



Designation: **D 3590—89 (Reapproved 1994)^{ε1} 3590 – 02**

Standard Test Methods for Total Kjeldahl Nitrogen in Water¹

This standard is issued under the fixed designation D 3590; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

^{ε1} ~~Note—Section 24, Keywords, was added in April 1994.~~

1. Scope*

1.1 These test methods cover the determination of total Kjeldahl nitrogen. The following test methods are included:

Test Method A—Manual Digestion/Distillation	Sections 8 to 14
Test Method B—Semiautomated Colorimetric Bertholt	15 to 23

1.2 The analyst should be aware that precision and bias statements included may not necessarily apply to the water being tested.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

¹ These test methods are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

Current edition approved ~~Aug. 25, 1989; Feb. 10, 2002~~. Published ~~January 1990; May 2002~~. Originally published as D 3590 – 77. Last previous edition D 3590 – 889 (1994)^{ε1}.

*A Summary of Changes section appears at the end of this standard.



- D 1129 Terminology Relating to Water²
- D 1193 Specification for Reagent Water²
- D 1426 Test Methods for Ammonia Nitrogen in Water²
- D 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water²
- D 3370 Practices for Sampling Water from Closed Conduits²
- D 5810 Guide for Spiking into Aqueous Samples²
- D 5847 Practice for the Writing Quality Control Specifications for Standard Test Methods for Water Analysis³

3. Terminology

3.1 *Definitions*—For definitions of terms used in these test methods, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *total Kjeldahl nitrogen*—the sum of the nitrogen contained in the free ammonia and other nitrogen compounds which are converted to ammonium sulfate [(NH₄)₂SO₄] under the specified digestion conditions.

4. Significance and Use

4.1 These test methods are useful for measuring organic nitrogen and ammoniacal nitrogen, which are essential growth nutrients.

4.2 Nitrogen compounds are widely distributed in the environment. Sources of nitrogen include surface-applied fertilizers, cleaning products, and drinking water treatment aids. Because nitrogen is a nutrient for photosynthetic organisms, it may be important to monitor and control discharge into the environment.

5. Interferences

5.1 Nitrate is known to cause a serious negative interference in the test. Reportedly, a concentration of 250 mg/L NO₃ results in zero recovery of ~~mg/L N~~ some level of N added as some N compound.

5.2 The analyst is cautioned that ammonia in the laboratory may easily become an interference in these test methods from contamination of reagents, caps, or from the laboratory atmosphere. Care should be taken that ammonium hydroxide, either as a reagent or as a cleaning substance, is not used in the same room.

6. Purity of Reagents

6.1 Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁴ Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean ~~Type III~~ reagent water conforming to Specification ~~D 1193~~ for D 1193, Type I. Other reagent water prepared by types may be used provided it is first ascertained that the passage through a strong, acid-cation exchange resin in water is of sufficiently high purity to permit its use without adversely affecting the precision and bias of the test method. Type III water was specified at the time of ~~rogund-robin testin~~ ~~fg~~ off this test method.

7. Sampling and Preservation

7.1 Collect the sample in accordance with applicable Practices D 3370.

7.2 Samples may be preserved up to 28 days by adding concentrated sulfuric acid to adjust to pH 2 or less and storing at 4°C. The preserved sample should be analyzed as soon as possible; data on decomposition are not available.

TEST METHOD A—MANUAL DIGESTION/DISTILLATION

8. Scope

8.1 This test method covers the determination of total Kjeldahl nitrogen in water. It measures free ammonia or ammonia formed from the conversion of nitrogen components of biological origin such as amino acids and proteins. However, the procedure may not convert the nitrogenous compounds of some wastes to ammonia. Examples of such compounds that may not be measured are nitro compounds, hydrozones, oximes, nitrates, semicarbazones, pyridines, and some refractory tertiary amines.

² Annual Book of ASTM Standards, Vol 11.01.

³ Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar ASTM Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD; Vol 11.02.

⁴ Micro-Kjeldahl steam distillation apparatus is commercially available.

⁴ Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

8.2 Three alternatives are described for the final determination of the ammonia: a titrimetric method, which is applicable to concentrations above 1 mg N/L; a Nesslerization method, which is applicable to concentrations below 1 mg N/L; and a potentiometric method which is applicable to the range from 0.04 to 1000 mg N/L.

8.3 This test method is described for micro and macro systems. Micro determination can be made on sample aliquots containing up to 10 mg of nitrogen.

9. Summary of Test Method

9.1 The sample is heated in the presence of concentrated H₂SO₄, K₂SO₄, and HgSO₄, and is digested until SO₃ fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and alkalized with a hydroxide-thiosulfate solution. The ammonia is distilled into a boric acid solution and total Kjeldahl nitrogen is determined by colorimetry, titrimetry, or potentiometry.

