A.1.06

AOAC Official Method 936.15
Standard Solution of Hydrochloric Acid

First Action 1936
Final Action

A. Preparation of Standard Solutions

Table **936.15** gives approximate volumes of 36.5–38% HCl required to make 10 L standard solutions.

B. Standard Sodium Hydroxide Method

Titrate 40 mL against standard alkali solution, 936.16C–E (*see* A.1.12), of ca same concentration as acid being standardized in 300 mL flask that has been swept free from CO_2, using CO_2-free H_2O and 3 drops phenolphthalein.

$$\text{Molarity} = \frac{\text{mL standard alkali} \quad \text{molarity of alkali}}{\text{mL HCl}}$$

If more concentrated than desired, dilute solution to required molarity value by following formula:

$$V_1 = V_2 \quad M_2/M_1$$

where M_2 and V_2 represent molarity and volume of stock solution, respectively, and V_1 = volume to which stock solution should be diluted to obtain desired molarity, M_1.

Check exact concentration of final solution by titration as above. Molarity will be exact only if same indicator is used in determination as in standardization. Restandardize if indicators other than phenolphthalein are used.

References: *JAOAC* **19**, 107, 194(1936); **49**, 250(1966).
Kolthoff & Stenger, "Volumetric Analysis," **II**, 52(1947).

B. Constant Boiling Method

Dilute 822 mL HCl (36.5–38% HCl) with 750 mL H_2O. Check specific gravity with spindle and adjust to 1.10. Place 1.5 L in 2 L flat-bottom distilling flask, add ca 10 SiC grains (ca "20 mesh"), and connect to long, straight inner-tube condenser. Heat on electric hot plate and distil at 5–10 mL/min, keeping end of condenser open to air. When 1125 mL has distilled, change receivers and catch next 225 mL, which is constant boiling HCl, in Erlenmeyer with end of condenser inserted into flask, but above surface of liquid. Read barometer to nearest mm at beginning and end of collection of 225 mL portion and note barometer temperature. Average readings.

Calculate air weight in g (G) of this constant boiling HCl required to give one equivalent weight of HCl from one of following equations:

For P_0 = 540–669 mm Hg:

$$G = 162.255 + 0.02415\ P_0$$

For P_0 = 670–780 mm Hg:

$$G = 164.673 + 0.02039\ P_0$$

where P_0 = barometric pressure in mm Hg corrected to 0°C for expansion of Hg and of barometer scale. For brass scale barometer, following correction is accurate enough:

$$P_0 = P_t(1 - 0.000162t)$$

where t = barometer temperature in C.

Table 936.15. Volumes of concentrated HCl required to prepare solutions of different molarities

Approximate molarity	mL HCl to be diluted to 10 L
0.01	8.6
0.02	17.2
0.10	86.0
0.50	430.1
1.0	860.1

Weigh required amount of constant boiling HCl in tared, stoppered flask to at least 1 part in 10 000. Dilute immediately, and finally dilute to volume with CO_2-free H_2O at desired temperature.

References: *JAOAC* **25**, 653(1942); **36**, 96, 354(1953);
37, 122, 462(1954).

Standard Borax Method

C. Reagents

(**a**) *Methyl red indicator*.—Dissolve 100 mg methyl red in 60 mL alcohol and dilute with H_2O to 100 mL.

(**b**) *Reference solution*.—Prepare reference solution of H_3BO_3, NaCl, and indicator corresponding to composition and volume of solution at equivalence point. For use in determination of end point of titration with 0.1M HCl, reference solution should be 0.1M in H_3BO_3 and 0.05M in NaCl.

(**c**) *Standard borax*.—Saturate 300 mL H_2O at 55 C (not higher) with $Na_2B_4O_7{\cdot}10H_2O$ (ACS) (ca 45 g). Filter at this temperature through folded paper into 500 mL Erlenmeyer. Cool filtrate to ca 10 C, with continuous agitation during crystallization. Decant supernate, rinse precipitate once with 25 mL cold water, and dissolve crystals in just enough water at 55 C to ensure complete solution (ca 200 mL). Recrystallize by cooling to ca 10 C, agitating flask during crystallization.

Filter crystals onto small Büchner with suction, wash precipitate once with 25 mL ice-cold water, and dry crystals by washing with two 20 mL portions alcohol, drying after each washing with suction. Follow with two 20 mL portions ether. (Just before use, free alcohol and ether from any possible reacting acids by vigorously shaking each with 2–3 g of the pure, dry $Na_2B_4O_7{\cdot}10H_2O$ and then filtering.) Spread crystals on watch glass, immediately place dried $Na_2B_4O_7{\cdot}10H_2O$ in closed container over solution saturated with respect to both sucrose and NaCl, and let it remain ≥24 h before using. Then transfer the pure $Na_2B_4O_7{\cdot}10H_2O$ to glass-stoppered container and store in closed container over solution saturated with respect to both sucrose and NaCl (stable under these conditions 1 year).

D. Standardization

Accurately weigh enough standard $Na_2B_4O_7{\cdot}10H_2O$ to titrate ca 40 mL and transfer to 300 mL flask. Add 40 mL CO_2-free H_2O, 936.16B(**a**) (*see* A.1.12), and stopper flask. Swirl gently until sample dissolves. Add 4 drops methyl red and titrate with solution that is being standardized to equivalence point as indicated by reference solution.

$$\text{Molarity (mol/L)} = \text{g } Na_2B_4O_7{\cdot}10H_2O \quad 1000/\text{mL acid} \quad 190.69$$

Reference: *JAOAC* **22**, 102, 563(1939).

Standard Sodium Carbonate Method

E. Reagents

(**a**) *Methyl orange indicator.*—0.1% in H_2O.

(**b**) *Reference solution*.—80 mL CO_2-free H_2O containing 3 or 4 drops methyl orange.

(**c**) *Anhydrous sodium carbonate*.—Heat 250 mL H_2O to 80 C and add $NaHCO_3$ (ACS), stirring until no more dissolves. Filter solution through folded paper (use of hot water funnel is desirable) into Erlenmeyer. Cool filtrate to ca 10 C, swirling constantly during crystallization. Fine crystals of trona that separate out have approximate composition: $Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$. Decant supernate, drain crystals by suction, and wash once with cold water.

Transfer precipitate, being careful not to include any paper fibers, to large flat-bottom Pt dish. Heat 1 h at 290 C in electric oven or furnace with pyrometer control. Stir contents occasionally with Pt wire. Cool in desiccator. Place the anhydrous Na_2CO_3 in glass-stoppered container and store in desiccator containing efficient desiccant. Dry at 120 C and cool just before weighing.

References: Kolthoff & Stenger, "Volumetric Analysis," **II**, 80(1947).
Ind. Eng. Chem., *Anal. Ed*. **9**, 141(1937).
JAOAC **22**, 563(1939).

F. Standardization

Accurately weigh enough anhydrous Na_2CO_3, **E**(**c**), to titrate ca 40 mL, transfer to 300 mL Erlenmeyer, and dissolve in 40 mL H_2O. Add 3 drops methyl orange and titrate until color begins to deviate from H_2O tint (reference solution). (Equivalence point has not been reached.) Boil solution gently 2 min and cool. Titrate until color is barely different from H_2O tint of indicator.

$$\text{Molarity HCl, moles/L} = \\ \text{g } Na_2CO_3 \quad 1000/\text{mL HCl} \quad (105.988/2)$$

Reference: *JAOAC* **22**, 102, 563(1939).

