Hach Company TNTplus™ Ammonia – Spectrophotometric Measurement of Ammonia Nitrogen and Total Kjeldahl Nitrogen in Water and Wastewater

Hach Method 10205
Spectrophotometric Measurement of Free Ammonia-nitrogen and for Determination of Total Kjeldahl Nitrogen in Water and Wastewater

Revision 1.1
June 2007

1.0 Scope and Application

1.1 These procedures cover the determination of ammonia-nitrogen and total Kjeldahl nitrogen in drinking water, surface and saline waters, domestic and industrial wastes.

1.2 The method is applicable in the range from 0.01 to 50 mg NH_3-N /L, depending on the test range of the TNTplus Ammonia kit.

1.3 This method is equivalent to EPA 350.1, 350.2, 351.1, and 351.2 and SM 4500-NH3 F, G, H for the purposes of regulatory reporting of Ammonia (as nitrogen) and Total Kjeldahl Nitrogen under 40 CFR 136.6.

2.0 Summary of Method

2.1 Ammonia and organic nitrogen-based compounds converted to ammonia are combined with hypochlorite to form monochloramine. Monochloramine further reacts with salicylate and nitroprusside to form the blue-colored compound, indosalicylate. The color is proportional to the ammonia or total Kjeldahl nitrogen concentration and is measured at 600 ± 5 nm.

2.2 Total Kjeldahl nitrogen samples are digested in the presence of sulfuric acid, potassium sulfate and mercuric sulfate to form ammonium sulfate.

2.3 Ammonia-nitrogen samples are prepared for analysis without digestion.

3.0 Interferences

3.1 High nitrate concentrations (10x or more than the TKN level) may result in low TKN values. The reaction between nitrate and ammonia can be prevented by the use of an anion exchange resin (chloride form) to remove the nitrate prior to TKN digestion.

4.0 Definitions

4.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$.

4.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.

5.0 Safety

5.1 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.

5.2 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.

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 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 16.6 and 16.7.

6.0 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.*

6.1 Sampling equipment

6.1.1 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.*

6.1.2 Cleaning

6.1.2.1 All glassware used should and rinsed with NH_3/NH_4^+-free distilled water.

6.2 Equipment for glassware cleaning

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

6.3 Equipment for sample analysis

6.3.1 Hach DR 5000/6000, DR 4800, DR 2800/3900 spectrophotometer.

6.3.2 DRB200 Digital Reactor Block for TNTplus: 30x13mm vial wells, 115 Vac (P/N DRB200-03)

6.4 Equipment for standard preparation

6.4.1 Volumetric flask – Glass, 1000-mL.

6.4.2 Volumetric flask – Glass, 50-mL.

6.4.3 Volumetric pipette – glass, assorted sizes.

7.0 Reagent and Standards

7.1 Reagent water – Water in which ammonia nitrogen or Kjeldahl nitrogen 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 Ammonia Kits (TNTplus 830, 0.015 – 2.000 mg NH_3-N/L; TNTplus 831, 1 - 12 mg NH_3-N/L, TNTplus 832, 2 – 47 mg/L NH_3-N).

7.3 Hach Company Ammonia Nitrogen Standard Solution, 1.0 mg/L as NH_3-N/L, Cat. No. 1891-49

7.4 Method detection limit solution

7.4.1 Prepare 7 or more replicate MDL solutions by diluting 1.5 mL of standard spiking solution (Section 7.3) to 50 mL. Final concentration = 0.03 mg NH_3-N/L.

7.5 Initial precision and recovery solution

7.5.1 TNTplus 830, 0.015 – 2.000 mg NH_3-N/L.

7.5.1.1 Prepare 4 or more replicate IPR solutions by diluting 20.0 mL of standard spiking solution (Section 7.3) to 50 mL. Final concentration = 0.40 mg NH_3-N/L.

7.6 On-going precision and recovery

7.6.1 TNTplus 830, 0.015 – 2.000 mg NH_3-N/L.

7.6.1.1 Prepare 1 or more solutions by diluting 2.0 mL of standard spiking solution (Section 7.3) to 50 mL. Final concentration = 0.40 mg NH_3-N/L.

