Designation: ~~D6406 – 99 (Reapproved 2009)~~ D6406 – 99 (Reapproved 2014)

Standard Test Method for Analysis of Sugar in Vegetable Tanning Materials[1]

This standard is issued under the fixed designation D6406; 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 covers determining the sugars present in vegetable tanning materials.

1.2 The values stated in SI units are to be regarded as the standard. The inch-pound units given in parentheses are for information only.

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]
D4901 Practice for Preparation of Solution of Liquid Vegetable Tannin Extracts
D4905 Practice for Preparation of Solution of Solid, Pasty and Powdered Vegetable Tannin Extracts
D6401 Test Method for Determining Non-Tannins and Tannin in Extracts of Vegetable Tanning Materials
D6403 Test Method for Determining Moisture in Raw and Spent Materials
D6404 Practice for Sampling Vegetable Materials Containing Tannin
D6405 Practice for Extraction of Tannins from Raw and Spent Materials
D6408 Test Method for Analysis of Tannery Liquors
2.2 *ALCA Methods:*
A30 Sugar in Tanning Materials[3]

3. Terminology

3.1 *Definitions:*
3.1.1 *dextrose*—d-glucose.

3.1.2 *glucose*—a simple sugar with formula $C_6H_{12}O_6$, and known to exist in d-, l-, and racemic forms. The term commonly refers to the sweet, colorless, water-soluble dextrorotatory form that occurs widely in nature and is the usual form in which carbohydrate is assimilated by animals. The term glucose can also refer to a light-colored syrup made from corn starch.

3.1.3 *sugar*—any of various water-soluble compounds that vary widely in sweetness and comprise the oligosaccharides including sucrose.

4. Summary of Test Method

4.1 An analytical strength solution (that is, 4.00 ± 0.25 g tannin per litre) of the tanning material is analyzed for reducing sugars and total sugars by the Munson and Walker procedure.

5. Significance and Use

5.1 This test method is used to determine the quantity of sugar present in vegetable tanning materials or vegetable tannin extracts. The amount of the reducing sugars, total sugars, and non-reducing sugars in a sample of material or extract can be determined by this method.

[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 A30 of the Official Methods of the American Leather Chemists Association.

Current edition approved ~~April 1, 2009~~Nov. 1, 2014. Published ~~July 2009~~December 2014. Originally approved in 1999. Last previous edition approved in ~~2004~~2009 as D6406 – 99 ~~(2004).~~(2009). DOI: ~~10.1520/D6406-99R09.~~10.1520/D6406-99R14.

[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.

5.2 Because of the possibility of errors in this test method it is 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 *Saturated Solution of Normal Lead Acetate.*

6.2 *Dipotassium Hydrogen Phosphate, Anhydrous* (K_2HPO_4),dried in an oven at 100°C for 16 h then stored in a tightly stoppered bottle.

6.3 *Toluene,* assay ≥ 99.5 %.

6.4 *Fehling's Solutions, A and B.*

6.5 *Hydrochloric Acid,* concentrated (sp.gr. 1.18).

6.6 *Kerosene,* commercial grade.

6.7 *Saturated Solution of Sodium Hydroxide.*

6.8 *Phenolphthalein Solution,* 0.5 g dissolved in 100 mL of 95 % ethanol.

6.9 *Tartaric Acid,* powdered.

6.10 *Copper Sulfate Solution,* prepared by dissolving 69.278 g of $CuSO_4 \cdot 5H_2O$ in 1 L of distilled water and filtering through asbestos.

6.11 *Alkaline Tartrate Solution,* prepared by dissolving 346 g of Rochelle salt (sodium potassium tartrate tetrahydrate) and 100 g of sodium hydroxide in 1 L of distilled water. After standing for two days the solution shall be filtered through asbestos.

6.12 *Alcohol,* 95 % ethyl alcohol.

6.13 *Ether,* diethyl ether.

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

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

6.16 *Watch Glasses,* a suitable size (approximately 150 mm diameter) to be used as a cover for the funnel and filter paper.

6.17 *Graduated Cylinder,* standard laboratory grade with 500 mL capacity.

6.18 *Pipets,* capable of measuring and transferring 100 mL, 50 mL, and 7.5 mL.

6.19 *Beakers,* 400 mL, low form.

6.20 *Erlenmeyer Flasks,* 500 mL capacity.

6.21 *Reflux Condensers,* to connect to the top of the Erlenmeyer flasks.

6.22 *Heat Source,* either a Bunsen burner or a hotplate.

6.23 *Volumetric Flasks,* 200 mL capacity.

6.24 *Filtering Crucibles,* either porcelain crucibles of Fine porosity or Gooch-asbestos crucibles prepared as follows:

6.24.1 Digest finely divided long fibered asbestos with nitric acid (diluted 1 to 3) for 2 to 3 days.

6.24.2 Wash the asbestos free from acid.

6.24.3 Digest the asbestos with 10 % sodium hydroxide solution for two to three days.

6.24.4 Wash the asbestos free from alkali.

6.24.5 Prepare the Gooch crucible by making a bottom layer of 6.4 mm (¼ in.) thickness using the coarser particles of asbestos on the bottom and dress off the mat with the finer asbestos particles.

