



Designation: D6406 – 99 (Reapproved 2020)

Standard Test Method for Analysis of Sugar in Vegetable Tanning Materials¹

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 standard. The values given in parentheses after SI units are provided 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

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

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.

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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.

2.2 *ALCA Methods:*

A30 Sugar in Tanning Materials³

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 dextro-rotatory 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.

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 $100^\circ C$ for 16 h then stored in a tightly stoppered bottle.

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.

6.3 *Toluene*, assay $\geq 99.5\%$.

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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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 ($\frac{1}{4}$ 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 $100 \pm 2.0^\circ\text{C}$.

6.28 *Thermometer*, accurate to $\pm 0.2^\circ\text{C}$ 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

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.

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 desiccator, 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 delead 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 ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)
10	8.9	4.0	55	48.9	23.5	100	88.8	43.3
11	9.8	4.5	56	49.7	23.9	101	89.7	43.8
12	10.7	4.9	57	50.6	24.3	102	90.6	44.2
13	11.5	5.3	58	51.5	24.8	103	91.5	44.7
14	12.4	5.7	59	52.4	25.2	104	92.4	45.1
15	13.3	6.2	60	53.3	25.6	105	93.3	45.5
16	14.2	6.6	61	54.2	26.1	106	94.2	46.0
17	15.1	7.0	62	55.1	26.5	107	95.0	46.4
18	16.0	7.5	63	56.0	27.0	108	95.9	46.9
19	16.9	7.9	64	56.8	27.4	109	96.8	47.3
20	17.8	8.3	65	57.7	27.8	110	97.7	47.8
21	18.7	8.7	66	58.6	28.3	111	98.6	48.2
22	19.5	9.2	67	59.5	28.7	112	99.5	48.7
23	20.4	9.6	68	60.4	29.2	113	100.4	49.1
24	21.3	10.0	69	61.3	29.6	114	101.3	49.6
25	22.2	10.5	70	62.2	30.0	115	102.2	50.0
26	23.1	10.9	71	63.1	30.5	116	103.0	50.5
27	24.0	11.3	72	64.0	30.9	117	103.9	50.9
28	24.9	11.8	73	64.8	31.4	118	104.8	51.4
29	25.8	12.2	74	65.7	31.8	119	105.7	51.8
30	26.6	12.6	75	66.6	32.2	120	106.6	52.3
31	27.5	13.1	76	67.5	32.7	121	107.5	52.7
32	28.4	13.5	77	68.4	33.1	122	108.4	53.2
33	29.3	13.9	78	69.3	33.6	123	109.3	53.6
34	30.2	14.3	79	70.2	34.0	124	110.1	54.1
35	31.1	14.8	80	71.1	34.4	125	111.0	54.5
36	32.0	15.2	81	71.9	34.9	126	111.9	55.0
37	32.9	15.6	82	72.8	35.3	127	112.8	55.4
38	33.8	16.1	83	73.7	35.8	128	113.7	55.9
39	34.6	16.5	84	74.6	36.2	129	114.6	56.3
40	35.5	16.9	85	75.5	36.7	130	115.5	56.8
41	36.4	17.4	86	76.4	37.1	131	116.4	57.2
42	37.3	17.8	87	77.3	37.5	132	117.3	57.7
43	38.2	18.2	88	78.2	38.0	133	118.1	58.1
44	39.1	18.7	89	79.1	38.4	134	119.0	58.6
45	40.0	19.1	90	79.9	38.9	135	119.9	59.0
46	40.9	19.6	91	80.8	39.3	136	120.8	59.5
47	41.7	20.0	92	81.7	39.8	137	121.7	60.0
48	42.6	20.4	93	82.6	40.2	138	122.6	60.4
49	43.5	20.9	94	83.5	40.6	139	123.5	60.9
50	44.4	21.3	95	84.4	41.1	140	124.4	61.3
51	45.3	21.7	96	85.3	41.5	141	125.2	61.8

TABLE 1 Continued

(Expressed in Milligrams)