10. Apparatus

10.1 *Digestion Apparatus*—A Kjeldahl digestion apparatus with 800 to 100-mL flasks and suction takeoff to remove SO₃ fumes and water.

10.2 *Distillation Apparatus*⁵—A macro Kjeldahl flask connected to a condenser and an adaptor so that the distillate can be collected.

10.3 *Spectrophotometer or Colorimeter*, for use at 425 nm with a spectral band path of not more than ± 20 nm and a light path of 1 cm or longer.

10.4 *Electrometer (pH Meter)*, with expanded millivolt scale, or a specific ion meter.

10.5 *Ammonia Selective Electrode*.⁶

10.6 *Magnet Stirrer*, thermally insulated.

11. Reagents and Materials

11.1 *Ammonia Solution Stock*, (1.0 mL = 1.0 mg ammonia nitrogen)—Dissolve 3.819 g of ammonium chloride (NH₄Cl) in water and dilute to 1 L in a volumetric flask with water.

11.2 *Ammonia Solution, Standard* (1.0 mL = 0.01 mg ammonia nitrogen)—Dilute 10.0 mL of the stock solution (see 11.1) with water to 1 L in a volumetric flask.

11.3 *Boric Acid Solution (2 %)*—Dissolve 20 g of boric acid (H₃BO₃) in water and dilute to 1 L with water in a volumetric flask.

11.4 *Mercuric Sulfate Solution*—Dissolve 8 g of red mercuric oxide (HgO) in a mixture of 10 mL of sulfuric acid (H₂SO₄, sp gr 1.84) and 40 mL of water, and dilute solution to 100 mL.

NOTE 1—Mercury is a toxic metal and requires special disposal requirements. See Occupational Health and Safety Act (OSHA) regulations for specific instructions on handling and disposal of mercury compounds. Alternate catalysts may be used but it is the users responsibility to determine the validity of other catalysts.

11.5 *Mixed Indicator Solution*—Mix 2 volumes of 0.2 % methyl red in 95 % ethanol with 1 volume of 0.2 % methylene blue in ethanol. Prepare fresh every 30 days.

11.6 *Methyl Purple Indicator Solution* (1 g/L)—Dissolve 0.4 g of dimethyl-aminoazobenzene-*o*-carboxylic acid, sodium salt, in approximately 300 mL of water. To this solution add 0.55 g of a water-soluble blue dyestuff, Color Index No. 714, ~~dissolve, and dilute to 1 L with water. This indicator is available commercially in a prepared form.~~⁷ dissolve, and dilute to 1 L with water. This indicator is available commercially in a prepared form.⁸

11.7 *Nessler Reagent*—Dissolve 100 g of mercuric iodide (HgI₂) and 70 g of potassium iodide (KI) in a small volume of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of sodium hydroxide (NaOH) in 500 mL of water. Dilute the mixture to 1 L. This solution is stable for at least one year if stored in a thick amber polyethylene bottle out of direct sunlight.

NOTE 2—Mercury is a toxic metal and requires special disposal requirements. See Occupational Health and Safety Act (OSHA) regulations for specific instructions on handling and disposal of mercury compounds. Alternate reagents may be used but it is the users responsibility to determine the validity of other reagents.

⁴ EIL Model 8002-2 of Electronics Instruments Ltd. (U. S. Representative: Cambridge Instrument Co., 73 Spring St., Ossining, NY 10562) has been found satisfactory for this purpose. Also, Orion Model 95-12 has been found satisfactory for this purpose.

⁵ Micro Kjeldahl steam distillation apparatus is commercially available.

⁶ Refers to compounds, bearing such number, as described in "Color Index," Society

⁷ EIL Model 8002-2 of Dyers and Colourists, Yorkshire, England (1924). American Cyanamid Company's "Calcocid Blux AX Double" Electronics Instruments Ltd. (U. S. Representative: Cambridge Instrument Co., 73 Spring St., Ossining, NY 10562) has been found satisfactory for this purpose. Also, Orion Model 95-12 has been found satisfactory for this purpose.

⁸ TM Fleisher Methyl Purple indicator, U. S. Patent No. 241699, is available from Fleisher Chemical Co., P. O. Box 616, Ben Franklin Station, Washington, DC 20004, or from any chemical supply company handling Fleisher Methyl Purple.

⁹ Refers to compounds, bearing such number, as described in "Color Index," Society of Dyers and Colourists, Yorkshire, England (1924). American Cyanamid Company's "Calcocid Blux AX Double" has been found satisfactory for this purpose.

¹⁰ Digestion packet, Kel-Pak

¹¹ TM Fleisher Methyl Purple indicator, U. S. Patent No. 241699, is available from the Curtin-Matheson Scientific Fleisher Chemical Co., has been found satisfactory for this purpose. P. O. Box 616, Ben Franklin Station, Washington, DC 20004, or from any chemical supply company handling Fleisher Methyl Purple.