Revised: June 2003

4.2.11

AOAC Official Method 2001.11
Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds

Block Digestion Method Using Copper Catalyst and Steam Distillation into Boric Acid

First Action 2001
Final Action 2005

[Applicable to the determination of 0.5–50% Kjeldahl N (3–300% equivalent crude protein) in forage, animal feed and pet food, grain, and oilseeds, and applicable to the same matrixes as 976.05 (*see* 4.2.05), 976.06 (*see* 4.2.06), 984.13 (*see* 4.2.09), 988.05 (*see* 4.2.03), and 990.02 (*see* 4.2.07); the method does not measure oxidized forms of N or heterocyclic N compounds.]

See Tables **2001.11A** and **B** for the results of the interlaboratory study, expressed on a protein basis (N 6.25), supporting acceptance of the method.

A. Principle

The material is digested in H_2SO_4 to convert the protein N to $(NH_4)_2SO_4$ at a boiling point elevated by the addition of K_2SO_4 with a Cu catalyst to enhance the reaction rate. Ammonia is liberated by alkaline steam distillation and quantified titrimetrically with standardized acid. Aluminum block heaters increase the efficiency of the digestion.

The digest must contain residual H_2SO_4 to retain the NH_3. Water is added manually or automatically to the digest to avoid mixing concentrated alkali with concentrated acid and to prevent the digest from solidifying. Concentrated NaOH is added to neutralize the acid and make the digest basic, and the liberated NH_3 is distilled into a boric acid solution and titrated with a stronger standardized acid, HCl, to a colorimetric endpoint. The same endpoint detection system (e.g., indicator, wavelength) must be used for the standardization of the HCl and for the analyte.

The analyte is referred to as "crude" protein because the method determines N, a component of all proteins. In addition, N from sources other than true protein is also determined. (Additional digestion procedures must be used in order to include N from nitrate.) The amount of protein in most materials is calculated by multiplying % N by 6.25, because most proteins contain 16% N.

The H_2SO_4 and NaOH used are in concentrated form and are highly corrosive. Wear gloves and eye protection while handling the chemicals. Do not mix concentrated acid and NaOH directly. If chemicals are splashed on the skin or in the eyes, flush with copious amounts of water. Seek medical attention. Do not breathe the sulfur oxide fumes produced during digestion.

B. Apparatus

(**a**) *Digestion block*.—Aluminum alloy block with adjustable temperature device for measuring and controlling block temperature (Tecator Digestion System 20, 1015 Digestor, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, USA; Tel: +1-952-974-9892, Fax: +1-952-974-9823, info@fossnorthamerica.com; or equivalent).

(**b**) *Digestion tubes*.—250 mL.

(**c**) *Distillation units*.—(*1*) *For steam distillation*.—Foss Tecator 2200, or equivalent, to accept 250 mL digestion tubes and 500 mL titration flasks. (*2*) *For steam distillation and autotitration*.—Foss Tecator 2300, or equivalent.

(**d**) *Titration flask*.—500 mL graduated Erlenmeyer flask (for collection and titration of distillate).

(**e**) *Fume exhaust manifold*.—With Teflon ring seals, connected to a water aspirator in a hooded sink.

(**f**) *Weighing paper.*—Low N, Alfie Packers No. 201 (Alfie Packers, Inc., 8901 J St, Ste 10, Omaha, NE 68127, USA), or Fisher 09-898-12A, 3 3 in. (76 76 mm), or equivalent.

(**g**) *Pipetting dispenser.*—25 mL, adjustable volume, attached to a 5 pint (2.4 L) acid bottle.

C. Reagents

(**a**) *Sulfuric acid.*—Concentrated, 95–98% H_2SO_4, reagent grade.

(**b**) *Catalyst*.—7.0 g K_2SO_4 + 0.8 g $CuSO_4$. (Commercially available in tablet form as 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ per tablet.)

(**c**) *Sodium hydroxide solution.*—40% (w/w) NaOH, low N (5 g N/g).

(**d**) *Methyl red indicator solution.*—Dissolve 100 mg methyl red in 100 mL methanol.

(**e**) *Bromocresol green indicator solution.*—Dissolve 100 mg bromocresol green in 100 mL methanol.

(**f**) *Boric acid solution.*—4% (w/v). Dissolve 400 g H_3BO_3 in 5–6 L hot deionized water. Mix and add more hot deionized water to a volume of about 9 L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Adjust to obtain a positive blank of 0.05–0.15 mL with 30 mL H_3BO_3 solution, using 0.1M NaOH (to increase blank) or 0.1M HCl (to decrease blank). Commercially available.

(**g**) *Boric acid solution.*—1% (w/v). (Optional trapping solution for titrators that automatically begin titration when distillation begins.) Dissolve 100 g H_3BO_3 in 5–6 L hot deionized water, mix, and add more hot deionized water to a volume of about 9 L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Commercially available.

(**h**) *Hydrochloric acid standard solution.*—0.1000M. Prepare as in 936.15 (*see* A.1.06) or use premade solution of certified specification range 0.0995–0.1005M, and use 0.1000M for calculation. Commercially available.

(**i**) *Reference standards.*—Ammonium sulfate, tryptophan, lysine·HCl, or glycine *p*-toluenesulfonic acid, for use as standard; 99.9%.

(**j**) *Sucrose.*—N-free.

D. Preparation of Analytical Sample

Grind dry laboratory sample to fineness of grind (ca 0.7–1 mm), which gives a relative standard deviation (RSD) of 2.0% for 10 successive determinations of N in ground mixture of corn grain and soybeans (2 + 1). Fineness required to achieve this precision must be used for all dry mixed feeds and other nonuniform materials. Mix liquids to uniformity.

E. Determination

(**a**) *Digestion.*—Turn on block digestor and heat to 420 C. Weigh materials, as indicated below, recording each test portion weight (W) to the nearest mg for weights of 1 g, and to the nearest 0.1 mg for weights of <1.0 g. Do not exceed 1.2 g. For materials with 3–25% protein, weigh approximately 1.0 g test portion; with 25–50% protein, approximately 0.5 g test portion; and >50% protein, approximately 0.3 g test portion.

Table 2001.11A. Interlaboratory study results for the determination of crude protein by block digestion with a copper catalyst and distillation into 4% boric acid

ID	No. of labs[a]	Mean, %	RSD_r, %	RSD_R, %	HorRat
Protein block	10(1)	40.19	0.45	0.76	0.333
Swine pellets	10(1)	37.04	0.47	0.60	0.256
Corn silage	11	7.10	1.64	2.16	0.726
Grass hay	11	7.11	1.94	1.94	0.650
Fish meal	11	64.67	0.73	0.98	0.460
Dog food	11	24.50	0.87	0.91	0.369
Chinchilla food	11	18.01	0.89	0.99	0.383
Albumin	10(1)	79.14	0.40	0.44	0.212
Birdseed	11	13.48	0.88	1.29	0.475
Meat and bone meal	11	50.06	1.90	1.90	0.857
Milk replacer	11	20.78	1.39	1.39	0.550
Soybeans	9(2)	38.76	0.49	0.54	0.236
Sunflower seeds	11	17.43	2.38	2.38	0.916
Legume hay	11	18.81	1.45	1.45	0.565

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

(*1*) *Dry feed, forage, cereal, grain, oilseeds*.—Weigh 1 g test portion of ground, well-mixed test portion onto a tared, low N weighing paper. Fold paper around material and drop into a numbered Kjeldahl tube.

