National food safety standard

GB 5413.5-2010

Determination of lactose and sucrose in foods for infants and young children, milk and milk products

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Foreword

This standard replaces GB/T 5413.5—1997 < Milk powder and formula foods for infant and young children - Determination of lactose and sucrose contents >

Compared with GB/T 5413.5—1997, the following items are revised:
---- Evaporative light scattering detector was added in the test.

This standard replaces the following editions issued in the past:
---- GB 5413-1985、GB/T 5413.5-1997.

Determination of lactose and sucrose in foods for infants and young children, milk and milk products

1 Scope

This standard specifies the method for the determination of lactose and sucrose in foods for infants and young children and in dairy products.

The standard applies to the determination of lactose and sucrose in foods for infants and young children and in dairy products.

2 Normative references

The references to this standard are indispensable. For the reference documents with dates cited, only the date-cited editions apply to this standard. For the reference documents without dates cited, the latest editions including all the amendments apply to this standard.

Method 1 High Performance Liquid Chromatography Method (HPLC)

3 Principle

Extract the lactose/sucrose in sample, separate them with high performance liquid chromatography (HPLC). Detect by refractive index detector or evaporative light scattering detector. External standard is used for quantitative test.

4 Reagents and materials

All the reagents used in this method is analytical pure unless otherwise specified. Water used should meet the requirements of 1st grade regulated in GB/T 6682.

4.1 Acetonitrile

4.2 Acetonitrile: HPLC grade.

4.3 Standard solution

4.3.1 Lactose standard solution, 20mg/mL

Dry lactose in a desiccator set at 94℃±2℃ for 2 hours. Weigh 2g lactose with precision to 0.1mg Dissolve the lactose standard sample in water and dilute to 100mL. Put the solution in refrigerator at 4℃.

4.3.2 Lactose standard solution for test: take lactose standard solution (4.3.1) 0mL, 1mL, 2mL, 3mL, 4mL, 5mL respectively to 10mL volumetric flasks, use acetonitrile (4.1) to set the volume to the mark. The lactose contents of the standard solutions for test are respectively 0mg/mL, 2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL, 10mg/mL.

4.3.3 Sucrose standard solution, 10mg/mL.

Dry sucrose in a desiccator set at 105℃±2℃ for 2 hours. Weigh 1g sucrose with precision to 0.1mg Dissolve the sucrose standard sample in water and dilute to 100mL. Put the solution in refrigerator at 4℃.

4.3.4 Sucrose standard solution for test: take sucrose standard solution (4.3.3) 0mL, 1mL, 2mL, 3mL, 4mL, 5mL respectively to 10mL volumetric flasks, use acetonitrile (4.1) to set the volume to the mark. The sucrose contents of the standard solutions for test are respectively 0mg/mL, 1mg/mL, 2mg/mL, 3mg/mL, 4mg/mL, 5mg/mL.

5 Apparatus

5.1 Analytical Balance, capable of weighing to the nearest 0.1mg.
5.2 HPLC, with refractive index detector or evaporative light scattering detector.
5.3 Ultrasonic oscillator.

6 Procedure

6.1 Sample preparation
Weigh 1g of solid sample or 2.5 g of liquid sample to the nearest 0.1mg and put the sample to 50mL volumetric flask. Add 15mL water with temperature of 50℃-60□, viberate by ultrasonic oscillator for 10min, dilute to the mark with acetonitrile (4.1). Set still for several minutes and filter the solution. Transfer 5 mL filtrate into 10mL volumetric flask, dilute to the mark with acetonitrile (4.1). Filter with 0.45μm membrane and collect the filtrate for chromatography analysis. Dilute it if needed.

6.2 HPLC determination

6.2.1 Chromatographic conditions
Column: amino column 250 × 4.6mm, 5μm; or column with equivalent performance.
Mobile phase: Acetonitrile (4.2): water = 70:30 (volume fraction)
Flow rate: 1mL/min
Column temperature: 35 □
Injection volume: 10μL
Refractive index detector condition: 33℃-37℃
Evaporative light scattering detector: drift tube temperature 85℃-90℃
air volume: 2.5L/min
cascade impactor: off

6.2.2 Preparation of calibration curve
Inject the standard solutions for test into HPLC, determine the corresponding peak responses (peak area or peak height). Get the calibration curve with peak area or peak height as y-coordinate and standard solution content as x-coordinate.

