Designation: D2868 – 17

Standard Test Method for
Nitrogen Content (Kjeldahl) and Hide Substance Content of Leather, Wet Blue and Wet White[1]

This standard is issued under the fixed designation D2868; 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.

This standard has been approved for use by agencies of the U.S. Department of Defense.

1. Scope

1.1 This test method covers the determination of the nitrogen content of all types of leather, wet blue and wet white. The nitrogen content is used to calculate the hide substance (protein fiber) content of leather, wet blue and wet white.

NOTE 1—The original test method for leather was essentially a composite of Method 6441 of Federal Test Method Standard No. 311 and Method B5 of the American Leather Chemists Association.

NOTE 2—Melamine, if present in bonded leather, could give an artificially high value for the calculation of protein fiber.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D2813 Practice for Sampling Leather for Physical and Chemical Tests

D6659 Practice for Sampling and Preparation of Wet Blue and Wet White for Physical and Chemical Tests

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Summary of Test Method

3.1 The specimen prepared according to an accepted procedure (see Note 3) is digested with acid in the presence of a catalyst to convert the nitrogen to ammonium ion. The ammonium ion formed is nonvolatile under these highly acid conditions.

NOTE 3—For leather use specimen prepared per Practice D2813. For wet blue and wet white, use specimen prepared per Practice D6659.

3.2 The acid mixture is then made alkaline and the ammonia liberated is distilled into either a boric acid solution which absorbs the ammonia, or a sulfuric acid solution which absorbs the ammonia.

3.3 When the boric acid solution is used, the amount of ammonia in the boric acid is then determined by back titration with standardized acid using a sharp color change indicator (green to purple) to determine the end point. When the sulfuric acid solution is used, the amount of ammonia in the sulfuric acid solution is then determined by back titration with standardized base using a sharp color change indicator (purple to green-blue) to determine the end point.

4. Significance and Use

4.1 The nitrogen content as determined by this test method is normally considered to be related to the amount of hide substance (protein fiber) present in the leather sample. A factor of 5.62 is normally used to calculate the hide substance from the nitrogen content.

4.1.1 The 5.62 factor represents the average result of many analyses of animal hides, but it cannot be considered to be accurate since it varies somewhat from hide to hide of the same type, from type of hide to type of hide, and also with the thickness of hide retained in the final leather (split thickness as compared to original hide thickness). As a result of these

---

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.06 on Chemical Analysis. This test method was developed in cooperation with the American Leather Chemists Assn. (Standard Method B5 – 1954).

Current edition approved April 1, 2017. Published May 2017. Originally approved in 1970. Last previous edition approved in 2015 as D2868 – 10 (2015). DOI: 10.1520/D2868-17.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

variations, the true factor for any given leather may be expected to vary from 5.44 to 5.80 or about ±3 %.[3]

4.2 A given leather sample may contain nitrogenous substances other than hide substance (protein fiber) which will be analyzed for by this test method, such as resins, dyestuffs, etc., that contain nitrogen. Therefore, although this test method is fairly accurate for determining the nitrogen content of leather, its use for determining hide substance may result in large errors.

4.3 The hide substance value derived from this determination has a large bearing on other chemical determinations of a given leather. Any errors, such as those described in 4.1.1 and 4.2, will be carried over into these other analytical calculations.

5. Apparatus

5.1 *Kjeldahl Apparatus* consisting of:

5.1.1 *Kjeldahl Flask,* of 500 or 800-mL capacity for digestion of the sample.

5.1.2 *Heater,* (gas or electric) for the Kjeldahl flask with fume hood or other exhaust system.

5.1.3 *Distillation Apparatus,* consisting of an efficient vapor trap that can be sealed tightly in the top of the Kjeldahl flask and a condenser connected to the top of the trap. All elements of the distillation system shall be constructed of block tin, borosilicate glass, or other materials known not to react with hot ammonia vapor.

5.2 Semi-automated equipment (Kjeltec/micro-Kjeldahl) produce comparable results and may be substituted for Kjeldahl apparatus. See Precision and Bias (12.1 – 12.4).

