studies comparing the method against currently promulgated nitrate methods. The method was evaluated in low ionic strength (LIS) and high ionic strength (HIS) matrices as well as multiple geographically diverse finished drinking water samples obtained from both surface water and ground water sources.

Acceptance Criterion	Section	Limit
Method Detection Limit	9.2.1	0.05 mg/L NO ₃ ⁻ N
Method Limit	9.2.1	0.20 mg/L NO ₃ ⁻ N
Initial Recovery Range	9.3.1	90% - 110%
Initial Precision (RPD)	9.3.1	10%
Matrix Recovery Range	9.5.1	90- 110%

14.0 Pollution Prevention

14.1 Follow guidelines in Section 15.

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 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.

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

16.0 References

- 16.1 “Handbook of Analytical Quality Control in Water and Wastewater Laboratories,” USEPA, EMSL-CI, Cincinnati, OH 45268, EPA-600-4-79-019, March 1979.
- 16.2 40 CFR 136, Appendix B.
- 16.3 “OSHA Safety and Health Standards, General Industry,” (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976)
- 16.4 “Safety in Academic Chemistry Laboratories,” American Chemical Society, Committee on Chemical Safety, 3rd Edition, 1979.
- 16.5 “Water Analysis Handbook,” Hach Company, 5th Edition, 2008.

17.0 Tables

- 17.1 Acceptance Criteria for Performance tests – The QC performance criteria for this method was performed with a Hach Company DR2800 Spectrophotometer and TNTplus 835 Reagent.

Table 1. Initial Precision and Recovery Method Performance

IPR Spike Concentration	Maximum RSD (%)	Lower Limit of Recovery (%)	Upper Limit of Recovery (%)
5.0 mg/L NO ₃ ⁻ N	10	90	110

Table 2. Method Detection Limit (MDL) & Minimum Level (ML) of Quantitation

MDL Test Concentration	MDL	Rounded ML
0.30 mg/L NO ₃ ⁻ N	0.05	0.20

Table 3. Matrix Spike (MS) / Matrix Spike Duplicate (MSD) QC Acceptance Criteria

MS/MSD Spike Concentration	Maximum RPD (%)	Lower Limit of Recovery (%)	Upper Limit of Recovery (%)
5.0 mg/L NO ₃ ⁻ N	20	90.0	110

18.0 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.

18.1 Units of weight and measure and their abbreviations

18.1.1 Symbols

°C: degrees Celsius

18.1.2 Alphabetical characters

mg/L: milligram per liter

18.2 Definitions, acronyms, and abbreviations

18.2.1 LRB – Laboratory Reagent Blank

18.2.2 MDL: Method detection limit

18.2.3 ML: Minimum level of quantitation

18.2.4 IPR: Initial precision and recovery

18.2.5 OPR: On-going precision and recovery

18.2.6 MS: Matrix spike

18.2.7 MSD: Matrix spike duplicate