Hach TNTplus 880 Total Kjeldahl Nitrogen Method 10242 Revision 1.2 March 2022

Simplified Spectrophotometric Measurement of Total Kjeldahl Nitrogen in Water and Wastewater

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1.0 Scope and Application

1.1 This method is for the measurement of Total Kjeldahl Nitrogen (TKN) in water and wastewater.

1.2 The method may be used as equivalent under 40 CFR 136.6 to EPA Reference Method 351,2 and Hach Method 10242 Revision 1.1 (January 10, 2013) that is approved at 40 CFR 136 Table IB, for the purposes of regulatory compliance monitoring and reporting of TKN.

1.3 The method is for use in the United States Environmental Protection Agency's (EPA's) survey and monitoring programs for the measurement of TKN under the Clean Water Act.

1.4 The method is applicable in the range from 1.0 to 16.0 mg/L TKN. The combined nitrate/nitrite concentration must be between 0.23 and 13.5 mg/L N. The range of detection can be extended with dilution of samples that exceed 16.0 mg/L TKN.

2.0 Summary of Method

2.1 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$ and subsequently analyzed colorimetrically using salicylate or phenate chemistry with nitroprusside. Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value. Total nitrogen (TN) is the sum of TKN (free-ammonia and organic nitrogen) plus nitrate and nitrate.

$$[TKN] = [(NH_3 + NH_4^+)] + [Nitrogen_{org}]$$
$$[Nitrogen_{org}] = [TKN] - [(NH_3 + NH_4^+)]$$
$$[TN] = [TKN] + [NO_3^-] + [NO_2^-]$$

2.2 Hach Method 10242 Revision 1.2, also known as TNTplus 880 is a simplified green chemistry alternative to other methods approved at 40 CFR 136 for the purposes of regulatory reporting of TKN . In the simplified total Kjeldahl (s-TKN) method, inorganic (NH_3, NH_4^+, NO_2^-) and organic nitrogen are oxidized to nitrate by digestion with peroxodisulfate. The nitrate ions react with 2,6-dimethylphenol in a solution of sulfuric and phosphoric acid to form a nitrophenol. Oxidized forms of nitrogen in the original sample (nitrite + nitrate) are determined in a second test vial and then subtracted, resulting in TKN.

$$[TKN] = [TN] - [NO_3^- + NO_2^-]$$

3.0 Interferences

3.1 High levels of oxidizable organic substances (COD) affect the reagent color and give high results.

3.2 Nitrite concentrations of more than 2.0 mg/L interfere (high-bias results). Add 50 mg of sulfamic acid to 5.0 mL of sample, dissolve, and wait for 10 minutes. Analyze the prepared sample as described in Section 11.

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 nitrogen containing 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 6000, DR 5000, DR 3900, DR 3800, DR 2800, or DR1900 spectrophotometer, or equivalent

6.2 Block Digester, capable of heating to 120º C. Hach Catalog Number LTV082.53.30001, or equivalent

6.3 Light shield for Hach DR2800 spectrophotometer, Hach Catalog Number LZV646, or equivalent

6.4 Pipette, variable volume, 1 to 5 mL, Hach Catalog Number BBP065, or equivalent

6.5 Pipette tips, for BBP065 pipette, Hach Catalog Number BBP068, or equivalent

6.6 Pipette, variable volume, 0.2 to 1.0 mL, Hach Catalog Number BBP078, or equivalent

6.7 Pipette tips, for BBP078 pipette, Hach Catalog Number BBP079, or equivalent

7.0 Reagents and Standards

7.1 Reagent water – Water in which TKN 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 TNTplus Simplified TKN (s-TKNTM) Reagent - Hach Catalog Number TNT880.

7.3 Sulfuric Acid, ACS - Hach catalog Number 97949, or equivalent

7.4 Sodium Hydroxide, 5N - Hach Catalog Number 245053, or equivalent

7.5 Method Detection Limit Ammonia Standard Solution – 1.0 mg/L as NH_3-N, Hach Catalog Number 189149, or equivalent

7.6 Initial Precision and Recovery Ammonia Standard Solution – 1000 mg/L as NH_3-N, Hach Catalog Number 2354153, or equivalent

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 ≤6º C and analyze within 28 days of collection.

8.3 Adjust sample pH to less than 2 with sulfuric acid (about 2 mL per liter).

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 Method Detection Limit (MDL) - 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

9.2.1.1 Obtain the Method Detection Limit Ammonia Standard Solution in Section 7.5. The concentration of the MDL samples should be 1.0 mg/L NH^3-N. Analyze a minimum of seven replicate of this standard according to the procedure in Section 11.

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

9.2.2.1 Obtain the Initial Precision and Recovery Limit Ammonia Standard Solution in Section 7.6.

9.2.2.2 Add 0.5 mL of the Initial Precision and Recovery Ammonia Standard Solution into a 100-mL volumetric flask and bring to volume with reagent water. The concentration of the IPR solution should be 5 mg/L NH_3-N. Analyze four replicate volumes of this standard according to the procedure in Section 11.

9.2.2.3 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:

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

where:

n = Number of samples
x_i = % recovery in sample i

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

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

9.2.2.5 Compare RSD and x with the corresponding limits for initial precision and recovery in Table 2. If RSD and x meet the acceptance criteria, system performance is acceptable, and analysis of samples may begin. If, however, the RSD 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.

9.3 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 determine 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.