11.8 *Phenolphthalein Indicator Solution*—Dissolve 5 g of phenolphthalein in 500 mL of 95 % ethyl alcohol or isopropanol and add 500 mL of water. Add NaOH (0.8 g/L) solution dropwise until a faint pink color appears.

11.9 *Sodium Hydroxide Solution* (400 g/L)—Dissolve 400 g of NaOH in 800 mL of water, cool, and dilute to 1 L with water.

11.10 *Sodium Hydroxide Solution* (0.8 g/L)—Dilute 2 mL of NaOH solution (400 g/L) (see 11.9) with water to 1 L.

11.11 *Sodium Hydroxide-Sodium Thiosulfate Solution*—Dissolve 500 g of NaOH and 25 g of Na₂S₂O₃·5H₂O in water and dilute to 1 L.

11.12 *Sulfuric Acid Solution, Standard* (0.02 N, 1 mL = 0.28 mg ammonia nitrogen)—Prepare a stock solution of approximately 0.1 N acid by diluting 3 mL of concentrated H₂SO₄ (sp gr 1.84) to 1 L with water. Dilute 200 mL of this solution to 1 L with water. Standardize the approximately 0.02 N H₂SO₄ solution against 0.0200 N Na₂CO₃ solution. This last solution is prepared by dissolving 1.060 g of anhydrous Na₂CO₃, oven dried at 140°C, and diluting to 1 L with water.

11.13 *Digestion Solution*—Dissolve 267 g of K₂SO₄ in 1300 mL water and 400 mL of concentrated H₂SO₄. Add 50 mL of mercuric sulfate solution (see 11.4) and dilute to 2 L with water. A digestion packet ⁹ may be used in place of the digestion solution in the macro Kjeldahl system.

12. Procedure

12.1 Clean the distillation apparatus with steam before use by distilling a 1 + 1 mixture of water and sodium hydroxide-thiosulfate solution (see 11.11) until the distillate is ammonia-free. Repeat this operation each time the apparatus is out of service long enough to accumulate ammonia (usually 4 h or more).

12.2 Macro Kjeldahl System:

12.2.1 Place a measured sample into an 800-mL Kjeldahl flask and dilute to 500 mL. The sample size can be determined using the following table:

Kjeldahl Nitrogen in Sample, mg/L	Sample Size, mL
0 to 5	500
5 to 10	250
10 to 20	100
20 to 50	50.0
50 to 500	25.0

Prepare a 500-mL reagent water blank.

12.2.2 Add 100 mL of digestion solution (see 11.13) (see Note 1³) and digest the mixture in the Kjeldahl apparatus until SO₃ fumes are given off and the solution turns colorless or pale yellow. Continue heating for an additional 30 min. Cool the residue and add 300 mL of water. Mix well.

NOTE 1³—Digesting the sample with a packet ⁹ and 20 mL of concentrated H₂SO₄ is acceptable. Cut the end of the package and empty the contents into the digestion flask.

12.2.3 Alkalinize the digestate by careful addition of 100 mL of sodium hydroxide-thiosulfate solution (see 11.11). Do not mix until the digestion flask has been connected to the distillation apparatus (see 12.2.4).

NOTE 2⁴—Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause the heavier solution to underlay the aqueous H₂SO₄ without loss of free ammonia.

12.2.4 Connect the Kjeldahl flask to the condenser with the tip of the condenser (or an extension of the condenser tip) below the level of 50 mL of 2 % boric acid solution (see 11.3) contained in a 500-mL Erlenmeyer flask. Distill 300 mL at the rate of 6 to 10 mL/min.

12.2.5 Transfer the distillate to a 500-mL volumetric flask, dilute to volume with water, and mix. Transfer 250 mL to an Erlenmeyer flask and titrate with H₂SO₄ (see 12.4.1). If the concentration is found to be below 1 mg/L, determine the value colorimetrically. Use the remaining 250 mL for this determination.

12.3 Micro Kjeldahl System:

12.3.1 Place 50.0 mL of sample or an aliquot in a 100-mL Kjeldahl flask and add 10 mL of digestion solution (see 11.13). At the same time start a reagent blank. Evaporate the mixture in the Kjeldahl apparatus until SO₃ fumes are given off and the solution turns colorless or pale yellow. Digest for an additional 30 min. Cool the residue and add 30 mL of water.

12.3.2 Alkalinize the digestate by careful addition of 10 mL of sodium hydroxide-thiosulfate solution (see 11.11). Do not mix until the digestion flask has been connected to the distillation apparatus (see Note 2⁴).

12.3.3 Connect the Kjeldahl flask to the condenser with the tip of the condenser (or an extension of the condenser tip) below the level of 5 mL of 2 % H₃BO₃ solution (see 11.3) contained in a small Erlenmeyer flask. Distill 30 mL at the rate of 6 to 10 mL/min.