6.2.3 Determination of samples
Inject sample solution (6.1) into HPLC, determine peak area or peak height, calculate the sugar content from calibration curve.

7 Calculation and results expression

The sugar content in sample is calculated through equation 1:

$$X = \frac{c \times V \times 100 \times n}{m \times 1000} \qquad \ldots\ldots(1)$$

Where,
X – the sugar content in sample, g/100g
c - the sugar content in test sample, mg/mL;
V – the constant volume of the sample, mL;

n - dilution rate;
m – mass of test sample, g.

The result is expressed as arithmetic mean of duplicate samples of single test results, with 3 significant digits.

8 Precision

The absolute difference between duplicate samples of single test results shall not exceed 5% of arithmetic mean.

Method 2 Lane and Eynon's Method

9 Principle

Lactose: Deproteinize sample first, then directly titrate the calibrated Fehling solution with methylene blue as indicator. Calculate lactose content according to volume of sample solution used in titration.

Sucrose: Deproteinize sample first. Sucrose is hydrolyzed by hydrochloric acid to glucose and fructose with reduction capacity, then test the reducing sugar. The content of sucrose equals to the difference value of invert sugar between before and after hydrolysis multiplies corresponding coefficients.

10 Reagents and materials

All the reagents used in this method is analytical pure unless otherwise specified. Water used should meet the requirements of 3rd grade regulated in GB/T 6682.

10.1 Lead acetate
10.2 Potassium oxalate
10.3 Disodium hydrogen phosphate
10.4 Hydrochloric acid
10.5 Copper sulphate
10.6 Concentrated sulfuric acid
10.7 Potassium sodium tartrate
10.8 Sodium hydroxide
10.9 Phenolphthalein
10.10 Ethanol
10.11 Methylene blue
10.12 Lead acetate solution (200g/L): weigh 200g lead acetate and dissolve to 1000mL with water.
10.13 Potassium oxalate - disodium hydrogen phosphate solution: weigh 30g potassium oxalate, 70g disodium hydrogen phosphate, dissolve to 1000mL with water.
10.14 Hydrochloric acid solution (1+1): volume ratio to water is 1:1.
10.15 Sodium hydroxide solution (300g/L): weigh 300g sodium hydroxide, dissolve to 1000mL with water.
10.16 Fehling solution (A and B)
10.16.1 A: weigh 34.639g copper sulphate, dissolve in water, add 0.5mL concentrated sulfuric acid, and dilute to 500mL.
10.16.2 B: weigh 173g potassium sodium tartrate and 50g sodium hydroxide, dissolve to 500mL with water. Set still for two days and filter the solution.

10.17 Phenolphthalein solution: weigh 0.5g phenolphthalein and dissolve to 100mL with 95% ethanol(V/V)
10.18 Methylene blue solution (10g / L): weigh 1g methylene blue and dissolve with 100mL water.

11 Apparatus

11.1 Analytical Balance, capable of weighing to the nearest 0.1mg.
11.2 Water bath at 75℃±2℃

12 Procedure

12.1 Calibration of Fehling Solution

12.1.1 Calibration with lactose standard

12.1.1.1 Dry lactose in a desiccator set at 94℃±2℃ for 2 hours. Weigh 0.75g lactose with precision to 0.1mg. Dissolve to 250mL with water. Transfer part of the solution to a 50mL burette, prepared for titration.

12.1.1.2 Pre-titration: Transfer 10mL Fehling solution (5mL A and 5mL B) into a 250mL Erlenmeyer flask. Add 20mL distilled water and put in several glass balls. Release from the above burrette (12.1.1.1) 15mL solution into the flask. Place the flask on a preheated electric hot plate, let the solution boil within 2min, keep the boiling state for 15seconds, add 3 drops of methylene blue solution (10.18), titrate till blue color disappears, read out the volume of lactose solution used.