9.4 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:

9.4.1 Prepare a precision and recovery standard following the procedure in Section 9.2.2 and analyze at the end of each analytical batch according to the procedure in Section 11.

9.4.1.1 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.

9.4.1.2 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%.

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

9.5 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.

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

9.5.1.1 If, as in compliance monitoring, the concentration of TKN in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or one to five times higher than the background concentration determined in Section 10, whichever concentration would be larger.

9.5.1.2 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 11, whichever concentration would be larger.

9.5.2 Analyze one sample aliquot to determine the background concentration (B).

9.5.3 Analyze the spiked MS and MSD aliquots following the procedure in Section 11.

9.5.5 Calculate each percent recovery (P) as 100 (A-B)%/T, where A is the concentration of TKN in the spiked samples and T is the known true value of the spike.

9.5.5.1 Compare the percent recovery (P) TKN with the corresponding QC acceptance criteria found in Section 17, Table 3.

9.5.6 Calculate the relative percent difference (*RPD*) between two sample results using the following equation:

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

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

9.5.6.1 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 TKN properly, analyze a 1.0 mg/L and10.0 mg/L NH_3-N standard. Results shall be within 10 percent of the actual value. Perform this calibration verification daily while instrument is in use.

11.0 Sample Analysis Procedure

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

11.2 Heating Block - Turn on heating block and heat to 120 °C.

11.3 Analysis – Using TNTplus s-TKN Reagent Set Revision 1.2

11.3.1 Add 1.3 mL of sample, 1.3 mL of Solution A and 1 Reagent B tablet in quick succession to a dry 20-mm reaction tube. Close the reaction tube immediately. Do not Invert tube.

11.3.2 Insert the reaction tube in the reactor and heat for 30 minutes.

11.3.3 After 30 minutes, remove the reaction tube from the heating block and cool to room temperature (15-20^o C).

11.3.4 Pipette 0.5 mL of the digested sample from the reaction tube into a Test Vial 1 (red label).

11.3.5 Pipette 0.2 mL of Solution D into the test vial. Quickly cap and invert the test vial 2-3 times until no more streaks can be seen in the vial solution. Immediately proceed to step 11.3.7.

11.3.6 Pipette 1.0 mL of undigested sample into a Test Vial 2 (green label).

11.3.7 Pipette 0.2 mL of Solution D into the test vial. Quickly cap and invert the test vial 2-3 times until no more streaks can be seen in the vial solution and let react for 15 minutes.

11.3.8 After 15 minutes, wipe the Test Vial 1 with a clean tissue or cloth and insert the prepared vial into the cell holder of the spectrophotometer. The instrument will read the barcode on the Test Vial 1 and display E1. Remove the vial and proceed immediately to step 11.3.9.

11.3.9 Wipe the Test Vial 2 with a clean tissue or cloth and insert the prepared vial into the cell holder of the spectrophotometer. The instrument will read the barcode on the Test Vial 2.

12.0 Data Analysis and Calculations

12.1 The sample result will be displayed in mg/L Total N, mg/L NO_3-N + NO_2-N and mg/L TKN.

13.0 Method Performance

Acceptance Criterion	Section	Limit
Method Detection Limit	9.2.1, Table 1	0.08 mg/L NH_3-N as TKN
Minimum Level Rounded	9.2.1, Table 1	1.0 mg/L NH_3-N as TKN
Initial Recovery Range	9.2.2, Table 2	90% - 110%
Matrix Recovery Range	9.5, Table 3	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.

16.6 Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water, United States Environmental Protection Agency, Office of Water, Washington, D.C., EPA-821-B-98-003, March 1999.

17.0 Tables

17.1 Acceptance Criteria for Performance Tests

Table 1. Method Detection Limit (MDL) & Minimum Level (ML) of Quantitation

MDL Test Concentration	MDL	ML	ML Rounded
1.0 mg/L NH_3-N as TKN	0.08 mg/L NH_3-N as TKN	0.26 mg/L NH_3-N as TKN	1.0 mg/L NH_3-N as TKN

Table 2. Initial Precision and Recovery QC Acceptance Criteria

IPR Spike Concentration	Average Recovery (%)	Standard Deviation of Recovery (%)	97.5 %Lower Limit of Recovery (%)	97.5 % Upper Limit of Recovery (%)
5.0 mg/L NH_3-N as TKN	96.9	2.2	90	110

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

Matrix Spike Concentration	Average Recovery (%)	Standard Deviation of Recovery	97.5 % Lower Limit of Recovery (%)	97.5 % Upper Limit of Recovery (%)
5.0 mg/L NH_3-N as TKN	95.6	2.2	90	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
^{o}C: degrees Celsius

18.1.2 Alphabetical characters
mg/L: milligram per liter

18.2 Definitions, acronyms, and abbreviations

18.2.1 TKN – Total Kjeldahl Nitrogen

18.2.2 s-TKN – simplified Total Kjeldahl Nitrogen

18.2.3 LRB – Laboratory Reagent Blank

18.2.3 MDL: Method detection limit

18.2.4 ML: Minimum level of quantitation

18.2.5 IPR: Initial precision and recovery

18.2.6 OPR: On-going precision and recovery

18.2.7 MS: Matrix spike

18.2.8 MSD: Matrix spike duplicate

18.2.9 RSD: Relative Standard Déviation

18.3.0 RPD: Relative Percent Difference