12.3.4 Transfer to a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 25 mL to an Erlenmeyer flask and titrate with H₂SO₄ (see 12.4.1). If the concentration is found to be below 1 mg/L determine the value colorimetrically. Use 20 mL

⁹Supporting data are

⁹Digestion packet, Kel Pak No. 5, available from ASTM Headquarters. Request RR-D19-1041; the Curtin-Matheson Scientific Co., has been found satisfactory for this purpose.

of the remaining solution for this determination.

12.4 *Determination of Ammonia Distillate*—Determine the ammonia content of the distillate titrimetrically, colorimetrically, or potentiometrically.

12.4.1 *Titrimetric Determination*—Add 3 drops of the mixed indicator (see 11.5) to the distillate and titrate the ammonia with 0.02 N H₂SO₄ (see 11.12), matching the end point against a blank containing the same volume of water and H₃BO₃ solution (see 11.3). If a pH meter is preferred, titrate to pH 6.2.

NOTE 35—As an alternative, 2 drops of methyl purple indicator solution (see 11.6) may be used and the titration carried out to the intermediate gray end point.

12.4.2 *Colorimetric Determination (Samples)*—To a 20-mL aliquot from the macro procedure (see 12.2.5) or micro procedure (see 12.3.4) diluted to 50 mL, add 1 mL of Nessler reagent (see 11.7), and mix. After 20 min, read the absorbance at 425 nm against the blank using 1-cm cells. Read the ammonia nitrogen in milligrams for the samples from the standard curve.

12.4.2.1 *Calibration Curve*—Prepare a series of standards on a daily basis in 50-mL volumetric flasks and dilute as follows:

Millilitres of Standard (see 11.2) 1.0 mL = 0.01 mg NH ₃ -N	Milligrams of NH ₃ -N/50.0 mL
0.0	0.0
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
5.0	0.050
8.0	0.080
10.0	0.10

To the standards diluted to 50 mL add 1 mL of Nessler reagent (see 11.7) and mix. After 20 min read the absorbance at 425 nm against the blank using 1-cm cells. From the values obtained for the standards plot a standard curve of absorbance versus milligrams of NH₃-N.

12.4.2 *Colorimetric Determination (Samples)*—To a 20-mL aliquot from the macro procedure (see 12.2.5) or micro procedure (see 12.3.4) diluted to 50 mL, add 1 mL of Nessler reagent (see 11.7), and mix. After 20 min, read the absorbance at 425 nm against the blank using 1-cm cells. Read the ammonia nitrogen in milligrams for the samples from the standard curve.

12.4.3 *Potentiometric Determination*—Test Method B of Test Methods D 1426 should be used for this determination.

12.4.3.1 It is recommended that at least two standards (a high and a low) be digested, distilled, and compared to similar values on the calibration curve to ensure that the digestion-distillation technique is reliable. If treated standards do not agree with untreated standards, the operator should find the cause of the apparent error before proceeding.

13. Calculation

13.1 If the titrimetric procedure is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 1:

$$\begin{aligned} \text{total Kjeldahl nitrogen, mg/L} \\ = (A - B)N \times F \times 1000/S \times D/C \end{aligned} \tag{1}$$

where:

- A = standard 0.02 N H₂SO₄ solution used in titrating sample, mL,
- B = standard 0.02 N H₂SO₄ solution used in titrating blank, mL,
- N = normality of H₂SO₄ solution,
- F = milliequivalent weight of nitrogen (14 mg),
- S = sample digested, mL,
- C = distillate taken for titration, mL, and
- D = final adjusted distillate volume, mL.

If the H₂SO₄ is exactly 0.0200 N and exactly one half of the distillate is taken for measurement, the equation is shortened, as shown in Eq 2:

$$\text{total Kjeldahl nitrogen, mg/L} = (A - B) \times 560/S \tag{2}$$

13.2 If the Nessler procedure is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 3:

$$\text{total Kjeldahl nitrogen, mg/L} = E \times 1000/S \times D/C \tag{3}$$

where:

- E = NH₃-H read from curve, corrected for blank, mg,
- D = final adjusted distillate volume, mL,
- C = distillate taken for Nesslerization, mL, and
- S = sample digested, mL.

13.3 If the potentiometric determination is used, calculate the total Kjeldahl nitrogen in the original sample using Eq 4:

$$\text{total Kjeldahl nitrogen, mg/L} = E \times 1000/S \times D/C \tag{4}$$

where:

E = NH_3 -N/L as determined using Test Method B of Test Methods D 1426,

S = sample digested, mL,

D = final adjusted volume, mL, and

C = distillate taken for measurement, mL.