Cuprous oxide (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Cuprous oxide (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)
52	46.2	22.2	97	86.2	42.0	142	126.1	62.2
53	47.1	22.6	98	87.1	42.4	143	127.0	62.7
54	48.0	23.0	99	87.9	42.9	144	127.9	63.1
145	128.8	63.6	192	170.5	85.3	239	212.3	107.5
146	129.7	64.0	193	171.4	85.7	240	213.2	108.0
147	130.6	64.5	194	172.3	86.2	241	214.1	108.4
148	131.5	65.0	195	173.2	86.7	242	215.0	108.9
149	132.4	65.4	196	174.1	87.1	243	215.8	109.4
150	133.2	65.9	197	175.0	87.6	244	216.7	109.0
151	134.1	66.3	198	175.9	88.1	245	217.6	110.4
152	135.0	66.8	199	176.8	88.5	246	218.5	110.8
153	135.9	67.2	200	177.7	89.0	247	219.4	111.3
154	136.8	67.7	201	178.5	89.5	248	220.2	111.8
155	137.7	68.2	202	179.4	89.8	249	221.2	112.3
156	138.6	68.6	203	180.3	90.4	250	222.1	112.8
157	139.5	69.1	204	181.2	90.9	251	223.0	113.2
158	140.3	69.5	205	182.1	91.4	252	223.8	113.7
159	141.2	70.0	206	183.0	91.8	253	224.7	114.2
160	142.2	70.4	207	183.9	92.3	254	225.6	114.7
161	143.0	70.9	208	184.8	92.8	255	226.5	115.2
162	143.9	71.4	209	185.6	93.2	256	227.4	115.7
163	144.8	71.8	210	186.5	93.7	257	228.3	116.1
164	145.7	72.3	211	187.4	94.2	258	229.2	116.6
165	146.6	72.8	212	188.3	94.6	259	230.1	117.1
166	147.5	73.2	213	189.2	95.1	260	231.0	117.6
167	148.3	73.7	214	190.1	95.6	261	231.8	118.1
168	149.2	74.1	215	191.0	96.1	262	232.7	118.6
169	150.1	74.6	216	191.9	96.5	263	233.6	119.0
170	151.0	75.1	217	192.8	97.0	264	234.5	119.5
171	151.9	75.5	218	193.6	97.5	265	235.4	120.0
172	152.8	76.0	219	194.5	98.0	266	236.3	120.5
173	153.7	76.4	220	195.4	98.4	267	237.2	121.0
174	154.6	76.9	221	196.3	98.9	268	238.1	121.5
175	155.5	77.4	222	197.2	99.4	269	238.9	122.0
176	156.3	77.8	223	198.1	99.9	270	239.8	122.5
177	157.2	78.3	224	199.0	100.3	271	240.7	122.9
178	158.1	78.8	225	199.9	100.8	272	241.6	123.4
179	159.0	79.2	226	200.7	101.3	273	242.5	123.9
180	159.9	79.7	227	201.6	101.8	274	243.4	124.4
181	160.8	80.1	228	202.5	102.2	275	244.3	124.9
182	161.7	80.6	229	203.4	102.7	276	245.2	125.4
183	162.6	81.1	230	204.3	103.2	277	246.1	125.9
184	163.4	81.5	231	205.2	103.7	278	246.9	126.4
185	164.3	82.0	232	206.1	104.1	279	247.8	126.9
186	165.2	82.5	233	207.0	104.6	280	248.7	127.3
187	166.1	82.9	234	207.9	105.1	281	249.6	127.8
188	167.0	83.4	235	208.7	105.6	282	250.5	128.3
189	167.9	83.9	236	209.6	106.0	283	251.4	128.8
190	168.8	84.3	237	210.5	106.5	284	252.3	129.3
191	169.7	84.8	238	211.4	107.0	285	253.0	129.8
286	254.0	130.3	333	295.8	153.7	380	337.5	177.9
287	254.9	130.8	334	296.7	154.2	381	338.4	178.4
288	255.8	131.3	335	297.6	154.7	382	339.3	178.9
289	256.7	131.8	336	298.5	155.2	383	340.2	179.4
290	257.6	132.3	337	299.3	155.8	384	341.1	180.0
291	258.5	132.7	338	300.2	156.3	385	342.0	180.5
292	259.4	133.2	339	301.1	156.8	386	342.9	181.0
293	260.3	133.7	340	302.0	157.3	387	343.8	181.5
294	261.2	134.2	341	302.9	157.8	388	344.6	182.0
295	262.0	134.7	342	303.8	158.3	389	345.5	182.6
296	262.9	135.2	343	304.7	158.8	390	346.4	183.1
297	263.8	135.7	344	305.6	159.3	391	347.3	183.6
298	264.7	136.2	345	306.5	159.8	392	348.2	184.1
299	265.6	136.7	346	307.3	160.3	393	349.1	184.7
300	266.5	137.2	347	308.2	160.8	394	350.0	185.2
301	267.4	137.7	348	309.1	161.4	395	350.9	185.7
302	268.3	138.2	349	310.0	161.9	396	351.8	186.2
303	269.1	138.7	350	310.9	162.4	397	352.6	186.8
304	270.0	139.2	351	311.8	162.9	398	353.5	187.3
305	270.9	139.7	352	312.7	163.4	399	354.4	187.8
306	271.8	140.2	353	313.6	163.9	400	355.3	188.4