

#### 44.1.15

### AOAC Official Method 923.09 Invert Sugar in Sugars and Syrups Lane-Eynon General Volumetric Method First Action 1923 Final Action

#### A. Reagents

*Soxhlet modification of Fehling solution.*—Prepare by mixing equal volumes of (a) and (b) immediately before use.

(a) *Copper sulfate solution.*—Dissolve 34.639 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute to 500 mL, and filter through glass wool or paper. Determine Cu content of solution [preferably by electrolysis, 929.09F (see 44.1.17)] and so adjust that it contains 440.9 mg Cu/25 mL.

(b) *Alkaline tartrate solution.*—Dissolve 173 g potassium sodium tartrate  $4\text{H}_2\text{O}$  (Rochelle salt) and 50 g NaOH in  $\text{H}_2\text{O}$ , dilute to 500 mL, let stand 2 days, and filter through prepared Fiberglas or porous (0.45  $\mu\text{m}$ ) filter with Fiberglas prefilter.

(c) *Invert sugar standard solution.*—1%. To solution of 9.5 g pure sucrose, add 5 mL HCl and dilute with  $\text{H}_2\text{O}$  to ca 100 mL. Store several days at room temperature (ca 7 days at 12–15 °C or 3 days at 20–25 °C); then dilute to 1 L. (Acidified 1% invert sugar solution is stable for several months.) Neutralize aliquot with ca 1M NaOH and dilute to desired concentration immediately before use.

#### B. Standardization

Accurately pipet 10 or 25 mL mixed Soxhlet reagent, or 5 or 12.5 mL each of Soxhlet solutions, A(a) and (b), into 300–400 mL Erlenmeyer. (Amount of Cu taken differs slightly between 2 methods of pipetting, and method used must be consistent in standardization and determination.) Prepare standard solution of pure sugar of such concentration that >15 mL and <50 mL is required to reduce all the Cu. Dispense from buret with offset tip to keep tube out of steam. To determine mg sugar required to completely reduce Cu at different concentrations, consult Table 930.44 (see Appendix C) and Table 930.45 (see Appendix C). Add sugar solution within 0.5–1.0 mL of total required, heat cold mixture to bp on wire gauze over burner, and maintain moderate boiling 2

min (coarse grains of C or other suitable inert material may be used to prevent bumping). Without removing flame add 1 mL 0.2% aqueous methylene blue solution (or 3–4 drops 1% solution) and complete titration within total boiling time of ca 3 min by small additions (2–3 drops) of sugar solution to decoloration of indicator. (Maintain continuous evolution of steam to prevent reoxidation of Cu or indicator.) After complete reduction of Cu, methylene blue is reduced to colorless compound and solution resumes orange  $\text{Cu}_2\text{O}$  color which it had before addition of indicator.

Multiply titer by mg/mL standard solution to obtain total sugar required to reduce the Cu. Compare with tabulated value in Table 930.44 (see Appendix C) or Table 930.45 (see Appendix C) to determine correction, if any, to be applied to table. Small deviations from tabulated values may arise from variations in technique or composition of reagents. If only approximate results (within 1%) are required, standardization may be omitted, provided specifications of analysis are rigidly observed.

#### C. Determination

(a) *Incremental method.*—If approximate concentration of sugar in test sample is unknown, proceed by incremental method of titration. To 10 or 25 mL mixed Soxhlet solution, add 15 mL sugar solution and heat to bp over wire gauze. Boil ca 15 s and rapidly add further amounts of sugar solution until only faintest perceptible blue remains. Then add 1 mL 0.2% aqueous methylene blue solution (or 3–4 drops 1% solution) and complete titration by adding sugar solution dropwise. (Error resulting from this titration will generally be 1%.)

(b) *Standard method.*—For higher precision repeat titration, adding almost entire sugar solution required to reduce all Cu and proceed as in B. From Table 930.44 (see Appendix C) or Table 930.45 (see Appendix C) find total reducing sugar corresponding to titer and apply correction previously determined. Calculate as follows: total reducing sugar required =  $100/\text{titer} = \text{mg sugar in } 100 \text{ mL}$ .