8.0 Sample Collection Preservation and Storage

8.1 If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

8.2 Sample containers may be of plastic material, such as cubitainers, or of Pyrex glass.

8.3 If samples cannot be analyzed within 15 minutes of collection, sample should be preserved by the addition of 2 mL conc. H_2SO_4 per liter and refrigeration at 4°C.

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 (Section 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. This procedure is described in Sections 8.3.

9.1.2 Accompanying QC for the determination of ammonia-nitrogen and ammonia-nitrogen from TKN digestions is required per analytical batch. An analytical batch is a set of up to 20 samples processed during a contiguous 8-hour period. Each analytical batch should 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).

9.2 Initial demonstration of laboratory capability.

9.2.1 To establish the ability to detect ammonia the analyst shall determine the MDL and ML per the procedure in 40 CFR 136, Appendix B (Section 16.5) 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 13.0 is recommended prior to the practice of this method.

9.2.1 Prepare and measure seven replicates of the MDL standard according to the procedure beginning in Section 7.4.1.

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:

9.2.3.1 Prepare and measure four samples of the IPR standard according to the procedure beginning in Section 7.5.

9.2.3.2 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

9.2.3.3 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.

9.3 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:

9.3.1 Prepare a precision and recovery standard with each analytical batch according to the procedure beginning in Section 7.6.

9.3.2 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.

9.3.3 The laboratory should add results that pass the specification in Section 13.0 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%.

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

10.0 Calibration and Standardization

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

11.0 Procedure

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

11.2 Sample Preparation

11.2.1 Remove the protective foil lid from the DosiCap™ **Zip**. Unscrew the cap from the vial.
11.2.2 Pipet 5.0 mL of sample into the vial.

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

11.2.4 Shake the capped vial 2-3 times to dissolve the reagent cap. Verify that the reagent has dissolved by looking down through the open end of the DoisCap **Zip**.

11.2.5 React for 15 minutes.

11.2.6 After 15 minutes, invert the sample an additional 2-3 times to mix. The color remains stable for 15 minutes.

11.3 Analysis

11.3.1 Thoroughly clean the outside of the vial. Install the Light Shield in Cell Compartment #2.

11.3.2 Insert the prepared vial into the cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L NH_3-N.

11.3.3 No instrument zero is required.

12.0 Data Analysis and Calculations

12.1 Ammonia-N (NH_3-N) concentration is calculated automatically against internal instrument calibration.

13.0 Method Performance

Acceptance Criterion	Section	Limit
Method Detection Limit	9.2.1	0.004 mg/L NH_3-N
Method Limit	9.2.1	0.01 mg/L NH_3-N
Initial Accuracy	9.2.2	90% - 110%
On-going Accuracy	9.3	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 International Oceanographic Tables, Vol. 1, National Institute of Oceanography of Great Britain, Womley, Godaming, Surrey, England and Uncesco, Paris 1971.

16.3 40 CFR 136, Appendix A, Methods 1624 and 1625.

16.4 40 CFR 136, Appendix B.

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

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

17.0 Tables

17.1 Acceptance Criteria for Performance tests – The QC performance criteria for this method was performed with a Hach Company DR 5000 spectrophotometer using Hach Company TNT830 Ammonia Kit.

Table 1. Initial Precision and Recovery Method Performance

IPR Concentration	Average Recovery (%)	Relative Standard Deviation (%)
0.40 mg/L NH_3-N	97	0.67

Table 2. Minimum Method Limit Performance

MDL Test Concentration	MDL	ML
0.01 mg NH_3-N/L	0.004 mg NH_3-N/L	0.01 mg NH_3-N/L