12.1.1.3 Accurate titration: Transfer 10mL Fehling solution (5mL A and 5mL B) in another 250mL Erlenmeyer flask. Add 20mL distilled water and put in several glass balls, release the volume of solution 0.5-1.0mL less than the volume used in pre-titration into the flask. Place the flask on a preheated electric hot plate, let the solution boil within 2min, keep the boiling state for 2min, add 3 drops of methylene blue solution (10.18), titrate with speed of one drop every two seconds till blue color disappears, read out the volume of lactose solution used and record the volume.

12.1.1.4 Correction value of lactose to Fehling solution is calculated according to equation 2 and equation 3 showed as below:

$$A_1 = \frac{V_1 \times m_1 \times 1000}{250} = 4 \times V_1 \times m_1 \quad \cdots\cdots (2)$$

$$f_1 = \frac{4 \times V_1 \times m_1}{AL_1} \quad \cdots\cdots (3)$$

Where,
A_1—— the lactose factor tested， mg；
V_1—— volume of lactose solution used while titration， mL；
m_1——the mass of lactose weighed， g；
f_1—— the correction value of lactose to Fehling solution
AL_1——the lactose factor obtained from table 1, mg.

Table 1 Lactose and invert sugar factors（10mL Fehling Solution）

Titration volume, mL	Lactose, mg	Invert sugar, mg	Titration volume, mL	Lactose, mg	Invert sugar, mg
15	68.3	50.5	33	67.8	51.7

16	68.2	50.6	34	67.9	51.7
17	68.2	50.7	35	67.9	51.8
18	68.1	50.8	36	67.9	51.8
19	68.1	50.8	37	67.9	51.9
20	68.0	50.9	38	67.9	51.9
21	68.0	51.0	39	67.9	52.0
22	68.0	51.0	40	67.9	52.0
23	67.9	51.1	41	68.0	52.1
24	67.9	51.2	42	68.0	52.1
25	67.9	51.2	43	68.0	52.2
26	67.9	51.3	44	68.0	52.2
27	67.8	51.4	45	68.1	52.3
28	67.8	51.4	46	68.1	52.3
29	67.8	51.5	47	68.2	52.4
30	67.8	51.5	48	68.2	52.4
31	67.8	51.6	49	68.2	52.5
32	67.8	51.6	50	68.3	52.5
Note: The "factor" means the value corresponding to the volume of titration, and it can be referred to table 1. If the ratio of sucrose content to lactose content is above 3:1, then the titration volume should plus the corrective factor showed in table 2.					

12.1.2 Calibration with sucrose

12.1.2.1 Dry sucrose in a desiccator set at 105℃±2℃ for 2 hours. Weigh 0.2g lactose with precision to 0.1mg. Dissolve with 50mL water and transfer the solution into a 100mL volumetric flask. Add 10mL water and 10mL hydrochloric acid solution (10.14). Put the flask in a water bath of 75℃, shake constantly, increase the inner temperature of solution in flask to 67.0℃-69.5℃, keep 5min and cool it down. Add 2 drops of phenolphthalein solution (10.17), neutralize with sodium hydroxide solution (10.15) to light pink color and set the volume to the mark. Process according to the procedure stated in 12.1.1.2 and 12.1.1.3.

12.1.2.2 Correction value of sucrose to Fehling solution is calculated according to equation 4 and equation 5 showed as below:

$$A_2 = \frac{V_2 \times m_2 \times 1000}{100 \times 0.95} = 10.5263 \times V_2 \times m_2 \quad \cdots\cdots (4)$$

$$f_2 = \frac{10.5263 \times V_2 \times m_2}{AL_2} \quad \cdots\cdots (5)$$

Where:

A_2—— the invert sugar factor tested, mg;

V_2——volume of sucrose solution used while titration, mL;

m_2——the mass of sucrose weighed, g;

0.95 – molecular weight ratio between sum of fructose and glucose to that of sucrose

f_2—— the correction value of sucrose to Fehling solution

AL_2——the invert sugar factor obtained from table 1 according to the read volume of sucrose solution used, mg.

12.2 Determination of lactose
12.2.1 Preparation of sample
12.2.1.1 Weigh 2g of infant food or skimmed milk powder, 2.5 g of whole milk powder fortified with sugar or whole milk powder, 1g of whey powder on the analytical balance to the nearest 0.1mg, dissolve with 100mL water, transfer into a 250mL volumetric flask.

