

11.1.11

**AOAC Official Method 973.48
Nitrogen (Total) in Water
Kjeldahl Method
First Action 1973**

A. Principle

Specimen is digested with H₂SO₄ to convert organic N to NH₃, which is distilled after alkalization and determined by nesslerization or titrimetry. Preserve specimens by addition of 40 mg HgCl₂/L and store at 4 C. Analyze as soon as possible, as conversion of organic N to NH₃ may occur even with preservation.

Method is applicable to surface and saline waters and domestic and industrial wastes. Some industrial wastes containing materials such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines may not be converted to NH₃.

Natural water analyzed by 31 analysts in 20 laboratories showed results in Table 973.48 on exact increments of organic N.

B. Apparatus

(a) *Digestion apparatus.*—See 955.04B(a) (see 2.4.03)

(b) *Distillation apparatus.*—See 955.04B(b) (see 2.4.03) or use all-glass apparatus with 800 or 1000 mL distilling flask and 500 mL glass-stoppered Erlenmeyers, marked at 350 and 500 mL, as receivers. Prepare for use by distilling mixture of NaOH–Na₂S₂O₃ solution and H₂O (1 + 1) until distillate is NH₃-free by Nessler reagent, (j). Repeat each time apparatus is out of service 4 h.

(c) *Nessler tubes.*—Matched, ca 300 mm long, 17 mm id, and marked at 225 1.5 mm inside measurement from bottom.

(d) *Spectrophotometer or filter photometer.*—For use at 425 nm.

C. Reagents

(a) *Water.*—Distilled, NH₃-free. Pass through ion exchange column of mixed strongly acidic cation and strongly basic anion exchange resins. Regenerate resins according to manufacturer's instructions.

(b) *Mercuric sulfate solution.*—Dissolve 8 g red HgO in 50 mL H₂SO₄ (1 + 5) and dilute to 100 mL with H₂O.

(c) *Digestion solution.*—Dissolve 267 g K₂SO₄ in 1300 mL H₂O and add 400 mL H₂SO₄. Add 50 mL HgSO₄ solution, (b), and dilute to 2 L.

(d) *Sodium hydroxide–sodium thiosulfate solution.*—Dissolve 500 g NaOH and 25 g Na₂S₂O₃ 5H₂O in H₂O and dilute to 1 L.

(e) *Phenolphthalein indicator solution.*—Dissolve 5 g phenolphthalein in 500 mL alcohol or isopropanol and add 500 mL H₂O. Add 0.02M NaOH until faint pink.

(f) *Sulfuric acid standard solution.*—0.01M. Prepare and standardize as in 890.01 (see A.1.14). 1.00 mL = 0.28 mg N.

(g) *Ammonia standard solutions.*—(1) *Stock solution.*—1.00 mg N/mL. Dissolve 3.819 g NH₄Cl in H₂O and dilute to 1 L. (2) *Working solution.*—0.01 mg N/mL. Dilute 10 mL stock solution to 1 L.

(h) *Boric acid solution.*—Dissolve 20 g H₃BO₃ in H₂O and dilute to 1 L.

(i) *Mixed indicator.*—Mix 2 volumes 0.2% alcoholic methyl red with 1 volume 0.2% alcoholic methylene blue. Prepare fresh every 30 days. SDA 3-A or SDA 30 denatured alcohol may be used.

(j) *Nessler reagent.*—Dissolve 100 g HgI₂ and 70 g KI in small amount H₂O. Add slowly, with stirring, to cooled solution of 160 g NaOH in 500 mL H₂O, and dilute to 1 L. Reagent is stable 1 year if stored in Pyrex container out of direct sunlight. Reagent should give characteristic color, but no precipitate, with 0.04 mg NH₃-N in 50 mL H₂O within 10 min.

D. Digestion and Distillation

Determine specimen size as follows:

N/L, mg	Specimen, mL
0–5	500
5–10	250
10–20	100
20–50	50.0
50–100	25.0

Place specimen, or residue from NH₃ determination (for organic Kjeldahl N only), into 800 mL Kjeldahl flask. Dilute, if necessary, to 500 mL and add 100 mL digestion solution, (c). Boil until SO₃ fumes are evolved and test solution becomes colorless or pale yellow. Cool, and dilute with 300 mL H₂O. Add NaOH–Na₂S₂O₃ solution slowly down neck of tilted flask to underlay acid solution in amount sufficient to make final solution strongly alkaline as shown by phenolphthalein (60 mL NaOH–Na₂S₂O₃ solution will neutralize 20 mL H₂SO₄). Connect flask to condenser, with tip of condenser dipping into 50 mL 2% H₃BO₃ solution in 500 mL glass-stoppered Erlenmeyer. If test solution is to be titrated, 100 or 200 mL H₃BO₃ may be used. Mix solutions and distil 300 mL at 6–10 mL/min. If NH₃ concentration is 1 mg/L, determine titrimetrically, E; if less, determine colorimetrically, F.

E. Titrimetric Determination

Add 3 drops mixed indicator, C(i), to distillate and titrate with 0.01M H₂SO₄, (f), matching end point against blank containing same volume NH₃-free H₂O, H₃BO₃ solution, and indicator.

$$\text{mg Total N/L} = \frac{[(\text{mL standard H}_2\text{SO}_4 \text{ for test portion} - \text{mL standard H}_2\text{SO}_4 \text{ for reagent blank}) \times 2 \text{ molarity standard H}_2\text{SO}_4 \times 14.01 \times 1000]}{\text{mL test portion digested}}$$

Table 973.48. Statistical results for water containing exact increments of organic nitrogen

Method	Added, mg N/L	Standard deviation		Bias	
		%	mg N/L	%	mg N/L
Colorimetric	0.20	100	0.20	+15.5	0.03
Colorimetric	0.31	81	0.25	+5.5	0.02
Titrimetric	4.10	26	1.06	+1.0	0.04
Titrimetric	4.61	26	1.19	-1.7	-0.08

F. Colorimetric Determination

Prepare series of standards containing 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mL NH₃ working standard solution, (g)(2), diluted to 50 mL with NH₃-free H₂O (contains 0.0, 0.04, 0.10, 0.20, 0.30, 0.40, 0.60, and 0.80 mg NH₃ N/L). Add 1 mL Nessler reagent, (j), and mix. After 20 min, read A at 425 nm against 0.0 (blank) standard, and plot A against concentration to obtain standard curve. Distil one or

more high and low standard solutions daily to ensure adequate recoveries.

As estimated by preliminary determination, determine NH_3 in 50 mL aliquot, or aliquot diluted to 50 mL, as above, and read NH_3 concentration from standard curve.

$$\text{mg Total N/L} = \frac{[(\text{mg NH}_3\text{-N from curve} \times 1000)/\text{mL specimen taken for distillation}] \times (\text{mL final distillate, including H}_3\text{BO}_3 \text{ solution})}{\text{mL distillate taken for nesslerization}}$$

References: *Methods for Chemical Analysis of Water and Wastes* (1983) (available from National Technical Information Service, 5285 Port Royal Rd, Springfield, VA 22161, USA, Stock No. NTIS PB84-128677).
Method Study No. 2.
Nutrient Analyses, Manual Methods (1970) (available from National Technical Information Service, 5285 Port Royal Rd, Springfield, VA 22161, USA, NTIS PB230828/BE).
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