(*2*) *Liquid feed.*—Weigh slightly >1 g test portion of well-mixed analytical sample into a small tared beaker. Quantitatively transfer to a numbered Kjeldahl tube with <20 mL deionized water. Alternatively, weigh slightly >1 g well-mixed test portion into a small tared beaker. Transfer to a numbered Kjeldahl tube and reweigh beaker. The differential weight loss corresponds to the amount of test portion actually transferred to the tube.

(**b**) *Standards.*—Perform quality control analysis and analyses of standards with each batch. The standards available from Hach Co. (PO Box 389, Loveland, CO 80539, USA; Tel: +1-800-227-4224 or +1-970-669-3050), Sigma (St. Louis, MO, USA), J.T. Baker (Phillipsburg, NJ, USA), the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) are listed in Table **2001.11C**.

The various ammonium salts and glycine *p*-toluenesulfonate serve primarily as a check on distillation efficiency and accuracy in titration steps because they are digested very readily. Lysine and nicotinic acid *p*-toluenesulfonate serve as a check on digestion efficiency because they are difficult to digest.

Include a reagent blank tube containing a folded low N weighing paper with each batch.

(**c**) *Digestion.*—Add 2 catalyst tablets to each tube. Add 12 mL H_2SO_4 to each tube, using pipetting dispenser; add 15 mL for high fat materials (>10% fat). Mixtures may be held overnight at this point. If mixture foams, slowly add 3 mL 30–35% H_2O_2. Let reaction subside in perchloric acid fume hood or in exhaust system.

Attach heat side shields to tube rack. Place fume manifold tightly on tubes, and turn water aspirator on completely. Place rack of tubes in preheated block. After 10 min, turn water aspirator down until acid fumes are just contained within exhaust hood. A condensation zone should be maintained within the tubes. After bulk of sulfur oxide fumes are produced during initial stages of digestion, reduce vacuum source to prevent loss of H_2SO_4. Digest additional 50 min. Total digestion time is approximately 60 min.

Turn digestor off. Remove rack of tubes with exhaust still in place, and put in the stand to cool for 10–20 min. Cooling can be increased by using commercial air blower or by placing in hood with hood sash pulled down to increase airflow across tubes. When fuming has stopped, remove manifold, and shut off aspirator. Remove side shields. Let tubes cool. Wearing gloves and eye protection, predilute digests manually before distilling. Carefully add a few milliliters of deionized water to each tube. If spattering occurs, the tubes are too hot. Let cool for a few more minutes. Add water to each tube to a total volume of approximately 80 mL (liquid level should be about half way between the 2 shelves of the tube rack). This is a convenient stopping point.

Table 2001.11B. Interlaboratory study results for the recovery of nitrogen from standard compounds by block digestion with a copper catalyst and distillation into boric acid

Compound	No. of labs[a]	Theoretical yield, % N	Avg. found, % N	Avg. rec., %	RSD_R, %	HorRat
Acetanilid	10(0)	10.36	10.37	100.1	1.50	0.53
Lysine HCl	10(0)	15.34	13.32	86.8	4.16	1.53
Tryptophan	10(0)	13.72	13.55	98.8	1.04	0.39

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

Table 2001.11C. Standards

Standard	Approximate weight, g	Theoretical yield, % N
Ammonium *p*-toluenesulfonate (Hach 22779-24)	0.5	7.402
Glycine *p*-toluenesulfonate (Hach 22780-24)	0.6	5.665
Nicotinic acid *p*-toluenesulfonate (Hach 22781-24)	0.2	4.743
Lysine monohydrochloride (Sigma L-5626)	0.1	15.34
Acetanilide (Baker A068-05)	0.3	10.36
Tryptophan (Sigma T 8659)	0.2	13.72
Ammonium salts		
Diammonium hydrogen phosphate (100% assay)	0.2	21.21
Ammonium chloride (100% assay)	0.2	26.18
Ammonium sulfate (100% assay)	0.2	21.2
Ammonium dihydrogen phosphate (NIST 200)	0.3	12.18
Citrus leaves (NIST 1572)	1.0	2.86
Urea (NIST 2141)	0.1	46.63

If digest solidifies, place tube containing diluted digest in block digester, and carefully warm with occasional swirling until salts dissolve. If distilling unit equipped with steam addition for equilibration is used, the manual dilution steps can be omitted. About 70 mL deionized water is then automatically added during the distillation cycle.

(**e**) *Distillation.*—Place 40% NaOH in alkali tank of distillation unit. Adjust volume dispensed to 50 mL. Attach digestion tube containing diluted digest to distillation unit, or use automatic dilution feature, if available. Place graduated 500 mL Erlenmeyer titration flask containing 30 mL H_3BO_3 solution with indicator on receiving platform, and immerse tube from condenser below surface of H_3BO_3 solution. (When an automatic titration system is used that begins titration immediately after distillation starts, 1% H_3BO_3 may be substituted.) Steam distill until 150 mL distillate is collected (180 mL total volume). Remove receiving flask. Titrate H_3BO_3 receiving solution with standard 0.1000M HCl to violet endpoint (just before the solution goes back to pink). Lighted stir plate may aid visualization of endpoint. Record milliliters of HCl to at least the nearest 0.05 mL.

This is done automatically by using a steam distiller with automatic titration. Follow the manufacturer's instructions for operation of the specific distiller or distiller/titrator.

F. Verification of Nitrogen Recovery

Run N recoveries to check accuracy of procedure and equipment.

(**a**) *Nitrogen loss.*—Use 0.12 g $(NH_4)_2SO_4$ and 0.67 g sucrose per flask. Add all other reagents as in **E**, and distill under same conditions as in **E**. Recoveries must be 99%.

(**b**) *Distillation and titration efficiency.*—Distill 0.12 g $(NH_4)_2SO_4$, omitting digestion. Recoveries must be 99.5%.

(**c**) *Digestion efficiency.*—Use 0.3 g acetanilide or 0.18 g tryptophan, with 0.67 g sucrose per flask. Add all other reagents as stated in **E**. Digest and distill under same conditions as used for a determination. Recoveries must be 98%.

G. Calculations

$$\text{Kjeldahl nitrogen, \%} = \frac{(V_S \quad V_B) \quad M \quad 14.01}{W \quad 10}$$

$$\text{Crude protein, \%} = \text{\% Kjeldahl N} \quad F$$

where V_S = volume (mL) of standardized acid used to titrate a test; V_B = volume (mL) of standardized acid used to titrate reagent blank; M = molarity of standard HCl; 14.01 = atomic weight of N; W = weight (g) of test portion or standard; 10 = factor to convert mg/g to percent; and F = factor to convert N to protein.

F factors are 5.70 for wheat, 6.38 for dairy products, and 6.25 for other feed materials.

Reference: J. AOAC Int. 85, 309(2002).