12.2.1.2 Slowly add 4mL lead acetate solution(10.12), 4mL potassium oxalate - disodium hydrogen phosphate solution (10.13) into the flask, shake and set the volume to the mark. Set still and filter. Abandon the first 25mL filtrate. Keep the rest of the filtrate for use.

12.2.2 Titration
12.2.2.1 Pre-titration: same procedure as described in 12.1.2.

12.2.2.2 Accurate titration: same procedure as described in 12.1.3.

12.3 Determination of sucrose: transfer 50mL of the sample to 100mL volumetric flask. Add 10mL water and 10mL hydrochloric acid solution (10.14). Put the flask in a water bath of 75℃, shake constantly, increase the inner temperature of solution in flask to 67.0℃-69.5℃, keep 5min and cool it down. Add 2 drops of phenolphthalein solution (10.17), neutralize with sodium hydroxide solution (10.15) to light pink color and set the volume to the mark. Process according to the procedure stated in 12.1.1.2 and 12.1.1.3.

13 Calculation and results expression
13.1 Lactose: the lactose content X, as percentage is calculated according to equation 6.

$$X = \frac{F_1 \times f_1 \times 0.25 \times 100}{V_1 \times m} \quad \cdots\cdots (6)$$

Where:
X - mass fraction of lactose in sample, g/100g;
F_1 - the lactose factor obtained from table 1 according to the read volume of lactose solution used, mg;
f_1 – correction value to Fehling solution;
V_1 - volume of solution used in titration, mL;
m - the mass of sample weighed, g;

The result is expressed as arithmetic mean of duplicate samples of single test results, with 3 significant digits.

13.2 Sucrose

The mass fraction of invert sugar before inversion, X_1(g/100g), is calculated according to equation 7 using the volume in lactose titration,.

$$X_1 = \frac{F_2 \times f_2 \times 0.25 \times 100}{V_1 \times m} \quad \cdots\cdots (7)$$

Where:
X_1 – mass fraction of invert sugar before inversion, g/100g

F_2 - the invert sugar factor obtained from table 1 according to the read volume of sample solution used while determine lactose, mg;
f_2 – correction value of sucrose to Fehling solution
V_1 - volume of solution used in titration, mL;
m - the mass of sample weighed, g.

The mass fraction of invert sugar after inversion, X_2(g/100g), is calculated according to equation 8 using volume in sucrose titration..

$$X_2 = \frac{F_3 \times f_2 \times 0.50 \times 100}{V_2 \times m} \quad \cdots\cdots (8)$$

Where:
X_2 – mass fraction of invert sugar after inversion, g/100g
F_3- the invert sugar factor obtained according toV_2, mg;
f_2 – correction value of sucrose to Fehling solution
m - the mass of sample weighed, g.
V_2 – inversion solution volume used in titration, mL;

The content of sucrose in sample, expressed in mass fraction X(g/100g), is calculated according to equation 9.

$$X = (X_2 - X_1) \times 0.95 \quad \cdots\cdots (9)$$

Where:
X—mass fraction of sucrose in sample, g/100g;
X_1—mass fraction of invert sugar before inversion, g/100g;
X_2 – mass fraction of invert sugar after inversion, g/100g.

The result is expressed as arithmetic mean of duplicate samples of single test results, with 3 significant digits.

13.3 If the ratio of sucrose content to lactose content in sample is beyond 3:1, correction factor showed on table 2 should be added to the titration volume for calculation of lactose, then check from table 1 and calculate.

Table 2 Correction factor of lactose titration volume

Sugar solution volume used at titration end, mL	The content ratio of sucrose to lactose,10mL Fehling solution used	
	3:1	6:1
15	0.15	0.30
20	0.25	0.50
25	0.30	0.60
30	0.35	0.70
35	0.40	0.80
40	0.45	0.90
45	0.50	0.95
50	0.55	1.05

14 Precision

Absolute difference of two independent results under repetitive condition should not exceed 1.5% of arithmetic mean value.

15 Others

The detection limit of method 1 in this standard is 0.3g/100g; the detection limit of method 2 in this standard is 0.4g/100g.