Designation: D6401 – 99 (Reapproved 2020)

# Standard Test Method for Determining Non-Tannins and Tannin in Extracts of Vegetable Tanning Materials[1]

This standard is issued under the fixed designation D6401; 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.

## 1. Scope

1.1 This test method is intended for use in determining the quantity of soluble non-tannins and tannin in solutions of tannin extracts, water extracts of vegetable tanning materials, or tanning liquors. The method is applicable to the analysis of liquid, solid, pasty, and powdered extracts and to extracts of raw or spent materials.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses after SI units are for information only and are not considered 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, health, and environmental 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]

D2617 Test Method for Total Ash in Leather

D3790 Test Method for Volatile Matter (Moisture) of Leather by Oven Drying

D4901 Practice for Preparation of Solution of Liquid Vegetable Tannin Extracts

D4902 Test Method for Evaporation and Drying of Analytical Solutions

D4903 Test Method for Total Solids and Water in Vegetable Tanning Material Extracts

D4905 Practice for Preparation of Solution of Solid, Pasty and Powdered Vegetable Tannin Extracts

D6402 Test Method for Determining Soluble Solids and Insolubles in Extracts of Vegetable Tanning Materials

D6405 Practice for Extraction of Tannins from Raw and Spent Materials

2.2 *ALCA Method:*

A22 Non-Tannins and Tannin[3]

## 3. Terminology

3.1 *Definitions:*

3.1.1 *soluble non-tannins*—non-volatile materials present in tannin extracts and raw or spent materials that are dissolved or suspended in water, are part of the soluble solids determined by Test Method D6402, and do not react with or bind to hide powder when mixed as in this test method.

3.1.2 *tannins*—non-volatile materials present in tannin extracts and raw or spent materials that are dissolved or suspended in water, are part of the soluble solids determined by Test Method D6402, and do react with or bind to hide powder when mixed as in this test method.

## 4. Summary of Test Method

4.1 An aliquot of the analytical solution prepared from tannin extracts (Practices D4901 or D4905) or of the water extract from raw or spent materials (Practice D6405) is dried overnight in a forced-air oven (Test Method D4902) and the solid residue remaining is determined and defined as the total solids for that sample (Test Method D4903). Another aliquot of the same solution is passed through a specified filtering procedure and the quantity of solid residue remaining in the filtrate is determined and defined as the soluble solids for that sample; the calculated difference between the total solids and the soluble solids is defined as the insolubles for that sample (Test Method D6402). Another aliquot of the original analytical solution or water extract is mixed with a prepared hide

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.01 on Vegetable Leather. This method has been adapted from, and is a replacement for, Method A22 of the Official Methods of the American Leather Chemists Association.

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[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.

[3] Official Methods of the American Leather Chemists Association. Available from the American Leather Chemists Association, University of Cincinnati, P.O. Box 210014, Cincinnati, OH 45221-0014.

powder material, with the resulting solution being passed through the same filtering procedure as for the soluble solids analysis. The quantity of solid residue remaining in the filtrate is determined and defined as the soluble non-tannins for that sample; the calculated difference between the total soluble solids and the soluble non-tannins is defined as the tannin content for that sample.

## 5. Significance and Use

5.1 This test method is used to determine the proportion of the total soluble solids which are soluble non-tannins and the proportion which are tannins in a solution of tannin extract or in the water extract from raw or spent materials prepared for tannin analysis.

5.2 The specimens are aliquots from the analytical solutions prepared from tannin extracts or the water extract solutions prepared from raw or spent materials.

5.3 The soluble non-tannins are defined as the portion of the soluble solids which are not absorbed or bound by a prepared hide powder material.

5.4 The tannins are defined as the portion of the soluble solids which are absorbed or bound by a prepared hide powder material.

5.5 The results of this test method are dependent on a great many variables, but particularly upon:

5.5.1 The temperature conditions under which the solutions were prepared and stored and the temperature at which the current analysis is performed;

5.5.2 The uniformity and consistency of the Kaolin paste layer deposited onto the filter paper;

5.5.3 The rate of solution run-out from the pipette;

5.5.4 Conditions related to the properties of the hide powder used to react with the tannin content of the solution; etc. It is, therefore, essential that the method be followed exactly in order to obtain reproducible results both among specimens within a laboratory and for analyses between laboratories.