14. Precision and Bias ¹⁰

14.1 Thirty-one analysts in 20 laboratories used titration and Nesslerization to analyze natural water samples containing exact increments of organic nitrogen and obtained the following results:

Amount Added as Nitrogen, Kjeldahl, mg N/L	Amount Found as Nitrogen, Kjeldahl, mg N/L	Precision as Standard De- viation, mg N/L	Bias, %
0.20	0.23	0.197	+ 15.54
0.31	0.33	0.247	+ 5.45
4.10	4.14	1.056	+ 1.03
4.61	4.53	1.191	- 1.67

14.2 The potentiometric test method has not been validated in conjunction with the digestion-distillation procedure described in this standard. However, since the procedure provides a relatively clean sample, it is thought that the user may be guided by the precision and bias information presented in Test Method B of Test Methods D 1426 and by 12.4.3 of this test method. ¹¹

14.3 The data in Section 14 may not apply to types of water other than those tested. It is the responsibility of the analyst to ensure the validity of this test method for untested matrices.

14.4 Precision and bias for this test method conforms to Practice D 2777-77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of D 2777-98, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D-19 test methods.

TEST METHOD B—SEMIAUTOMATED COLORIMETRIC BERTHØELOT

15. Scope

15.1 This test method covers the automated determination of total Kjeldahl nitrogen in water and wastewater and is based on the same principle and subject to the same limitations as the manual method (see 8.1).

15.2 This test method is a semiautomated procedure applicable to drinking water, surface water, and domestic and industrial wastes containing from 0.3 to 5 mg/L of nitrogen.

16. Summary of Test Method

16.1 This test method consists of digesting the sample in a block digester in the presence of H_2SO_4 , K_2SO_4 , and HgSO_4 at approximately 200°C for 1 h and then at 380°C for 1½ h. (The block digester is an electrically-heated metal block designed to hold block-digester reaction tubes).^{11,13} During the digestion, organic nitrogen is converted to ammonium ion. After digestion, the residue is cooled, dissolved in water, and an aliquot is transferred to a segmented-flow automated analysis system. The ammonia nitrogen content is determined by means of the automated analysis system using a modification of the Berthøelot reaction.

16.2 Ammoniacal nitrogen is determined by means of the salicylate/nitroprusside Bertholt reaction method.

¹⁰ The Results Advisor of Committee D-19 on Water has reviewed and approved this statement for conformity with the requirements of Practice D 2777 – 85; the Technical Operations Section of Executive Subcommittee D19.90 has supported this approval.

¹⁰ Supporting data are available from ASTM Headquarters. Request RR D19-1041.

¹¹ The block digester Results Advisor of Committee D-19 on Water has reviewed and continuous flow instruments capable approved this statement for conformity with the requirements of performing Practice D 2777 – 85; the digestion procedure are available from Scientific Instrument Corp., Hawthorne, NY or The Technicon Instrument Corp., Tarrytown, NY. Technical Operations Section of Executive Subcommittee D19.90 has supported this approval.

17. Interferences

17.1 Within the analytical range of this test method, there are no significant interferences, with the exception of nitrate at the 200 mg/L level.^{12,13}

17.2 Contamination from airborne nitrogen compounds will cause high results. Washing floors with ammonia solutions in the Kjeldahl nitrogen-analysis laboratory should be avoided.

18. Apparatus

18.1 *Continuous Segmented-Flow Automated Analysis System*, consisting of the following:

18.1.1 *Sampler*—40 samples per hour, 6 + 1, sample time 77 s, wash time 13 s.

18.1.2 *Proportioning Pump*.

18.1.3 *Analytical Cartridge*, with glassware and tubing per Fig. 1.

18.1.4 *Colorimeter*, equipped with a 50-mm tubular flow cell and 660 ± 10-nm filters.

18.1.5 *Recorder*.

18.2 *Block Digester*, capable of operating at 200 and 380°C.¹¹

18.3 *Mixer*, vortex.

NOTE 46—All connection nipples and the sample probe used within the automated system should be inert to acid solutions.

NOTE 7—Flow Injection Analysis has been shown to give comparable results to continuous Segmented-Flow Analysis. It is the users responsibility to validate the FIA method. See Appendix A for single laboratory supportive data.

19. Reagents and Materials

19.1 *Ammonia Solution, Stock* (1.0 mL = 1.0 mg ammonium nitrogen)—See 11.1.

19.2 *Ammonia Solution, Standard* (1.0 mL = 0.01 mg ammonium nitrogen)—See 11.2.

19.3 *Buffer Solution, Stock*—Dissolve 134 g of sodium phosphate, dibasic (Na₂HPO₄)·H₂O, or 71.0 g sodium phosphate anhydrous Na₂HPO₄, in 800 mL of water. Add 20 g of sodium hydroxide and dilute to 1 L.

19.4 *Buffer Solution, Working*—Combine the reagents in the following order: add 250 mL of stock sodium potassium tartrate solution (see 19.10) to 200 mL of stock buffer solution (see 19.3) and mix. Add 100 mL of sodium hydroxide (200 g/L solution) (see 19.7) and dilute to 1 L with water. Add 1 mL of Brij-35¹⁴, 30 % solution.