6. Reagents

6.1 *Purity of Reagents*—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 sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water, deionized water, or water of equal purity.

6.3 *Boric Acid Indicator Solution*—Dissolve 40 g of boric acid (H_3BO_3) (borax-free) in water, add 10 mL of mixed indicator solution (6.5) and dilute to 1 L.

6.4 *Catalyst Digestion Mixture*[5,6]— 20.0 g $K_2SO_4 + 0.6$ g $CuSO_4 + 0.2$ g pumice.

6.5 *Mixed Indicator Solution*[7]—Dissolve 0.060 g of methyl red indicator and 0.040 g of methylene blue indicator in 100 mL of 95 % ethyl alcohol.

6.6 *Sodium Hydroxide, Concentrated Solution* (450 g/L)—Dissolve 450 g of sodium hydroxide (NaOH) pellets (98 %) in water and dilute to 1 L.

6.7 *Sodium Hydroxide, Standard Solution* (0.1 N)—Dissolve 10 mL of the concentrated NaOH solution (6.6) in 1 L of boiled and cooled water. Determine the exact normality by titration against the standard sulfuric acid (6.10) using the mixed indicator (6.5) for the end point.

6.8 *Sucrose* ($C_{11}H_{22}O_{11}$).

6.9 *Sulfuric Acid* (sp gr 1.84)—Concentrated sulfuric acid (H_2SO_4), free from nitrogen.

6.10 *Sulfuric Acid, Standard* (0.3 N)—Dissolve 9 mL of concentrated H_2SO_4 (6.9) in water and dilute to 1 L. Determine the exact normality by titration against an equivalent solution of a primary standard such as anhydrous sodium carbonate or tris (hydroxymethyl) amino methane.

6.11 *Sulfuric Acid, Standard (0.5 N)*—Available commercially. Determine the exact normality by titration against an equivalent solution of a primary standard such as anhydrous sodium carbonate or tris (hydroxymethyl) amino methane.

6.12 *Sodium Hydroxide (0.5 N)*—Available commercially. Determine the exact normality by titration against a known solution of a primary standard such as potassium hydrogen phthalate.

6.13 When using semi-automatic equipment, follow the guidelines provided by the manufacturer.

7. Hazards

7.1 All reagents and chemicals should be handled with care. Before using any chemical, read and follow all safety precautions and instructions on the manufacturer's label or SDS (Safety Data Sheet).

8. Standardization

8.1 *Blanks*—Run a blank determination substituting 1.0 g of sucrose in place of the leather specimen by the procedure shown in Section 9. Calculate the blank results, as shown in Section 9.3.

8.2 *Standard*—Tris (hydroxymethyl) amino methane can be used as an internal nitrogen standard for the method. Weigh out to 0.001 g approximately 1 g of tris (hydroxymethyl) amino methane and transfer to the Kjeldahl flask. Run this standard by the same procedure shown in Section 9. One gram of this reagent is equal to 0.1156 g of N_2 or 8.255 meq of N_2.

9. Procedure

9.1 *Procedure A – Kjeldahl Apparatus*

9.1.1 *Sample and Specimen:*

---

[3] Dahl, S., "Determination of Hide Substance in the Kjeldahl Method," in *Chemistry and Technology of Leather*, Vol 4, Reinhold Publishing Co., New York, NY, 1965.

[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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[5] Dahl, S., and Oehler, R., "The Determination of Nitrogen in Leather by the Kjeldahl Method," JALCA, Vol 46, 1951, pp. 317–355.

[6] Available as a prepared catalyst mixture from some laboratory supply companies, for example, Alfie Packers, #20P.

[7] Available as prepared solution from some laboratory supply companies. Hach Bromcresol Green Methyl Red Indicator.

9.1.1.1 *Leather*—Weigh out two specimens from the prepared sample of 0.5 ± 0.05 g accurately to 0.001 g and record the weight of each specimen.

9.1.1.2 *Wet Blue or Wet White*—Weigh out two specimens from the prepared sample of 0.5 – 1.0 g accurately to 0.001 g and record the weight of each specimen.