## 6. Apparatus and Reagents

6.1 *Tannin Dish,* crystallizing dish, borosilicate glass, 50 mm tall, 70 mm outside diameter. The bottom corner shall be rounded to a radius of 6 mm, the bottom shall be flat and not cupped in the center, and the top edge shall be rounded and polished.

6.2 *Watch Glass,* a suitable size (approximately 150 mm diameter) to be used as a cover for the funnel and filter paper and a suitable size (75 mm) to be used as a cover for the tannin dishes.

6.3 *Pipet,* 100 mL capacity, preferably with a wide orifice approximately 2.4 mm (3⁄32 in.) diameter and 15-25 s delivery time.

6.4 *Volumetric Flasks,* graduated to deliver 200 mL borosilicate flask (Pyrex No. 5840 works well).

6.5 *Filter Paper*[4], 21.5 cm diameter, pleated to contain 32 evenly divided creases.

6.6 *Funnel,* 100 to 125 mm top diameter, 60° angle bowl, and 150 mm stem length.

6.7 *Shake Bottles,* 0.95 L (32 oz), with rubber stoppers. The bottles shall be approximately 21.6 cm (8.5 in.) overall height and 8.9 cm (3.5 in.) diameter. One-quart canning jars (Mason-type) with plastic screw-on lids work well.

6.8 *Shaking Machine,* rotating type, equipped to hold 0.95 L (32 oz) bottles for end-over-end agitation of hide powder and analytical solution. The speed of rotation shall be 60 ± 2 rpm, and the machine shall be so constructed that the side of the shake bottle adjacent to the rotating shaft shall be not less than 5.1 cm (2 in.) nor more than 7.6 cm (3 in.) from the center of the shaft.

6.9 *Cloth,* The cloth shall be of cotton, with a nominal (unbleached) thread count of 48 by 48 and a weight of 1.65 yards per 454 g (1 lb). When bleached, the thread count will be approximately 52 to 53 by 43 to 48.

6.10 *Kaolin*[5], acid-washed kaolin clay which conforms to the following specifications:

6.10.1 Suspend 1.0 g kaolin in 100 mL distilled water. The pH value should be between 4.5 and 6.0 after 5 min.

6.10.2 A mixture of 2.0 g kaolin and 200 mL distilled water are shaken for 10 min and the mixture filtered through the standard filter paper (see 6.5). A100 mL aliquot of the clear filtrate should have less than 0.001 g of residue after evaporation and oven-drying in a platinum dish.

6.11 *Chrome Alum Solution,* a 3 % solution, prepared by dissolving $CrK(SO_4)_2$ 12 $H_2O$ in distilled water at room temperature (3 g per 100 mL of solution). The solution shall not be more than 30 days old when used.

6.12 *Hide Powder*[5]—The hide powder shall meet the following specifications:

6.12.1 The hide powder shall be finely ground and uniform in texture with no lumps present.

6.12.2 The moisture content should be 12 to 15 % as determined by Test Method D4902.

6.12.3 The ash content shall be less than 0.3 % as determined by Test Method D2617.

6.12.4 A mixture prepared from 7.0 g of air-dry hide powder suspended in 100 mL of 0.1 *N* KCl and set aside for 24 h with occasional shaking shall have a pH value not less than 5.0 nor greater than 5.4.

6.12.5 A sample of the hide powder shall be carried through the analytical procedure for the determination of non-tannins

[4] The sole source of supply of S&S No. 610 filter paper known to the committee at this time is Schleicher & Schuell, 10 Optical Avenue, P.O. Box 2012, Keene, NH 03431. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

[5] The sole source of supply of Kaolin and hide powder known to the committee at this time is L. H. Lincoln & Son, Inc., 203 Cherry Street, Coudersport, PA 16915. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

described below using distilled water in place of the analytical solution. The water-soluble content of the resulting filtrate shall not exceed 0.004 g.

6.12.6 Each new shipment of hide powder should be tested against the hide powder of the previous shipment using certain standard extracts. The new hide powder shall give an average non-tannin percentage which differs by less than ± 0.2 from the comparable non-tannin percentage obtained with the hide powder from the previous shipment.

6.12.7 Hide powder, prepared as described below, shall be used for the analysis of tanning materials on the same day that it is prepared.