19.5 *Digestion Solution*—See 11.13.

19.6 *Mercuric Sulfate Solution*—See 11.4.

19.7 *Sodium Hydroxide Solution* (200 g/L)—Dissolve 200 g of NaOH in 800 mL of water, cool, and dilute to 1 L with water.

¹² EPA-600/7-77-017 report

¹² The block digester and continuous flow instruments capable of performing the digestion procedure are available from Environmental Protection Agency, 401 "M" St., S.W., Washington, DC 20406; Scientific Instrument Corp., Hawthorne, NY or The Technicon Instrument Corp, Tarrytown, NY.

¹³ Brij-35, a registered trademark product of Atlas Chemical Co., is

¹³ EPA-600/7-77-017 report available from Fisher Scientific, Pittsburgh, PA 15238; Environmental Protection Agency, 401 "M" St., S.W., Washington, DC 20406.

¹⁴ Supporting data are

¹⁴ Brij-35, a registered trademark product of Atlas Chemical Co., is available from ASTM Headquarters. Request RR:D19-1104; Fisher Scientific, Pittsburgh, PA 15238.

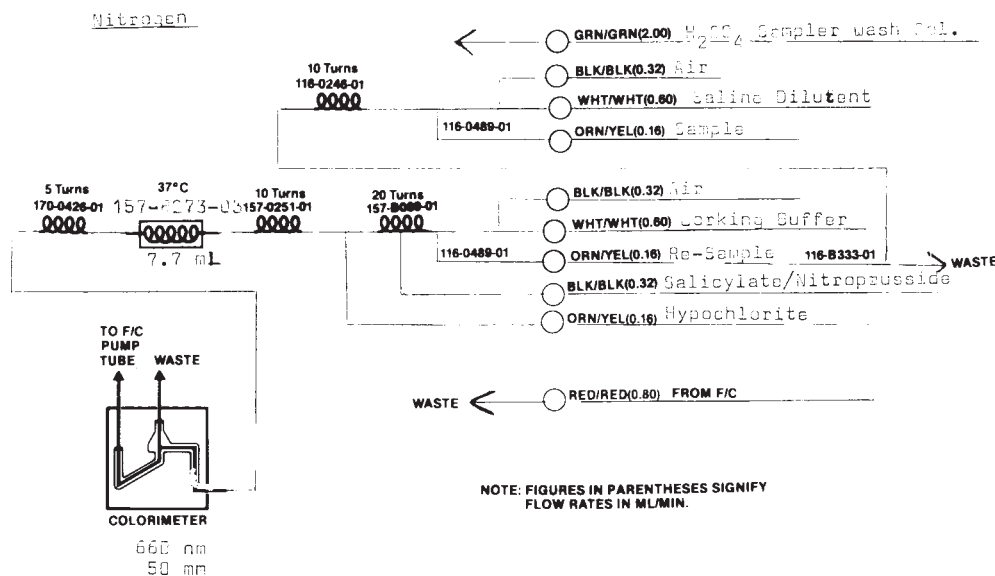


FIG. 1 Digestion Tube

19.8 *Sodium Hypochlorite Solution*—Dilute 6 mL of sodium hypochlorite (household bleach 5.25 %) solution to 100 mL with water. Add 0.2 mL (5 drops) of Brij-35 (30 % solution). Alternatively, 1 g of dichloroisocyanuric acid sodium salt dissolved in 1 L can be used as the chlorinating agent.

19.9 *Sodium Chloride Saline Diluent* (20 g/L)—Dissolve 20 g NaCl in 600 mL of water. Add 1 mL Brij-35, mix, and dilute to 1 L with water and mix.

19.10 *Sodium Potassium Tartrate Solution, Stock* (200 g/L)—Dissolve 200 g of sodium potassium tartrate in 800 mL of water and dilute to 1 L.

19.11 *Sodium Salicylate/Sodium Nitroprusside Solution*—Dissolve 75 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 mL of water and dilute to 1 L. Add 1 mL of Brij-35, 30 % solution.

19.12 *Sulfuric Acid Wash Solution*—Dissolve 31.7 g of potassium sulfate in 800 mL of water. Add slowly 48 mL of concentrated sulfuric acid (sp gr 1.84) and dilute to 1 L with water.

20. Calibration

20.1 Prepare standard solutions containing 0, 2.5, and 5 mg/L N by diluting appropriate aliquots of the ammonia standard solution to 100 mL with water.

NOTE 58—The analytical curve obtained by this procedure is linear to 5.0 mg/L.

20.2 Transfer by pipet 20 mL of the blank and each of the prepared standard solutions to separate block-digestor tubes. Digest solutions as described in Section 21.

20.3 Prepare an analytical curve by plotting peak heights of blank and standards versus concentration (mg/L TKN) on linear graph paper. Alternatively, electronic peak reading devices may be used to calibrate the instrument in concentration terms.