NOTE 4—The specimens for all chemical tests to be performed on the leather, wet blue or wet white, should be weighed at the same time to keep the moisture content constant among the specimens. If only the nitrogen content is being analyzed for, then specimens for moisture analysis should be weighed out at the same time as those for the nitrogen analyses.

9.1.2 *Digestion*—Transfer the specimen to a Kjeldahl flask, being careful that all the powder is shaken down into the main bulb of the flask. Add 10 ± 0.5 g of the catalyst digestion mixture, a few glass beads or other anti-bumping agents, and 25 mL of H_2SO_4. Mix the contents by gently swirling until all of the powder is wet by the acid. Place the flask over the heater in the fume hood with the flask inclined at about 45°. Adjust the heat so that the contents boil gently (the condensate line should be within the neck of the flask) and maintain this boiling for 1.5 h. Cool and dilute with 250 to 300 mL of water.

NOTE 5—For distillation, use either boric acid solution (9.1.3) or sulfuric acid (9.1.4) in the receiving flasks.

9.1.3 *Distillation (Boric acid solution):*

9.1.3.1 Measure out 125 mL of the H_3BO_3 solution with a graduated cylinder and transfer to a 500-mL Erlenmeyer receiving flask. Place the receiving flask under the outlet tube from the condenser so that the end of the tube dips below the surface of the H_3BO_3 solution.

9.1.3.2 Carefully pour an amount of the concentrated NaOH solution, sufficient to make the contents of the flask strongly alkaline, slowly down the side of the digestion flask so that the caustic settles to the bottom and does not mix with the acid layer. The amount of concentrated NaOH solution required is about 95 mL. Connect the Kjeldahl flask to the trap immediately and be sure that the rubber stopper is tightly in place. Swirl the contents gently to mix the two layers and then heat sufficiently to boil the solution in the flask. Continue heating until 150 to 200 mL has distilled over and been collected in the H_3BO_3 solution in the receiver. Disconnect the flask and trap before turning off the heat to prevent sucking the solution from the receiver back into the flask. Disconnect the condenser outlet tube and rinse it off into the receiver. Dilute the contents of the receiver to approximately 350 mL.

9.1.4 *Distillation (Sulfuric acid solution):*

9.1.4.1 Measure out 25.0 mL of the 0.5 N H_2SO_4 solution from a buret into a 500-mL Erlenmeyer receiving flask. Add 0.5 mL of the mixed indicator solution. Adjust the volume to the 100 mL mark with DI water. Place the receiving flask under the outlet tube from the condenser so that the end of the tube dips below the surface of the acidic receiving solution. Prepare a receiving flask for each sample, plus a separate flask for the blank.

9.1.4.2 Carefully pour an amount of the concentrated NaOH solution, sufficient to make the contents of the flask strongly alkaline, slowly down the side of the digestion flask so that the caustic settles to the bottom and does not mix with the acid layer. The amount of concentrated NaOH solution required is about 95 mL. Connect the Kjeldahl flask to the trap immediately and be sure that the rubber stopper is tightly in place. Swirl the contents gently to mix the two layers and then heat sufficiently to boil the solution in the flask. Continue heating until 150 to 200 mL has distilled over and been collected in the solution in the receiver. Disconnect the flask and trap before turning off the heat to prevent sucking the solution from the receiver back into the flask. Disconnect the condenser outlet tube and rinse it off into the receiver. Dilute the contents of the receiver to approximately 350 mL.

9.1.5 *Titration (Boric acid solution)*—Titrate the receiver contents of the blank distillate immediately with the 0.3 *N* H_2SO_4 (6.10) to a purple end point (pH about 4.9). Blank determinations run in accordance with 8.1 may require titration with alkali (if they are purple at the end of the distillation). In this case, titrate with the 0.1 *N* NaOH solution.

9.1.6 *Titration (Sulfuric acid solution)*—Titrate the receiver contents of the blank distillate immediately with the 0.5 *N* NaOH (6.12) to a blue-green end point. Record the volume of titrant for the blank. Titrate the receiving flasks for the samples to the same end-point. Record the volume of titrant for each sample.