References: *J. Soc. Chem. Ind.* **42**, 32T(1923).

*JAOAC* **9**, 35(1926); **12**, 38(1929).

CAS-8013-17-0 (invert sugar)

#### 4.7.02

### AOAC Official Method 925.05 Sucrose in Animal Feed

First Action 1925

Final Action 1958

Place 10 g test portion in 250 mL volumetric flask. If material is acid, neutralize by adding 1–3 g CaCO<sub>3</sub>. Add 125 mL 50% alcohol by volume, mix thoroughly, and boil on steam bath or by partially immersing flask in water bath 1 h at 83–87°C, using small funnel in neck of flask to condense vapor. Cool and let mixture stand several h, preferably overnight. Dilute to volume with neutral 95% alcohol, mix thoroughly, let settle or centrifuge 15 min at 1500 rpm, and decant closely. Pipet 200 mL supernate into beaker and evaporate on steam bath to 20–30 mL. Do not evaporate to dryness. Little alcohol in residue does no harm.

Transfer to 100 mL volumetric flask and rinse beaker thoroughly with H<sub>2</sub>O, adding rinsings to flask. Add enough saturated neutral Pb(CH<sub>3</sub>COO)<sub>2</sub> solution (ca 2 mL) to produce flocculent precipitate, shake thoroughly, and let stand 15 min. Dilute to volume with H<sub>2</sub>O, mix thoroughly, and filter through dry paper. Add enough anhydrous Na<sub>2</sub>CO<sub>3</sub> or potassium oxalate to filtrate to precipitate all Pb, again filter through dry paper, and test filtrate with little anhydrous Na<sub>2</sub>CO<sub>3</sub> or potassium oxalate to make sure that all Pb has been removed.

Place 50 mL prepared test solution in 100 mL volumetric flask, add piece of litmus paper, neutralize with HCl, add 5 mL HCl, and let inversion proceed at room temperature as in [925.48\(c\)](#) (*see* 44.1.09). When inversion is complete, transfer solution to beaker, neutralize with Na<sub>2</sub>CO<sub>3</sub>, return solution to 100 mL flask, dilute to volume with H<sub>2</sub>O, filter if necessary, and determine reducing sugars in 50 mL solution (representing 2 g test portion) as in [906.03B](#) (*see* 44.1.16). Calculate results as invert sugar.

$$\text{Sucrose, \%} = [\text{percent total sugar after inversion} \\ - \text{percent reducing sugars before inversion} \\ (\text{both calculated as invert sugar})] \quad 0.95$$

Because insoluble material of grain or cattle food occupies some space in flask as originally made up, correct by multiplying all results by factor 0.97, as results of large number of determinations on various materials show average volume of 10 g material to be 7.5 mL.

References: *USDA Bur. Chem. Circ.* **71**.

*JAOAC* **41**, 276(1958); **42**, 39(1959).

CAS-57-50-1 (sucrose)

44.1.17

**AOAC Official Method [929.09](#)**  
**Invert Sugar in Sugars and Syrups**  
Determination of Reduced Copper  
First Action 1929  
Final Action

*See 31.039–31.044, 14th Ed.*

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(10th Ed.)**

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930.44 Total reducing sugar required for complete reduction of 10 ml Soxhlet solution to be used in conjunction with Lane-Eynon general volumetric method