21. Procedure

21.1 Digestion:

21.1.1 Add 5 mL of digestion solution and 20 or 25 mL of a well-mixed sample into a digestion tube and mix, using a vortex mixer. Add 4 or 5 acid-washed TFE-fluorocarbon boiling stones.

NOTE 69—The same acid concentration is used in the sampler wash receptacle as in the digestion tube. See Fig. 1.

21.1.2 Place tube in digester that has been preheated to 200°C. Heat at 200°C for 1 h, raise the temperature to 380°C, and heat for an additional 1 ½ h (1½ h digestion at 380°C).

21.1.3 After 21.1.2 has been completed, cool sample (25 ± 5°C), add 20 mL of water, and mix thoroughly. Place an aliquot of this digested sample in a sample cup.

NOTE 710—High-temperature digestion of aqueous sample in sulfuric acid requires the use of an efficient fume hood with wash-down facilities.

21.2 Colorimetric Determination:

21.2.1 Check the level of all reagent containers to ensure an adequate supply of reagent to the automated system.

21.2.2 Except for the sodium salicylate/sodium nitroprusside solution line, place all reagent lines in their respective containers, connect the sample probe to the sampler, and start the proportioning pump.

21.2.3 Flush the sampler wash receptacle with sulfuric acid wash solution (see 19.12).

21.2.4 When reagents have been pumping for at least 5 min, place the sodium salicylate/sodium nitroprusside solution line in its respective container and allow the system to equilibrate.

21.2.5 After a stable baseline has been obtained, initiate sampler and analyze blank, standards, and samples.

22. Calculation

22.1 Determine the concentration of TKN (mg/L N) by referring the peak heights obtained for each sample to the prepared analytical curve (see 20.3).

22.2 Calculate the sample concentration using Eq 5:

$$\text{total Kjeldahl nitrogen, mg/L} = (A \times F) - B \quad (5)$$

where:

A = concentration mg/L, found in sample aliquot, mg/L,

B = concentration mg/L, found in blank, mg/L, and

F = dilution factor, if any.

23. Precision and Bias¹⁵

23.1 The collaborative test of the semiautomated colorimetric Berthelot test method was performed at three levels in a water

¹⁵ These test methods are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

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of choice and at four levels in reagent water (Type II). The analyses were conducted by six laboratories (six operators), each performing replicates for each level and water type of three separate days.

23.2 Recoveries of known amounts of total Kjeldahl nitrogen from reagent water Type II and selected water matrices for the same laboratories are described in Table 1.

23.3 These collaborative test data were obtained on reagent-grade water and various other selected water types (that is, sewage wastewater (municipal and industrial), estuary waters, and well water). For other matrices, these data may not apply.

23.4 Precision and bias for this test method conforms to Practice D 2777-77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of D 2777-98, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D-19 test methods.

24. Quality Control

24.1 In order to be certain that analytical values obtained using these test methods are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing total Kjeldahl nitrogen.

24.2 Calibration and Calibration Verification:

24.2.1 Analyze at least three working standards containing concentrations of total Kjeldahl nitrogen that bracket the expected sample concentration prior to analysis of samples to calibrate the instrument.

24.2.2 Verify instrument calibration after standardization by analyzing a standard at the concentration of one of the calibration standards. The absorbance shall fall within 4% of the absorbance from the calibration. Alternately, the concentration of a mid-range standard should fall within $\pm 10\%$ of the known concentration.

24.2.3 If calibration cannot be verified, recalibrate the instrument.

24.3 Initial Demonstration of Laboratory Capability:

24.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability.

24.3.2 Analyze seven replicates of a standard solution prepared from an Independent Reference Material containing a mid-range concentration of total Kjeldahl nitrogen. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps. The replicates may be interspersed with samples.

24.3.3 Calculate the mean and standard deviation of the seven values and compare to the acceptable ranges of bias in Table 1. This study should be repeated until the recoveries are within the limits given in Table 1. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

24.4 Laboratory Control Sample (LCS) :

24.4.1 To ensure that the test method is in control, analyze two LCS samples containing a mid-range concentration of total Kjeldahl nitrogen with each batch or 10 samples. If large numbers of samples are analyzed in the batch, analyze the LCS after every 10 samples. The LCS must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the LCS shall fall within $\pm 15\%$ of the known concentration.

NOTE 11—The first LCS sample should be ammonia, for which a low recovery would indicate the samples were over-digested. The second LCS sample should be a difficult to digest material, for which low recovery would indicate the samples were inadequately digested.