6.24.6 Wash the mat with boiling Fehling's solution.

6.24.7 Wash the mat with nitric acid diluted 1 to 3.

6.24.8 Wash and rinse the mat with hot distilled water.

6.24.9 Crucibles so prepared can be used for a long time.

6.25 *Suction Flask and Crucible Holder,* with connections to a vacuum.

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

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

[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.

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

6.29 *Dessicator,* any convenient form or size, using any normal desiccant.

7. Test Specimen

7.1 The specimen for the sugar analysis shall consist of 400 mL of a solution of the tanning material of analytical strength (4.00± 0.25 g tannin per L).

8. Procedure

8.1 Sample the tanning material using Practice D6404, and prepare the analytical solution as described in Practices D4901, D4905, D6405, or D6408.

8.2 *Detannization of Analytical Solution:*

8.2.1 Add to 400 mL of the analytical solution 50 mL of a saturated lead acetate solution. Shake the mixture well and allow to stand for 5 to 10 min.

NOTE 1—It is important that the mixture of liquor and lead acetate solution be very well shaken. Good results are obtained by placing the solution mixture in shake bottles and running in the shake machine for 10 min (as described in Test Method D6401) to ensure complete detannization of the liquor. The mixture filters better after complete detannization. Complete detannization also results in less danger of residual quantities of unreacted lead which may exceed the capacity of the potassium phosphate to remove and which could then interfere in the final copper precipitation step.

8.2.2 Then filter the mixture through a folded filter paper and return the filtrate to the filter until it is clear. Continue filtration until 360 to 380 mL of the clear filtrate has been collected; this may take an hour or more to accomplish. Cover the funnel during the filtration.

8.2.3 Measure the volume of the collected filtrate in a graduated cylinder. Remove the excess lead from this filtrate by adding dried dipotassium hydrogen phosphate (K_2HPO_4) at the rate of 2.5 g (± 0.1 g) phosphate per 100 mL of the filtrate. After addition of the phosphate shake the mixture well for 4 to 5 min and then filter through a folded filter paper. Allow time for the solution to drain completely from the lead phosphate. Cover the funnel during the filtration.

8.3 *Determination of Reducing Sugars:*

8.3.1 Add to 100 mL of the clarified (de-tanned) and de-leaded filtrate solution obtained from 8.2.3 33.3 mL of distilled water. If the reduction is not to be made at once also add eight to ten drops of toluene. Shake this mixture well and stopper with a plug of cotton. Keep the prepared solution in a cool place and make the reduction within 24 h. When ready for reduction, filter the solution if toluene has been added. Determine reducing sugars by the Munson and Walker procedure in 8.4 using duplicate 50 mL aliquots.

8.4 *Munson and Walker Method for Sugar Analysis:*

8.4.1 Measure a 50 mL aliquot by pipet into a 400 mL beaker containing a mixture of 25 mL of the alkaline tartrate solution and 25 mL of the copper sulfate solution and cover the beaker. Heat this mixture to 100°C, as indicated by a thermometer, in exactly 4 min and continue boiling for exactly 2 min.

8.4.1.1 Regulate the rate of heating before the determination is started by adjusting the burner or hotplate so that 50 mL of water, 25 mL of the tartrate solution, and 25 mL of the copper sulfate solution in a 400 mL beaker will be heated to 100°C in exactly 4 min.

8.4.2 Filter the solution, without dilution, immediately through a tared crucible. Wash the residue thoroughly with hot water, then with alcohol, and finally with ether. Prepare the tared crucibles ahead of time by oven drying and weighing as described in Test Method D6403.

8.4.3 Dry the crucible and contents for 30 min in the oven, cool in a dessicator, and weigh.

8.5 *Determination of Total Sugars:*

8.5.1 To a 500 mL Erlenmeyer flask add 150 mL aliquot of the clarified (de-tanned) and deleaded filtrate solution obtained from 8.2.3 and 7.5 mL of concentrated hydrochloric acid. Connect a reflux condenser to the Erlenmeyer flask and boil the mixture under refluxing conditions for exactly 1 h to hydrolyze the sugars. If the solution foams at the start, which is unusual, add five to ten drops of kerosene to the mixture. Then remove the flask from the heat source, loosely stopper when moderately cool, and allow to stand until ready for reduction, usually overnight.

8.5.2 When ready for reduction, cool the hydrolyzed solution in ice-water for 20 to 30 min and add two drops of phenolphthalein solution as an indicator. Neutralize the cooled solution carefully with a saturated solution of sodium hydroxide. Then add concentrated hydrochloric acid, drop by drop, until the red or pink color of the indicator is just discharged.

TABLE 1 Munson and Walker's Table[A]

(Expressed in Milligrams)

Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu_2O)	Copper (Cu)	Dextrose (d-glucose)
10	8.9	4.0	55	48.9	23.5	100	88.8	43.3