Titer	Invert Sugar, No Sucrose	g Sucrose/100 ml Invert Sugar				Glu- cose	Fruc- tose	Maltose		Lactose	
		1	5	10	25			Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O	Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O
Required for Reduction of 10 ml Soxhlet Soln											
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	.6	50.0	.6	.1	.4	.2	.3	.1	.2	.8	.2
17	.7	.1	.6	.1	.4	.3	.3	.0	.1	.8	.2
18	.8	.1	.6	.1	.3	.3	.4	.0	.0	.7	.1
19	.8	.2	.6	.1	.3	.4	.5	76.9	80.9	.7	.1
20	.9	.2	.6	.1	.2	.5	.5	.8	.8	.6	.0
21	51.0	.2	.6	.1	.2	.5	.6	.7	.7	.6	.0
22	.0	.3	.6	.1	.1	.6	.7	.6	.6	.6	.0
23	.1	.3	.6	.1	.0	.7	.7	.5	.5	.5	67.9
24	.2	.3	.6	.1	.9	.8	.8	.4	.4	.5	.9
25	.2	.4	.6	.0	.8	.8	.8	.4	.4	.5	.9
26	.3	.4	.6	.0	.8	.9	.9	.3	.3	.5	.9
27	.4	.4	.6	.0	.7	.9	.9	.2	.2	.4	.8
28	.4	.5	47.7	.0	.7	50.0	53.0	.1	.1	.4	.8
29	.5	.5	.7	.0	.6	.0	.1	.0	.0	.4	.8
30	.5	.5	.7	.0	.5	.1	.2	.0	.0	.4	.8
31	.6	.6	.7	45.9	.5	.2	.2	75.9	79.9	.4	.8
32	.6	.6	.7	.9	.4	.2	.3	.9	.9	.4	.8
33	.7	.6	.7	.9	.3	.3	.3	.8	.8	.4	.8
34	.7	.6	.7	.8	.2	.3	.4	.8	.8	.4	.9
35	.8	.7	.7	.8	.2	.4	.4	.7	.7	.5	.9
36	.8	.7	.7	.8	.1	.4	.5	.6	.6	.5	.9
37	.9	.7	.7	.7	.0	.5	.5	.6	.6	.5	.9
38	.9	.7	.7	.7	.0	.5	.6	.5	.5	.5	.9
39	52.0	.8	.7	.7	41.9	.6	.6	.5	.5	.5	.9
40	.0	.8	.7	.6	.8	.6	.6	.4	.4	.5	.9
41	.1	.8	.7	.6	.8	.7	.7	.4	.4	.6	68.0
42	.1	.8	.7	.6	.7	.7	.7	.3	.3	.6	.0
43	.2	.8	.7	.5	.6	.8	.8	.3	.3	.6	.0
44	.2	.9	.7	.5	.5	.8	.8	.2	.2	.6	.0
45	.3	.9	.7	.4	.4	.9	.9	.2	.2	.7	.1
46	.3	.9	.7	.4	.4	.9	.9	.1	.1	.7	.1
47	.4	.9	.7	.3	.3	51.0	.9	.1	.1	.8	.2
48	.4	.9	.7	.3	.2	.0	54.0	.1	.1	.8	.2
49	.5	.0	.7	.2	.1	.0	.0	.0	.0	.8	.2
50	.5	.0	.7	.2	.0	.1	.0	.0	.0	.9	.3

930.45 Total reducing sugar required for complete reduction of 25 ml Soxhlet solution to be used in conjunction with Lane-Eynon general volumetric method

Titer	Invert Sugar, No Sucrose	1 g Sucrose/100 ml Invert Sugar	Glu-cose	Fruc-tose	Maltose		Lactose	
					Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O	Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O
Required for Reduction of 25 ml Soxhlet Soln								
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	.6	.7	.2	.4	.4	207.8	.5	.1
17	.6	.7	.2	.5	.0	.4	.1	171.7
18	.7	.7	.2	.5	196.7	.1	162.8	.4
19	.7	.8	.3	.6	.5	206.8	.5	.1
20	.8	.8	.3	.6	.2	.5	.3	170.9
21	.8	.8	.3	.7	195.8	.1	.0	.6
22	.9	.9	.4	.7	.5	205.8	161.8	.4
23	.9	.9	.4	.8	.1	.4	.6	.2
24	124.0	.9	.5	.8	194.8	.1	.5	.0
25	.0	123.0	.5	.9	.5	204.8	.4	169.9
26	.1	.0	.6	.9	.2	.4	.2	.7
27	.1	.0	.6	128.0	193.9	.1	.0	.5
28	.2	.1	.7	.0	.6	203.8	160.8	.3
29	.2	.1	.7	.1	.3	.5	.7	.2
30	.3	.1	.8	.1	.0	.2	.6	.0
31	.3	.2	.8	.1	192.8	202.9	.5	168.9
32	.4	.2	.8	.2	.5	.6	.4	.8
33	.4	.2	.9	.2	.2	.3	.2	.6
34	.5	.3	.9	.3	191.9	.0	.1	.5
35	.5	.3	121.0	.3	.7	201.8	.0	.4
36	.6	.3	.0	.4	.4	.5	159.8	.2
37	.6	.4	.1	.4	.2	.2	.7	.1
38	.7	.4	.2	.5	.0	.0	.6	.0
39	.7	.4	.2	.5	190.8	200.8	.5	167.9
40	.8	.4	.2	.6	.5	.5	.4	.8
41	.8	.5	.3	.6	.3	.3	.3	.7
42	.9	.5	.4	.6	.1	.1	.2	.6
43	.9	.5	.4	.7	189.8	199.8	.2	.6
44	125.0	.6	.5	.7	.6	.6	.1	.5
45	.0	.6	.5	.8	.4	.4	.0	.4
46	.1	.6	.6	.8	.2	.2	.0	.4
47	.1	.7	.6	.9	.0	.0	158.9	.3
48	.2	.7	.7	.9	188.9	198.9	.8	.2
49	.2	.7	.7	129.0	.8	.7	.8	.2
50	.3	.8	.8	.0	.7	.6	.7	.1