AGRICULTURAL MATERIALS

Determination of Crude Protein in Animal Feed, Forage, Grain, and Oilseeds by Using Block Digestion with a Copper Catalyst and Steam Distillation into Boric Acid: Collaborative Study

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A collaborative study was conducted to evaluate the repeatability and reproducibility of an extension of AOAC Official Method 991.20, Nitrogen (Crude) in Milk, to animal feed, forage (plant tissue), grain, and oilseed materials. Test portions are digested in an aluminum block at 420°C in sulfuric acid with potassium sulfate and a copper catalyst. Digests are cooled and diluted, and concentrated sodium hydroxide is added to neutralize the acid and make the digest basic; the liberated ammonia is distilled by using steam distillation. The liberated ammonia is trapped in a weak boric acid solution and titrated with a stronger standardized acid, hydrochloric acid; colorimetric endpoint detection is used. Fourteen blind samples were sent to 13 collaborators in the United States, Denmark, Sweden, Germany, and the United Kingdom. Recoveries of nitrogen from lysine, tryptophan, and acetanilide were 86.8, 98.8, and 100.1%, respectively. The within-laboratory relative standard deviation (RSD_r, repeatability) ranged from 0.40 to 2.38% for crude protein. The among-laboratories (including within-) relative standard deviation (RSD_R, reproducibility) ranged from 0.44 to 2.38%. It is recommended that the method be adopted First Action by AOAC INTERNATIONAL. A lower concentration (1% H_3BO_3) of trapping solution was compared with the concentration specified in the original protocol (4% H_3BO_3) and was found comparable for use in an automatic titration system in which titration begins automatically as soon as distillation starts. The Study Directors recommend that 1% H_3BO_3 as an optional alternative to 4% boric acid trapping solution be allowed for automatic titrators that titrate throughout the distillation.

The Danish chemist Johan Kjeldahl (1849–1900) developed what today is well known as the Kjeldahl method for determining nitrogen in organic substances. The original method as presented by Kjeldahl has been continuously improved. These developments have improved environmental and personal safety aspects, increased the speed and versatility of the method, and simplified the entire analytical procedure.

The Kjeldahl method has 3 different steps: digestion, distillation, and titration. Traditionally Kjeldahl flasks with a capacity for 500–800 mL and gas or electric heating have been used for the digestion. For distillation, the addition of water and alkali to the digested sample has been followed by the heating of the flask in order to distill >150 mL distillate. The distilled ammonia has been captured in standardized acid, and back titration with standardized sodium hydroxide has been used.

In 1970, the Swedish chemist Roger Mossberg introduced the modern concept of using a block digestor, which decreased the use of chemicals and improved the efficiency of the digestion. The method was further improved in 1974 by the introduction of direct steam distillation, which drastically decreased the time needed for distillation. Because the distillate was captured in a boric acid receiver, the need to standardize 1 reagent was eliminated.

Today, this method is universally accepted and used in tens of thousands of laboratories throughout the world. However, when it comes to the official status of the method, the situation is somewhat complicated. In selected cases, official standard methods are available, describing the Kjeldahl method (Inter-

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Submitted for publication September 2001.

The recommendation was approved by the Methods Committee on Feeds, Fertilizers, and Related Agricultural Topics as First Action. *See* “Official Methods Program Actions,” (2001) *Inside Laboratory Management*, November/December issue.

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national Organization for Standardization, Deutsches Institut fur Normung, American National Standards Institute, American Society for Testing and Materials, and AOAC INTERNATIONAL). A review of the available AOAC procedures for the Kjeldahl method shows that the laboratory that wants to follow an official procedure closely has a problem when trying to use this method. Basically it is approved only for dairy products and meat and meat products (1). For most other sample types, traditional Kjeldahl flask digestion followed by back titration of standard acid or block digestions followed by colorimetric detection are still the only approved Kjeldahl methodologies (2–9). For most agricultural sample types, the use of copper catalysts instead of mercury catalysts has been approved, thereby minimizing the environmental hazards of the method.

Today most laboratories implement quality assurance/quality control procedures to improve both laboratory efficiency and the quality of the data they generate. To support this improvement, it is important that officially approved methods are updated so that available technology can be used in the laboratory.

A method based on block digestion/steam distillation/boric acid trapping solution having a wide scope of applicability to agricultural products would definitely fulfill a need in the international laboratory community.

Comparability Study

Results of comparability studies of the proposed method and methods having AOAC Official status are provided in Table 1.

Table 1. Results (%) of the comparability studies of the copper/boric acid Kjeldahl method

Sample	Proposed method	AOAC **976.06H**[a]	AOAC **990.03**[b]
	Nitrogen		
Lysine	14.99	15.266	
Tryptophan	13.58	13.67	
Nicotinic acid	2.37	3.04	
	Crude protein		
Protein block	39.74	39.44	40.53
Meat and bone meal	50.75	48.53	50.86
Albumin	80.73	79.92	79.99
Grass hay	7.3	7.03	7.11
Milk replacer	21.31	21.68	21.21
Birdseed	13.52	13.35	13.2
Dog food	24.62	24.64	24.35
Soybeans	38.42	38.40	38.3
Chinchilla food	18.19	18.14	18.01
Corn silage green chop	7.26	7.17	6.95
Legume hay	18.92	18.91	18.78
Swine pellets	37.32	37.05	37.26
Sunflower seed	16.55	17.05	17.19
Fish meal	64.48	64.24	65.40
Avg. for crude protein	31.37	31.11	31.37

[a] Ref. 3.

[b] Ref. 10.

Collaborative Study

The Study Director with the cooperation of 9 additional collaborating laboratories conducted the collaborative study. These laboratories represented a variety of laboratory types, including research laboratories, commercial laboratories, manufacturers/industry laboratories, and state regulatory laboratories. Four laboratories were from outside the United States (2 Swedish, 1 Danish, and 1 Scottish). Participants received no compensation.

Collaborating laboratories provided the digestion block, digestion block tubes, distillation unit, burette/titrator, sulfuric acid, and sodium hydroxide. Collaborators were provided with sufficient portions of the test samples, Kjeldahl catalyst, boric acid trapping solution, standardized hydrochloric acid titrant, and weighing paper.

Collaborating laboratories were asked to analyze 14 animal feed, pet food, and feed ingredient materials (including forages, grain, and oilseed materials) as blind duplicate pairs. A blank material (weighing paper) and 3 recovery materials were also shipped, for a total of 32 samples. Familiarization samples were sent to each collaborator to be analyzed before the study samples to acquaint collaborators with the methods and to ensure that each laboratory was capable of handling the study samples.

Study materials were chosen to be representative of different animal feeds, pet foods, feed ingredients, forage, cereal grains, and oilseeds. The categories that each material represents are provided in Table 2. The samples were also chosen to be robust with respect to carbohydrate and fiber concentrations, with respect to species, and with respect to ingredients. All materials used for the study were natural or “real world”; none were spiked. The nitrogen content of the samples ranged from 1 to about 15% (7% crude protein to about 80% equivalent crude protein). Samples were coded at random so that there was no preselection from the order of presentation. Approximately 20 g of each sample material was supplied, which was more than the amount needed to complete the study.