9.2 *Procedure B – Kjeltec Apparatus*

9.2.1 *Sample and Specimen:*

9.2.1.1 *Leather*—Weigh out two specimens from the prepared sample of 0.5 ± 0.05 g accurately to 0.001 g and record the weight of each specimen (see Note 4).

9.2.1.2 *Wet Blue or Wet White*—Weigh out two specimens from the prepared sample of 0.5 – 1.0 g accurately to 0.001 g and record the weight of each specimen (see Note 4).

9.2.2 *Digestion*—Transfer the specimen to a Kjeltec tube, being careful that all the powder is shaken down into the bottom of the tube. Add 2 Kjeltabs (3.5 g K_2SO_4 + 0.4 g $CuSO_4 \times H_2O$), a few glass beads or other anti-bumping agents, and 12 mL of H_2SO_4. Mix the contents by gently swirling until all of the powder is wet by the acid. Place the tubes at ~200 °C, slowly raising the temperature to 420 °C and digest for 60 min.

9.2.3 *Distillation*—Follow the manufacturer's instructions.

9.2.4 *Titration*—Follow the manufacturer's instructions.

9.3 *Procedure C – Micro-Kjedahl Apparatus*

9.3.1 *Sample and Specimen:*

9.3.1.1 *Leather*—Weigh out five specimens from the prepared sample of 0.05 ± 0.01 g accurately to 0.001 g and record the weight of each specimen (see Note 4).

9.3.1.2 *Wet Blue or Wet White*—Weigh out five specimens from the prepared sample of 0.05 ± 0.01 g accurately to 0.001 g and record the weight of each specimen (see Note 4).

9.3.2 *Digestion*—Follow the manufacturer's instructions.

9.3.3 *Distillation*—Follow the manufacturer's instructions.

9.3.4 *Titration*—Follow the manufacturer's instructions.

10. Calculations

10.1 *Blank (Boric acid solution)*—If the blank was acid (purple) and required titration by the 0.1 *N* NaOH solution, then convert this value to equivalent millilitres of 0.3 *N* H_2SO_4 as follows:

$$B = \text{blank, mL of standard} = V_b N_b / N_a \qquad (1)$$

where:

V_b = millilitres of NaOH solution required for titration of the blank,

N_b = normality of the NaOH solution, and

N_a = normality of the H_2SO_4.

10.2 *Nitrogen in Sample (Boric acid solution)*—Calculate as follows for the as received basis:

$$\text{Nitrogen, } \% = \left[\left(\left(A \pm B\right) \times N \times 0.014\right)/W\right] \times 100 \qquad (2)$$

where:

A = millilitres of H_2SO_4 required for titration of the sample,

B = millilitres of H_2SO_4 required for titration of the blank (or equivalent millilitres of H_2SO_4 in terms of 0.1 N NaOH solution calculated in 10.1) (use plus B in the formula above if the blank was acidic and required titration by alkali. Use minus B if the blank was alkaline and required titration by acid),

N = normality of the H_2SO_4, and

W = grams of sample.

10.3 *Nitrogen in Sample (Sulfuric acid solution)*—Calculate as follows for the as received basis:

where:

A = millilitres of 0.5 N $NaOH_4$ required for titration of the blank,

C = millilitres of 0.5 N $NaOH_4$ required for titration of the sample,

N = actual normality of the 0.5 N NaOH solution, and

W = grams of sample.

10.4 Calculate the percent nitrogen on the moisture-free basis as follows:

$$\text{Nitrogen, } \% \left(\text{moisture} - \text{free basis}\right) = \left[D/\left(100 - M\right)\right] \times 100 \qquad (4)$$

where:

D = percent nitrogen (as-received basis), and

M = percent volatile matter or moisture determined according to an accepted procedure.[2]

10.5 Calculate the percent of hide substance (protein fiber) on the moisture free basis as follows:

$$\text{Hide Substance, } \% \left(\text{Moisture Free Basis}\right) \qquad (5)$$

$$= 5.62 \times \text{nitrogen} \left(\text{Moisture Free Basis}\right)$$

11. Report

11.1 Report the following information:

11.1.1 *Nitrogen Content*—Report the percent nitrogen on the moisture-free basis.