930.45 Total reducing sugar required for complete reduction of 25 ml Soxhlet solution to be used in conjunction with Lane-Eynon general volumetric method

Titer	Invert Sugar, No Sucrose	1 g Sucrose/100 ml Invert Sugar	Glu-cose	Fruc-tose	Maltose		Lactose	
					Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O	Anhyd.	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O
Required for Reduction of 25 ml Soxhlet Soln								
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	.6	.7	.2	.4	.4	207.8	.5	.1
17	.6	.7	.2	.5	.0	.4	.1	171.7
18	.7	.7	.2	.5	196.7	.1	162.8	.4
19	.7	.8	.3	.6	.5	206.8	.5	.1
20	.8	.8	.3	.6	.2	.5	.3	170.9
21	.8	.8	.3	.7	195.8	.1	.0	.6
22	.9	.9	.4	.7	.5	205.8	161.8	.4
23	.9	.9	.4	.8	.1	.4	.6	.2
24	124.0	.9	.5	.8	194.8	.1	.5	.0
25	.0	123.0	.5	.9	.5	204.8	.4	169.9
26	.1	.0	.6	.9	.2	.4	.2	.7
27	.1	.0	.6	128.0	193.9	.1	.0	.5
28	.2	.1	.7	.0	.6	203.8	160.8	.3
29	.2	.1	.7	.1	.3	.5	.7	.2
30	.3	.1	.8	.1	.0	.2	.6	.0
31	.3	.2	.8	.1	192.8	202.9	.5	168.9
32	.4	.2	.8	.2	.5	.6	.4	.8
33	.4	.2	.9	.2	.2	.3	.2	.6
34	.5	.3	.9	.3	191.9	.0	.1	.5
35	.5	.3	121.0	.3	.7	201.8	.0	.4
36	.6	.3	.0	.4	.4	.5	159.8	.2
37	.6	.4	.1	.4	.2	.2	.7	.1
38	.7	.4	.2	.5	.0	.0	.6	.0
39	.7	.4	.2	.5	190.8	200.8	.5	167.9
40	.8	.4	.2	.6	.5	.5	.4	.8
41	.8	.5	.3	.6	.3	.3	.3	.7
42	.9	.5	.4	.6	.1	.1	.2	.6
43	.9	.5	.4	.7	189.8	199.8	.2	.6
44	125.0	.6	.5	.7	.6	.6	.1	.5
45	.0	.6	.5	.8	.4	.4	.0	.4
46	.1	.6	.6	.8	.2	.2	.0	.4
47	.1	.7	.6	.9	.0	.0	158.9	.3
48	.2	.7	.7	.9	188.9	198.9	.8	.2
49	.2	.7	.7	129.0	.8	.7	.8	.2
50	.3	.8	.8	.0	.7	.6	.7	.1