The chinchilla food, birdseed, and swine pellets were ground to pass through a 0.75 mm screen in a Retsch ZM 100 Ultra Centrifugal mill (Retsch, Haan, Germany) with a flow-through attachment. The soybeans, dog food, sunflower seed, and meat and bone meal were ground in a Foss Tecator Knifetec mill (Foss North America, Eden Prairie, MN). The grass hay, alfalfa hay, and corn silage green chop were ground to pass through a 1.0 mm screen in a Tecator Cyclotec mill. The milk replacer, albumin, and fish meal were split without

Table 2. Categories represented by each collaborative study material and respective RSD values obtained for the determination of crude protein in duplicate analyses of 3 random bags of each study material

	Category				
Material	Animal feed	Forage	Grain	Oilseed	RSD, %
Meat and bone meal	X				0.25
Dog food	X		X		0.24
Chinchilla food	X	X	X		0.20
Birdseed	X		X		1.11
Soybeans	X			X	0.64
Corn silage green chop	X	X	X		0.90
Grass hay	X	X			0.91
Alfalfa hay	X	X			0.31
Milk replacer	X				0.79
Albumin	X				0.29
Swine pellets	X		X		0.65
Sunflower seed	X			X	1.82
Protein block (with urea)	X		X		0.39
Fish meal	X				0.09

grinding. No further grinding by the collaborating laboratories was necessary for any of the study materials.

The homogeneity/uniformity of the samples sets was verified by selecting 3 bags at random for each material and analyzing them in duplicate by the proposed method in the Study Director's laboratory. The relative standard deviations (RSDs) are reported in Table 2. An RSD of approximately 2.0% was considered acceptable for the study materials. Only the suitability of the meat and bone meal sample was questioned. When the tests were repeated on a second day, the results improved and the sample was retained for the collaborative study.

Collaborators were asked to perform single analyses, to report data on an "as is" basis, and to report data to 4 significant figures.

AOAC Official Method 2001.11
Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds

Block Digestion Method Using Copper Catalyst and Steam Distillation into Boric Acid
First Action 2001

[Applicable to the determination of 0.5–50% Kjeldahl N (3–300% equivalent crude protein) in forage, animal feed and pet food, grain, and oilseeds, and applicable to the same matrixes as **976.05** (4.2.05), **976.06** (4.2.06), **984.13** (4.2.09), **988.05** (4.2.03), and **990.02** (4.2.07); the method does not measure oxidized forms of N or heterocyclic N compounds.]

See Tables **2001.11A** and **B** for the results of the interlaboratory study, expressed on a protein basis (N × 6.25), supporting acceptance of the method.

A. Principle

The material is digested in H_2SO_4 to convert the protein N to $(NH_4)_2SO_4$ at a boiling point elevated by the addition of K_2SO_4 with a Cu catalyst to enhance the reaction rate. Ammonia is liberated by alkaline steam distillation and quantified titrimetrically with standardized acid. Aluminum block heaters increase the efficiency of the digestion.

The digest must contain residual H_2SO_4 to retain the NH_3. Water is added manually or automatically to the digest to avoid mixing concentrated alkali with concentrated acid and to prevent the digest from solidifying. Concentrated NaOH is added to neutralize the acid and make the digest basic, and the liberated NH_3 is distilled into a boric acid solution and titrated with a stronger standardized acid, HCl, to a colorimetric endpoint. The same endpoint detection system (e.g., indicator, wavelength) must be used for the standardization of the HCl and for the analyte.

The analyte is referred to as "crude" protein because the method determines N, a component of all proteins. In addition, N from sources other than true protein is also determined. (Additional digestion procedures must be used in order to include N from nitrate.) The amount of protein in most materials is calculated by multiplying % N by 6.25, because most proteins contain 16% N.

The H_2SO_4 and NaOH used are in concentrated form and are highly corrosive. Wear gloves and eye protection while handling the chemicals. Do not mix concentrated acid and NaOH directly. If chemicals are splashed on the skin or in the eyes, flush with copious amounts of water. Seek medical attention. Do not breathe the sulfur oxide fumes produced during digestion.

B. Apparatus

(**a**) *Digestion block*.—Aluminum alloy block with adjustable temperature device for measuring and controlling block temperature (Tecator Digestion System 20, 1015 Digestor, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, USA; +1-952-974-9892, Fax: +1-952-974-9823, info@fossnorthamerica.com; or equivalent).

(**b**) *Digestion tubes*.—250 mL.

(**c**) *Distillation units*.—(*1*) *For steam distillation*.—Foss Tecator 2200, or equivalent, to accept 250 mL digestion tubes and 500 mL titration flasks. (*2*) *For steam distillation and autotitration*.—Foss Tecator 2300, or equivalent.

(**d**) *Titration flask*.—500 mL graduated Erlenmeyer flask (for collection and titration of distillate).

(**e**) *Fume exhaust manifold*.—With Teflon ring seals, connected to a water aspirator in a hooded sink.

(**f**) *Weighing paper*.—Low N, Alfie Packers No. 201 (Alfie Packers, Inc., 8901 J St, Ste 10, Omaha, NE 68127, USA), or Fisher 09-898-12A, 3 × 3 in. (76 × 76 mm), or equivalent.

(**g**) *Pipetting dispenser*.—25 mL, adjustable volume, attached to a 5 pint (2.4 L) acid bottle.

Table 2001.11A. Interlaboratory study results for the determination of crude protein by block digestion with a copper catalyst and distillation into 4% boric acid

ID	No. of labs[a]	Mean, %	RSD_r, %	RSD_R, %	HORRAT
Protein block	10(1)	40.19	0.45	0.76	0.333
Swine pellets	10(1)	37.04	0.47	0.60	0.256
Corn silage	11	7.10	1.64	2.16	0.726
Grass hay	11	7.11	1.94	1.94	0.650
Fish meal	11	64.67	0.73	0.98	0.460
Dog food	11	24.50	0.87	0.91	0.369
Chinchilla food	11	18.01	0.89	0.99	0.383
Albumin	10(1)	79.14	0.40	0.44	0.212
Birdseed	11	13.48	0.88	1.29	0.475
Meat and bone meal	11	50.06	1.90	1.90	0.857
Milk replacer	11	20.78	1.39	1.39	0.550
Soybeans	9(2)	38.76	0.49	0.54	0.236
Sunflower seeds	11	17.43	2.38	2.38	0.916
Legume hay	11	18.81	1.45	1.45	0.565

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

C. Reagents

(**a**) *Sulfuric acid.*—Concentrated, 95–98% H_2SO_4, reagent grade.

(**b**) *Catalyst.*—7.0 g K_2SO_4 + 0.8 g $CuSO_4$. (Commercially available in tablet form as 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ per tablet.)

(**c**) *Sodium hydroxide solution.*—40% (w/w) NaOH, low N (≤5 μg N/g).

(**d**) *Methyl red indicator solution.*—Dissolve 100 mg methyl red in 100 mL methanol.

(**e**) *Bromocresol green indicator solution.*—Dissolve 100 mg bromocresol green in 100 mL methanol.

(**f**) *Boric acid solution.*—4% (w/v). Dissolve 400 g H_3BO_3 in 5–6 L hot deionized water. Mix and add more hot deionized water to a volume of about 9 L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Adjust to obtain a positive blank of 0.05–0.15 mL with 30 mL H_3BO_3 solution, using 0.1M NaOH (to increase blank) or 0.1M HCl (to decrease blank). Commercially available.