6.13 *Balance,* analytical balance which will weigh up to 100 g with an accuracy of ±0.1 mg (±0.0001 g).

6.14 *Drying Oven,* a forced-air convection oven (or mechanical-convection draft oven) capable of maintaining a temperature of 100 ± 2.0 °C.

6.15 *Thermometer,* accurate to ±0.2 °C, used to check and monitor the oven set point.

6.16 *Dessicator,* any convenient form or size, using any normal dessicant.

## 7. Test Specimen

7.1 The specimen shall consist of 100 mL of the solution prepared as described in Practices D6405, D4901, or D4905 and after mixing with the hide powder and filtering as described in this method.

## 8. Procedure

8.1 *Preparation of the Hide Powder:*

8.1.1 Transfer a 5 g specimen of the air-dry hide powder to a tared tannin dish and weighed to the nearest ±0.001 g. Place the dish with powder in the drying oven for a period of 16.0 ± 0.5 h. Remove the dish and dried powder from the oven, place in a dessicator, cool to room temperature, re-weigh, and calculate the percentage of moisture in the hide powder as in Test Method D3790.

8.1.2 The amount of oven-dry hide powder required will equal 12.5 g times the number of specimens to be analyzed, plus 12.5 g times 2 (for blanks), plus 5.5 g (for determination of moisture in the wet powder). That is, [(12.5 g) × (n + 2) + (5.5 g)] of oven-dry hide powder.

8.1.3 Digest an amount of air-dry hide powder that is equivalent to the weight of oven-dry hide powder calculated above, plus 10 to 15 g of air-dry powder to allow for mechanical loss, with ten times its weight of distilled water at 23 to 25 °C for 30 min, being stirred three or four times during this period.

8.1.4 Then add 1.0 mL of the 3 % chrome alum solution to the digest mixture for each gram of the air-dry hide powder taken, and the whole mixed well. Stir the mixture or mix every 15 min for 2 h and then allow to stand, covered, overnight at a temperature of not less than 23 nor greater than 28 °C.

8.1.5 Next morning, pour the mixture into a large piece of cloth (see 6.9), allow the excess liquid to drain off, and the wetted powder squeezed or pressed to approximately 75 % moisture. Break up the powder cake and wash by digesting with four successive portions of distilled water at 23 to 25 °C, each portion equal in amount to 15 times the weight of air-dry hide powder taken. The hide powder shall be well suspended in the water each time, all lumps being broken up, and each digestion shall last for 15 min. Squeeze the hide powder or press to approximately 75 % moisture after each digestion, except the last.

8.1.6 The wet hide powder used for the analysis shall contain as nearly as possible 72.5 % moisture (not less than 71 % nor more than 74 %).

8.2 *Preparation of the Hide Powder/Analytical Solution Reaction Mixture:*

8.2.1 While the hide powder is being washed, fill each 200 mL volumetric flask (see 6.4) to the mark with its appropriate, well-mixed analytical solution. Fill two of the 200 mL flasks to the mark with distilled water to provide blank control solutions. Maintain these aliquots at 23 to 25 °C.

8.2.2 Without delay, after washing, weigh out a quantity of wet hide powder (72.5 ± 2 % moisture), equivalent to 12.5 ± 0.3 g of oven-dry hide powder, and transfer to each of the shake bottles, and close the bottles immediately with rubber stoppers. Weigh a 20 g specimen of the wet powder into a tared dish for the determination of moisture in the prepared hide powder (Test Method D4902).

8.2.3 Without delay thereafter, add the 200 mL aliquots and blanks, previously measured out, and at a temperature of 23 to 25 °C, to the hide powder in the respective shake bottles and allow the flasks to drain for not less than 15 s nor more than 30 s. Then remove the flasks, stopper the bottles, and place in the shaking machine and shake for exactly 10 min.

8.2.4 At the end of this time, remove the bottles and empty the contents onto separate cloth filters (see 6.9) that are held in 125 mm funnels. Collect the filtrate in a suitable glass container (a 250 mL beaker works well) to which 2 g of kaolin has been added prior to the start of the filtration. Lightly squeeze the drained hide powder so that the volume of filtrate is approximately 135 mL. The total time of contact between the hide powder and the tannin solution shall be not less than 13 nor more than 15 min, from the time the solution first comes in contact with the hide powder until it is squeezed therefrom.