Table 3. On-going Recovery Performance

OPR Concentration	Average % Recovery
0.40 mg NH_3-N/L	100%

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

ºC degrees Celsius

18.1.2 Alphabetical characters

mg/L milligram per liter

18.2 Definitions, acronyms, and abbreviations

18.2.1 MDL: Method detection limit

18.2.2 ML: Method limit

18.2.3 IPR: Initial precision and recovery

18.2.4 OPR: On-going precision and recovery

18.2.5 MS: Matrix spike

18.2.6 MSD: Matrix spike duplicate

Appendix

	EPA 350.1/ SM 4500-NH_3 F, G, H	**EPA 351.1**	**EPA 351.2**	**Hach TNTplus Ammonia**
Scope and Application	0.01 to 21.00 mg/L NH_3-N Drinking Water	0.05 to 2.0 mg/L NH_3-N Wastewater	0.01 to 20 mg/L NH_3-N Wastewater	0.01 to 50 mg/L NH_3-N Drinking water and wastewater
Summary of Method	Ammonium ions react with hypochlorite ions and phenate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue.	Ammonium ions react with hypochlorite ions and phenate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue.	Ammonium ions react with hypochlorite ions and phenate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue.	Ammonium ions react with hypochlorite ions and phenate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue.
Equipment	CFA/Manual Spectroscopy/Colorimetry	CFA Automated Colorimetry	CFA Automated Colorimetry	Manual Spectrophotometry/Colorimetry
Reagents	Sodium phenolate Hypochrlorite (Clorox) Sodium nitroprusside	Sodium phenolate Hypochrlorite (Clorox) Sodium nitroprusside	Sodium phenolate Hypochrlorite (Clorox) Sodium nitroprusside	Sodium salicylate Hypochrlorite (Sodium-dichloroisocyanuric acid) Sodium nitroprusside
Method Performance	MDL – not provided IPR – not provided OPR – not provided Surface water spike recoveries: 107% and 99% Surface water spike recovery precision: ± 0.005	MDL – not provided IPR – not provided OPR – not provided Natural Water Spike Average % Bias: -24.7	MDL – not provided IPR – not provided OPR – not provided MS/MSD Recovery – not provided Sewage sample spike precision: 1.2, 2.6, and 1.7 mg N/L spike precision was ± 0.07, ± 0.03, and ± 0.15, respectively	MDL – 0.004 mg/L NH_3-N ML - 0.01 mg/L NH_3-N IPR @ 0.4 mg/L NH_3-N – 97% IPR Precision - 2.7% OPR @ 0.4 mg/L NH_3-N – 100% OPR @ 40 mg/L NH_3-N – 98% Laboratory Control Spike Recovery @ 1.0 mg/L NH_3-N– 99% Laboratory Control Spike Recovery @ 10 mg/L NH_3-N– 103% MS/MSD Spike Recovery of 3 different POTW wastewaters @ 1.0 mg/L NH_3-N – 99% Precision – 0.96% MS/MSD Spike Recovery of 3 different POTW wastewaters @ 25 mg/L NH_3-N – 100% Precision – 2.8% MS/MSD Spike Recovery of paper and pulp industrial effluent @ 1.0 mg/L NH_3-N – 102% Precision – 0.67%

	EPA 350.2
Scope and Application	0.05 - 1.0 mg NH_3-N; Nessler colorimetric procedure, 1.0 – 25 mg/L NH_3-N; titrimetric procedure, 0.05 – 1400 mg/L NH_3-N ; electrode procedure Wastewater
Summary of Method	The ammonia in the distillate can be determined colorimetrically by nesslerization, titrimetrically with standard sulfuric acid with the use of a mixed indicator, or potentiometrically by the ammonia electrode.
Equipment	Spectrophotometer
Reagents	Nessler colorimetric reagents – mercuric iodide, potassium iodide, sodium hydroxide
Method Performance	MDL – not provided IPR – not provided OPR – not provided Natural water spike recoveries:

Increment as Nitrogen,ammonia mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias %	Accuracy as Bias mg N/liter
0.21	0.122	-5.54	-0.01
0.26	0.070	-18.12	-0.05
1.71	0.244	0.46	0.01
1.92	0.279	-2.01	-0.04