(**g**) *Boric acid solution.*—1% (w/v). (Optional trapping solution for titrators that automatically begin titration when distillation begins.) Dissolve 100 g H_3BO_3 in 5–6 L hot deionized water, mix, and add more hot deionized water to a volume of about 9 L. Cool to room temperature, add 100 mL bromocresol green solution and 70 mL methyl red solution, and dilute to a final volume of 10 L. Commercially available.

(**h**) *Hydrochloric acid standard solution.*—0.1000M. Prepare as in **936.15** (*see* A.1.06) or use premade solution of certified specification range 0.0995–0.1005M, and use 0.1000M for calculation. Commercially available.

(**i**) *Reference standards.*—Ammonium sulfate, tryptophan, lysine·HCl, or glycine *p*-toluenesulfonic acid, for use as standard; 99.9%.

(**j**) *Sucrose.*—N-free.

D. Preparation of Analytical Sample

Grind dry laboratory sample to fineness of grind (ca 0.7–1 mm), which gives a relative standard deviation (RSD) of ≤2.0% for 10 successive determinations of N in ground

Table 2001.11B. Interlaboratory study results for the recovery of nitrogen from standard compounds by block digestion with a copper catalyst and distillation into boric acid

Compound	No. of labs[a]	Theoretical yield, % N	Avg. found, % N	Avg. rec., %	RSD_R, %	HORRAT
Acetanilid	10(0)	10.36	10.37	100.1	1.50	0.53
Lysine·HCl	10(0)	15.34	13.32	86.8	4.16	1.53
Tryptophan	10(0)	13.72	13.55	98.8	1.04	0.39

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

mixture of corn grain and soybeans (2 + 1). Fineness required to achieve this precision must be used for all dry mixed feeds and other nonuniform materials. Mix liquids to uniformity.

E. Determination

(**a**) *Digestion.*—Turn on block digestor and heat to 420°C. Weigh materials, as indicated below, recording each test portion weight (W) to the nearest mg for weights of ≥1 g, and to the nearest 0.1 mg for weights of <1.0 g. Do not exceed 1.2 g. For materials with 3–25% protein, weigh approximately 1.0 g test portion; with 25–50% protein, approximately 0.5 g test portion; and >50% protein, approximately 0.3 g test portion.

(*1*) *Dry feed, forage, cereal, grain, oilseeds*.—Weigh 1 g test portion of ground, well-mixed test portion onto a tared, low N weighing paper. Fold paper around material and drop into a numbered Kjeldahl tube.

(*2*) *Liquid feed.*—Weigh slightly >1 g test portion of well-mixed analytical sample into a small tared beaker. Quantitatively transfer to a numbered Kjeldahl tube with <20 mL deionized water. Alternatively, weigh slightly >1 g well-mixed test portion into a small tared beaker. Transfer to a numbered Kjeldahl tube and reweigh beaker. The differential weight loss corresponds to the amount of test portion actually transferred to the tube.

(**b**) *Standards.*—Perform quality control analysis and analyses of standards with each batch. The standards available from Hach Co. (PO Box 389, Loveland, CO 80539, USA; +1-800-227-4224 or +1- 970-669-3050), Sigma (St. Louis, MO), J.T. Baker (Phillipsburg, NJ), the National Institute of Standards and Technology (NIST; Gaithersburg, MD) are listed in Table **2001.11C**.

The various ammonium salts and glycine *p*-toluenesulfonate serve primarily as a check on distillation efficiency and accuracy in titration steps because they are digested very readily. Lysine and nicotinic acid *p*-toluenesulfonate serve as a check on digestion efficiency because they are difficult to digest.

Include a reagent blank tube containing a folded low N weighing paper with each batch.

(**c**) *Digestion.*—Add 2 catalyst tablets to each tube. Add 12 mL H_2SO_4 to each tube, using pipetting dispenser; add 15 mL for high fat materials (>10% fat). Mixtures may be held overnight at this point. If mixture foams, slowly add 3 mL 30–35% H_2O_2. Let reaction subside in perchloric acid fume hood or in exhaust system.

Attach heat side shields to tube rack. Place fume manifold tightly on tubes, and turn water aspirator on completely. Place rack of tubes in preheated block. After 10 min, turn water aspirator down until acid fumes are just contained within exhaust hood. A condensation zone should be maintained within the tubes. After bulk of sulfur oxide fumes are produced during initial stages of digestion, reduce vacuum source to prevent loss of H_2SO_4. Digest additional 50 min. Total digestion time is approximately 60 min.

Turn digestor off. Remove rack of tubes with exhaust still in place, and put in the stand to cool for 10–20 min. Cooling can be increased by using commercial air blower or by placing in hood with hood sash pulled down to increase airflow across tubes. When fuming has stopped, remove manifold, and shut off aspirator. Remove side shields. Let tubes cool. Wearing gloves and eye protection, predilute digests manually before distilling. Carefully add a few milliliters of deionized water to each tube. If spattering occurs, the tubes are too hot. Let cool for a few more minutes. Add water to each tube to a total volume of approximately 80 mL (liquid level should be about half way between the 2 shelves of the tube rack). This is a convenient stopping point.

Table 2001.11C. Standards

Standard	Approximate weight, g	Theoretical yield, % N
Ammonium *p*-toluenesulfonate (Hach 22779-24)	0.5	7.402
Glycine *p*-toluenesulfonate (Hach 22780-24)	0.6	5.665
Nicotinic acid *p*-toluenesulfonate (Hach 22781-24)	0.2	4.743
Lysine monohydrochloride (Sigma L-5626)	0.1	15.34
Acetanilide (Baker A068-05)	0.3	10.36
Tryptophan (Sigma T 8659)	0.2	13.72
Ammonium salts		
Diammonium hydrogen phosphate (100% assay)	0.2	21.21
Ammonium chloride (100% assay)	0.2	26.18
Ammonium sulfate (100% assay)	0.2	21.2
Ammonium dihydrogen phosphate (NIST 200)	0.3	12.18
Citrus leaves (NIST 1572)	1.0	2.86
Urea (NIST 2141)	0.1	46.63

If digest solidifies, place tube containing diluted digest in block digester, and carefully warm with occasional swirling until salts dissolve. If distilling unit equipped with steam addition for equilibration is used, the manual dilution steps can be omitted. About 70 mL deionized water is then automatically added during the distillation cycle.

(**e**) *Distillation.*—Place 40% NaOH in alkali tank of distillation unit. Adjust volume dispensed to 50 mL. Attach digestion tube containing diluted digest to distillation unit, or use automatic dilution feature, if available. Place graduated 500 mL Erlenmeyer titration flask containing 30 mL H_3BO_3 solution with indicator on receiving platform, and immerse tube from condenser below surface of H_3BO_3 solution. (When an automatic titration system is used that begins titration immediately after distillation starts, 1% H_3BO_3 may be substituted.) Steam distill until ≥150 mL distillate is collected (≥180 mL total volume). Remove receiving flask. Titrate H_3BO_3 receiving solution with standard 0.1000M HCl to violet endpoint (just before the solution goes back to pink).