11.1.2 *Hide Substance*—Report the hide substance on the moisture-free basis.

12. Precision and Bias[8]

12.1 The precision of this test method is based on an interlaboratory study of D2868 conducted in 2008. Four laboratories tested ground leather material at one or more of three different test sizes using Kjeldahl digestion. Three laboratories tested ground leather material at one or more of three different test sizes using Kjeltec digestion. Some laboratories were able to perform both digestions. Every "test result" represents an individual determination. Each laboratory was asked to submit up to six replicate test results, from a single operator, for each analysis and material size. Except for the limited number of reporting laboratories, Practice E691 was followed for the design and analysis of the data; the details are given in an ASTM Research Report.[8]

12.1.1 *Repeatability limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the " *r*" value for that material: "*r*" is the interval representing the critical difference between two test results for the same material obtained by the same operator using the same equipment on the same day in the same laboratory.

12.1.1.1 Repeatability limits are listed in Tables 1-3.

12.1.2 *Reproducibility limit (R)*—Two test results shall be judged not equivalent if they differ more than the "*R*" value for that material. "*R*" is the interval representing the critical difference between two test results for the same material obtained by different operators using different equipment in different laboratories.

12.1.2.1 Reproducibility limits are listed in Tables 1-3.

12.1.3 The terms (repeatability limit and reproducibility limit) are as specified in Practice E177.

12.1.4 Any judgment in accordance with 12.1.1 and 12.1.2 would normally have an approximate 95 % probability of being correct, however the precision statistics obtained in this ILS must not be treated as exact mathematical quantities which are applicable to all circumstances and uses. The limited number of materials tested and laboratories reporting results guarantees that there will be times when differences greater than predicted by the ILS results will arise, sometimes with considerably greater or smaller frequency than the 95 % probability limit would imply. The repeatability limit and the reproducibility

TABLE 1 Kjeldahl Digestion ***Hide Substance*****, % (MFB)**

Material	Average[A] $\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
Leather, ground, 0.5 g, nominal	73.47	0.39	0.89	1.08	2.48
Leather, ground, 1.0 g, nominal	72.93	0.21	_	0.58	_

[A] The average of the laboratories' calculated averages.

---

[8] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR: D31–1014. An earlier study comparing copper and mercury catalysts is also available from ASTM International Headquarters: request RR: D31–1008.

TABLE 2 Kjeltec Digestion *Hide Substance*, **% (MFB)**

Material	Average[A] $\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
Leather, ground, 0.5 g, nominal	72.23	0.46	0.51	1.28	1.42
Leather, ground, 1.0 g, nominal	72.40	0.26	_	0.74	_
Leather, ground, 0.05 g, nominal	74.51	2.12	_	5.93	_

[A] The average of the laboratories' calculated averages.

TABLE 3 Combined Data from both Kjeldahl and Kjeltec Digestions *Hide Substance* **% (MFB)**

Material	Average[A] $\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
Leather, ground, 0.5 g, nominal	73.14	0.44	1.06	1.22	2.98
Leather, ground, 1.0 g, nominal	72.67	0.36	_	1.01	_
Leather, ground, 0.05 g, nominal	74.51	2.12	_	5.93	_

[A] The average of the laboratories' calculated averages.

limit should be considered as general guides, and the associated probability of 95 % as only a rough indicator of what can be expected.

12.2 *Bias*—At the time of this study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

12.3 The precision statement was determined through statistical examination of 29 analytical results from four laboratories, on three material sizes.

12.4 To judge the equivalency of two test results, it is recommended to choose the particle size closest in characteristics to the test material size.

13. Keywords

13.1 hide substance; Kjeldahl; nitrogen; wet blue; wet white

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, Tel: (978) 646-2600; http://www.copyright.com/