8.3 *Filtration of the Reacted Tannin Solution:*

8.3.1 Thoroughly mix the filtrate and kaolin and then filter through a standard filter paper (that is, S&S No. 610 paper, 21.5 cm diameter, folded with 32 evenly spaced pleats as in Test Method D6402) into the original container in which they were mixed. After approximately 40 mL of the filtrate has been collected, swirl it to pick up kaolin remaining on the sides and bottom of the container, and return to the funnel. During this operation, keep funnels and containers covered to avoid changes due to evaporation, and maintain the temperature of the solution and of the filtrates between 23 and 25 °C.

8.3.2 Repeat the operation of collecting and repouring 40 mL of filtrate as many times as is necessary until the filtrate becomes clear. Then substitute a clean container and collect the clear filtrate.

8.3.3 Normally, the filtrate must be returned three to five times before true clarity can be assured. Test the final filtrate for clarity by swirling and viewing against the light.

8.3.4 If the filtrate is clear, mix it with a clean glass rod and withdraw a 100 mL specimen with a pipet and transfer into a tared tannin dish. The pipet should be the one used for transferring the specimens for determining total solids (Test Method D4903) and soluble solids (Test Method D6402) and rinse with a portion of the filtrate before withdrawing and measuring the specimen.

8.3.5 Place the dish containing the specimen, together with the other tannin dishes containing the specimens for total solids and soluble solids, in the drying oven and evaporate and dry as specified in Test Method D4902.

## 9. Calculation

9.1 Calculate the amount of soluble non-tannins in the specimen as follows:

$$\text{non-tannins, \%} = \{[F \times (W_2 - W_1) \times 10]/(W_3)\} \times 100 \quad (1)$$

where:

$W_1$ = weight (grams), tare weight of tannin dish,
$W_2$ = weight (grams), tannin dish plus oven-dried specimen,
$W_3$ = weight (grams), specimen used to prepare 1 L of the analytical solution in Practices D4901, D4905, or D6405, and
$F$ = [200 + (grams of water added in wet hide powder to solution in 8.2)]/(200)

9.1.1 The residue from the blank specimens (that is, $W_2 - W_1$) shall not exceed 0.004 g, as specified in 6.12.

9.1.2 Where, as in Practice D6405, it has been necessary to reduce the quantity of prepared hide powder used in the detannization, the formula in 9.1 applies, provided the factor ($F$) be correctly calculated for the lesser quantity of water introduced in 8.2.

9.1.3 Two specimens of each sample material were taken in preparing the solutions (Practices D4901, D4905 or D6405); therefore two values for soluble non-tannins will be obtained for each extract or tanning material. The average (mean) of these values shall be taken as the percentage of soluble non-tannins in the sample under test.

9.1.4 Duplicates are considered to be in good agreement when the percent non-tannins differ by no more than 0.2.

9.2 The amount of tannin in the sample shall be calculated as follows:

$$\text{tannin, \%} = \text{soluble solids (\%)} - \text{non-tannins (\%)} \quad (2)$$

where:
soluble solids (%) is determined as in Test Method D6402, and non-tannins (%) is determined as in 9.1.

9.2.1 Duplicates are considered to be in good agreement when the duplicate values for tannin content differ by no more than 0.3.

9.2.2 The average (mean) of these duplicate values for tannin content shall be taken as the percentage of tannin in the sample under test.

## 10. Report

10.1 Record the non-tannin and tannin results to the nearest 0.01 %.

## 11. Precision and Bias

11.1 This test method is adopted from Method A22 of The Official Methods of the ALCA. This test method has long been in use and was approved for publication before the inclusion of precision and bias statements was mandated. The original inter-laboratory test data are no longer available. The user is cautioned to verify by the use of reference materials, if available, that the precision and bias (or reproducibility) of this test method is adequate for the contemplated use.

11.2 The non-tannin content obtained by this test method is operationally defined as that portion of the soluble solids of the specimen collected after reacting with hide powder and filtering through the specially constructed filter system. The tannin content is defined as the difference between the soluble solids and the non-tannins of the specimen. There is no independent measure of the true non-tannin or tannin content of a sample. Therefore the bias cannot be related to the true non-tannin or tannin content of the sample.

## 12. Keywords

12.1 non-tannins; soluble non-tannins; tannins; tannin analysis; vegetable tannin analysis