Table 3. Collaborative study results for the determination of crude protein in animal feed, forage (plant tissue), and oilseeds

Lab	Crude protein, %[a]																											
	Protein block		Swine pellets		Corn silage		Grass hay		Fish meal		Dog food		Chinchilla food		Albumin		Birdseed		Meat and bone meal		Milk replacer		Soybeans		Sunflower seed		Legume hay	
	A(1)	B(13)	A(2)	B(10)	A(3)	B(23)	A(4)	B(24)	A(5)	B(9)	A(6)	B(25)	A(7)	B(26)	A(8)	B(27)	A(11)	B(22)	A(12)	B(17)	A(14)	B(21)	A(15)	B(18)	A(16)	B(19)	A(20)	B(28)
1	39.93	40.31	36.95	37.14	7.23	7.04	7.18	7.11	64.63	64.81	24.75	24.53	18.15	18.08	79.09	79.59	13.62	13.46	50.41	50.65	20.86	20.56	38.77	38.74	17.58	17.42	18.77	18.79
2	41.25[b]	39.80[b]	36.86[b]	38.72[b]	7.09	7.22	7.15	6.98	63.79	63.73	24.05	24.58	17.75[b]	17.60[b]	79.65	79.58	13.71	13.67	50.01	48.22	20.54	20.93	38.90[b]	40.26[b]	18.21	17.08	18.56	18.94
3	40.51	40.70	37.17	36.88	6.93	6.95	6.97	7.09	65.40	65.14	24.36	23.96	17.91	18.07	79.28	79.57	13.43	13.42	50.27	50.06	20.69	21.03	38.87	39.07	17.05	17.03	18.84	18.58
4	39.62	39.96	36.83	36.90	7.33	7.03	7.15	7.07	65.65	64.04	24.48	24.44	18.05	18.15	79.10	79.32	13.42	13.48	49.42	49.86	20.70	20.52	38.87	38.69	17.48	17.51	19.14	18.90
5	40.33	40.22	36.62	36.61	6.88	6.95	7.24	6.88	65.01	65.24	24.77	24.69	18.16	17.95	80.58[c]	80.84[c]	13.11[d]	13.07[d]	51.17	48.85	21.07	20.17	38.01	39.36	17.58	17.11	18.34	18.80
6	40.12	39.93	36.92	37.38	7.42	7.34	7.36	7.21	63.36	64.02	24.51	24.67	17.71	18.28	79.14	78.31	13.68	13.27	49.47	50.65	20.70	21.19	38.92	39.16	17.49	17.48	18.83	19.12
7	39.65	40.03	37.16	37.11	7.14	6.93	7.01	7.19	65.07	64.90	24.41	24.58	18.06	18.13	78.81	78.99	13.55	13.43	49.26	50.34	21.08	20.91	38.74	38.70	17.91	17.44	18.63	19.00
8	40.22	40.40	37.22	37.04	7.22	6.92	7.22	7.13	64.55	64.92	24.48	24.49	18.16	18.20	78.96	78.90	13.46	13.60	49.03	51.72	20.77	20.80	38.70	38.74	17.19	17.46	18.76	18.94
8	40.24[e]	40.28[e]	37.07[e]	36.92[e]	7.09[e]	7.08[e]	7.04[e]	*7.14*[e]	63.69[e]	64.09[e]	24.67[e]	24.57[e]	18.11[e]	18.18[e]	78.48[e]	78.89[e]	13.50[e]	13.56[e]	50.33[e]	51.02[e]	20.89[e]	20.92[e]	38.49[e]	39.06[e]	17.33[e]	17.29[e]	18.85[e]	19.11[e]
9	34.71[f]	35.62[f]	32.59[f]	32.08[f]	6.89[f]	6.46[f]	6.11[f]	6.46[f]	56.08[f]	56.30[f]	21.44[f]	22.09[f]	15.98[f]	16.33[f]	68.73[f]	70.93[f]	12.28[f]	12.41[f]	44.04[f]	43.34[f]	18.24[f]	18.77[f]	33.22[f]	34.34[f]	15.44[f]	9.21[f]	16.75[f]	17.15[f]
10	40.45	40.14	37.16	37.18	7.06	7.10	7.24	6.83	65.07	65.37	24.44	24.93	17.71[c]	18.01[c]	79.34	79.07	13.80	13.53	49.56	49.90	20.56	20.99	38.91	38.22	17.01	18.36	19.44	18.63
11	40.63	40.58	37.00	37.48	7.21	7.17	7.14	7.11	64.53	65.15	24.63	24.51	17.99	18.03	79.37	78.56	13.45	13.44	51.37	50.30	20.96	20.63	38.49	38.55	17.41	17.11	18.61	19.11
12	39.99	40.16	37.06	36.95	7.06	6.99	7.05	7.07	63.64	64.71	24.20	24.63	17.98	18.16	79.30	78.91	13.44	13.41	50.50	50.32	20.94	20.65	38.68	38.83	17.11	17.36	18.87	18.81

[a] A and B are blind duplicates; sample numbers are shown in parentheses.

[b] Excluded by the Cochran test; *P* = 2.5% (1-tailed), both when 1% boric acid results are included and excluded.

[c] Excluded by the Grubbs test; *P* = 2.5% (2-tailed); *P* = 1.25% (1-tailed), both when 1% boric acid results are included and excluded.

[d] Excluded by the Grubbs test; *P* = 2.5% (2-tailed); *P* = 1.25% (1-tailed), only when 1% boric acid results are included.

[e] Value obtained by using 1% boric acid trapping solution in place of 4% boric acid trapping solution.

[f] Excluded by the laboratory ranking test described by Youden and Steiner (11).

Table 4. Results of the laboratory ranking tests

Laboratory	Laboratory ranking score	
	Excluding data using 1% boric acid	Including data using 1% boric acid
1	70	76
2	81	88
3	96	105
4	85	92
5	103	112
6	73	80
7	80	88
8	78	84
8	—	84[a]
9	168[b]	182[c]
10	80	87
11	73	80
12	105	116

[a] 1% boric acid trapping solution.

[b,c] Excluded by the laboratory ranking test (11).

Lighted stir plate may aid visualization of endpoint. Record milliliters of HCl to at least the nearest 0.05 mL.

This is done automatically by using a steam distiller with automatic titration. Follow the manufacturer's instructions for operation of the specific distiller or distiller/titrator.

F. Verification of Nitrogen Recovery

Run N recoveries to check accuracy of procedure and equipment.

(**a**) *Nitrogen loss.*—Use 0.12 g $(NH_4)_2SO_4$ and 0.67 g sucrose per flask. Add all other reagents as in **E**, and distill under same conditions as in **E**. Recoveries must be ≥99%.

(**b**) *Distillation and titration efficiency.*—Distill 0.12 g $(NH_4)_2SO_4$, omitting digestion. Recoveries must be ≥99.5%.

(**c**) *Digestion efficiency.*—Use 0.3 g acetanilide or 0.18 g tryptophan, with 0.67 g sucrose per flask. Add all other reagents as stated in **E**. Digest and distill under same conditions as used for a determination. Recoveries must be ≥98%.

G. Calculations

$$\text{Kjeldahl nitrogen, \%} = \frac{(V_S - V_B) \times M \times 14.01}{W \times 10}$$

$$\text{Crude protein, \%} = \text{\% Kjeldahl N} \times F$$

where V_S = volume (mL) of standardized acid used to titrate a test; V_B = volume (mL) of standardized acid used to titrate reagent blank; M = molarity of standard HCl; 14.01 = atomic weight of N; W = weight (g) of test portion or standard; 10 = factor to convert mg/g to percent; and F = factor to convert N to protein.