24.4.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all the samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

24.5 Method Blank:

24.5.1 Analyze a reagent water test blank with each batch. The concentration of total Kjeldahl nitrogen found in the blank should be less than 0.5 times the lowest calibration standard. If the concentration of total Kjeldahl nitrogen is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

TABLE 1 Recoveries of Known Amounts of Total Kjeldahl Nitrogen

	Amount Added, mg/L	Amount Found, mg/L	Precision, mg/L		% Bias	Statistically Significant (95 % Confidence Level)
			S_T	S_O		
Reagent water, Type II	0.10	0.17	0.13	0.07	+ 70	yes
	1.0	1.18	0.28	0.10	+ 18	yes
	2.0	2.05	0.13	0.11	+ 2.5	no
	4.0	3.94	0.13	0.17	-1.5	no
Water of choice	1.0	1.07	0.14	0.08	+ 7	no
	2.0	2.03	0.12	0.14	+ 1.5	no
	2.5	2.44	0.18	0.15	-2.4	no

24.6 Matrix Spike (MS):

24.6.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch by spiking an aliquot of the sample with a known concentration of total Kjeldahl nitrogen and taking it through the analytical method.

24.6.2 The spike concentration plus the background concentration of total Kjeldahl nitrogen must not exceed the high calibration standard. The spike must produce a concentration in the spiked sample that is 2 to 5 times the analyte concentration in the unspiked sample, or 10 to 50 times the detection limit of the test method, whichever is greater.

24.6.3 Calculate the percent recovery of the spike (P) using the following formula:

$$P = 100 [A(V_s + V) - B V_s] / C V \quad (6)$$

where:

- A = Analyte Concentration (mg/L) in Spiked Sample,
- B = Analyte Concentration (mg/L) in Unspiked Sample,
- C = Concentration (mg/L) of Analyte in Spiking Solution,
- V_s = Volume (mL) of Sample Used, and
- V = Volume (mL) added with Spike

24.6.4 The percent recovery of the spike shall fall within the limits, based on the analyte concentration, listed in Guide D 5810, Table 1. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. Note: acceptable spike recoveries are dependent on the concentration of the component of interest. See Guide 5810 for additional information.

24.7 Duplicate:

24.7.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch. If the concentration of the analyte is less than five times the detection limit for the analyte, a matrix spike duplicate (MSD) should be used.

24.7.2 Calculate the standard deviation of the duplicate values and compare to the precision in the collaborative study using an F test. Refer to 6.4.4 of Test Method D 5847 for information on applying the F test.

24.8 Independent Reference Material (IRM):

24.8.1 In order to verify the quantitative value produced by the test method, analyze an Independent Reference Material (IRM) submitted as a regular sample (if practical) to the laboratory at least once per quarter. The concentration of the reference material should be in the concentration mid-range for the method chosen. The value obtained must fall within the control limits established by the laboratory.

25. Keywords

245.1 analysis; colorimetric; distillation; TKN; water

APPENDIX

XI. FLOW INJECTION ANALYSIS SINGLE LABORATORY SUPPORTIVE DATA

X1.1 The following is a summary of LaChat Method 10-107-06-2-D. For the determination of Total Kjeldahl Nitrogen:

X1.1.1 The analytical range is 0.5 to 20.0 mg/L N.

X1.1.2 The method detection limit for Kjeldahl Nitrogen using a 0.5 mg/L N standard, MDL = 0.007 mg/L N. Standard Deviation (s) = 0.026 mg/L N, Mean (x) = 0.45 mg/L N, Known value = 0.50 mg/L N.

X1.1.3 Single laboratory precision study for Kjeldahl Nitrogen using a 5.00 mg/L N standard gives a % RSD = 1.1. Standard Deviation (s) = 0.056 mg/L N, Mean (X) = 5.15 mg/L N, Known Value = 5.00 mg/L N.

X1.1.4 The method detection limit and precision data does not conform to ASTM D 2777.

X1.1.5 It is the users responsibility to validate the FIA method.

X1.2 The following is a summary of LaChat Method 10-107-06-2-E. For the determination of Total Kjeldahl Nitrogen.

X1.2.1 The analytical range is 0.1 to 5.0 mg/L N.

X1.2.2 The method detection limit for Kjeldahl Nitrogen using a 0.1 mg/L N standard, MDL = 0.018 mg/L N. Standard Deviation = 0.0071, Mean (X) = 0.117 mg/L, Known Value = 0.1 mg/L

X1.2.3 Single laboratory precision study for Kjeldahl Nitrogen using a 1.00 mg/L N standard gives a % RSD = 0.63. Standard Deviation (s) = 0.0062, Mean (X) = 0.99, Known Value = 1.00 mg/L

X1.2.4 The method detection limit and precision data does not conform to Practice D 2777.

X1.2.5 It is the users responsibility to validate the FIA method.



SUMMARY OF CHANGES

This section identifies the locations of selected changes to these test methods that have been incorporated since the last issue. For the convenience of the user, Committee D-19 has highlighted those changes that may impact the use of these test methods. This section may also include descriptions of the changes or reasons for the changes, or both.

- (1) The potentiometric determination portion of Test Method A (Manual Digestion/Distillation) was reinstated.

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