F factors are 5.70 for wheat, 6.38 for dairy products, and 6.25 for other feed materials.

Ref.: *J. AOAC Int.* **85**, 311–315(2002)

Results and Discussion

Study materials were shipped December 11, 2000. Results were received from 12 laboratories (Table 3) over a period of 3 months, with the last set received on March 13, 2001. The study materials were sent to a 13th laboratory, but it could not provide data because of an in-house miscommunication. Weighing paper, which had been shipped with the collaborative study materials, was reported by some collaborators to produce a high blank when the familiarization samples were analyzed. After learning of the problem, the Study Directors instructed the collaborators not to use the weighing paper for the study samples. It was never determined where the contamination of the weighing paper occurred.

A laboratory ranking obtained by using the test described by Youden and Steiner (11) detected some bias among laboratories participating in the collaborative study. Overall, Laboratory 9 ranked highest (reported the lowest protein values) with a laboratory ranking score of 168 (Table 4). On the basis of the test results, the data of Laboratory 9 were considered invalid and were not included in the statistical analysis. All other laboratories appeared to have valid data and were retained in the study.

The results of the statistical analysis of the study data are shown in Table 3, recovery data are shown in Table **2001.11B**, and the statistical data are summarized in Table **2001.11A**. The average recoveries of nitrogen from lysine, tryptophan, and acetanilide were 86.8, 98.8, and 100.1%, respectively. The within-laboratory RSD (RSD_r, repeatability) ranged from 0.40 to 2.38% for crude protein. The among-laboratories (including within-) RSD (RSD_R, reproducibility) ranged from 0.44 to 2.38%. In general, the materials with the highest RSD in the collaborative study were the materials with the highest RSD in the homogeneity test (sunflower seeds, corn silage, grass hay). These results suggest that the greatest source of variability in the method is related to sampling when the test portion is obtained. Most previous Kjeldahl collaborative studies were completed before RSDs were included in reports of study results. However, the reproducibility standard deviation (s_R) values of this study compare favorably with the s_L (estimates of interlaboratory bias) values of the next most recent study (12), which led to the adoption of AOAC Method **988.05**. The Study Directors believe that the results of this study compare very favorably with those for other AOAC official Kjeldahl methods (2–9), and that the method performance is very acceptable.

Comparability Data for 1% Boric Acid Trapping Solution with Titration Beginning with Start of Distillation Process

One collaborator provided comparability data for 1% boric acid as an option to 4% boric acid trapping solution. This option may be desirable in laboratories using some types of auto-

Table 5. Recovery of nitrogen from lysine, tryptophan, and acetanilide

	Lysine	Tryptophan	Acetanilide	Lysine	Tryptophan	Acetanilide
Lab	Found, % N			Recovery, %		
1	13.47	13.65	10.34	87.81	99.49	99.81
2	13.35	13.50	10.68	87.03	98.40	103.09
3	13.17	13.63	10.25	85.86	99.34	98.94
4	14.05	13.67	10.22	91.59	99.64	98.65
5	NR[a]	NR	NR			
6	12.92	13.32	10.50	84.22	97.08	101.35
7	13.33	13.51	10.26	86.90	98.47	99.03
8	14.08	13.68	10.32	95.70	99.71	99.61
8	13.64[b]	13.71[b]	10.34[b]	88.92[b]	99.93[b]	99.81[b]
9	NR	NR	NR			
10	13.02	13.42	10.55	84.88	97.81	101.83
11	12.20	13.37	10.23	79.53	97.45	98.75
12	NR	NR	NR			
Mean	13.32	13.55	10.37	86.83	98.76	100.09

[a] NR = not reported.

[b] Value obtained by using 1% boric acid trapping solution (all other data were obtained by using 4% boric acid trapping solution).

mated equipment where the titration automatically begins as soon as the distillation process begins (thus the need for a less concentrated trapping solution). Adding an option of 1% trapping solution to the method falls into the category of "minor change."

Comparability data for the study including the 1% boric acid trapping solution data is provided in Table 4. A paired *t*-test found no significant difference between the 2 trapping solutions ($P = 0.1044$) in the collaborator's laboratory. The Pearson coefficient for data supplied by the collaborator for the 2 trapping solutions was 0.9999. A laboratory ranking using the test described by Youden and Steiner (11) showed that the 1% boric acid had no bias compared to 4% boric acid. The 1% boric acid modification performed by the collaborator had a laboratory ranking score of 84, which was identical to the score obtained by the collaborator with the 4% solution (Table 4). The values obtained with the 1% boric acid were not detected as outliers for laboratory variance using the Cochran's test (11). Recovery data for the 1% boric acid trapping solution are included in Table 5. Statistical results for the study including the 1% boric acid data are shown in Table 6. Comparing data in Tables 6 and **2001.11A**, the within-laboratory relative standard deviation (RSD_r, repeatability) ranged from 0.44 to 2.38% when excluding the 1% boric acid data and 0.40 to 2.28% when including it. Among-laboratory (including within-) relative standard deviation (RSD_R, reproducibility) ranged from 0.44 to 2.38% when excluding the 1% boric acid data and 0.46 to 2.28% when including it. Including 1% boric acid data did not affect collaborative study results, except to slightly improve them.

Recommendations

On the basis of the results of this study, the Study Directors recommend that this method for the determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid be adopted Official First Action. It is recommended that 1% boric acid be allowed as an optional alternative trapping solution only for automatic titration systems that begin titration immediately when distillation begins.

Acknowledgments

We thank Brian Steinlicht (Olson Biochemistry Laboratories, South Dakota State University, Brookings, SD) for assistance with preparing, splitting, and sealing familiarization and study samples. We would like to thank Foss North America for providing the Kjeldahl catalyst, boric acid trapping solution, standardized hydrochloric acid titrant, and weighing paper to collaborators and for supporting the study by the shipment of reagents and study materials. We also thank the following collaborators for their participation in this study:

Diana Budde and Gloria Bolek, Land O'Lakes, Arden Hills, MN

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Table 6. Interlaboratory study results for the determination of crude protein by block digestion with a copper catalyst and distillation into 4% boric acid and including one set of 1% boric acid results

Product	No. of labs[a]	Mean, %	RSD_r, %	RSD_R, %	HORRAT
Protein block	11(1)	40.20	0.43	0.73	0.32
Swine pellets	11(1)	37.03	0.46	0.57	0.24
Corn silage	12	7.10	1.57	2.07	0.69
Grass hay	12	7.11	1.88	1.88	0.63
Fish meal	12	64.60	0.72	1.00	0.47
Dog food	12	24.51	0.83	0.88	0.36
Chinchilla food	10(2)	18.08	0.83	0.83	0.32
Albumin	11(1)	79.10	0.40	0.46	0.22
Birdseed	11(1)	13.52	0.88	0.91	0.34
Meat and bone meal	12	50.11	1.84	1.84	0.83
Milk replacer	12	20.79	1.33	1.33	0.53
Soybeans	10(2)	38.76	0.57	0.57	0.25
Sunflower seeds	12	17.42	2.28	2.28	0.88
Legume hay	12	18.85	1.42	1.42	0.55

[a] Each value is the number of laboratories retained after elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

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