Japan Customs Analysis Methods

No. 108

Quantitative Analysis of Sucrose in Confectionary

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1. Scope

This analysis method is applied to confectionary for which a determination of the sucrose content is required.

2. Outline of the Test Method

This analytical method is applied for the determination of sucrose contained in confectionery, such as sugar confectionary, bakery products, chocolates and other products containing cocoa, and boiled red beans, and the analysis is to be performed by either a high performance liquid chromatographic (HPLC) method or a titration method according to the flow diagram below.

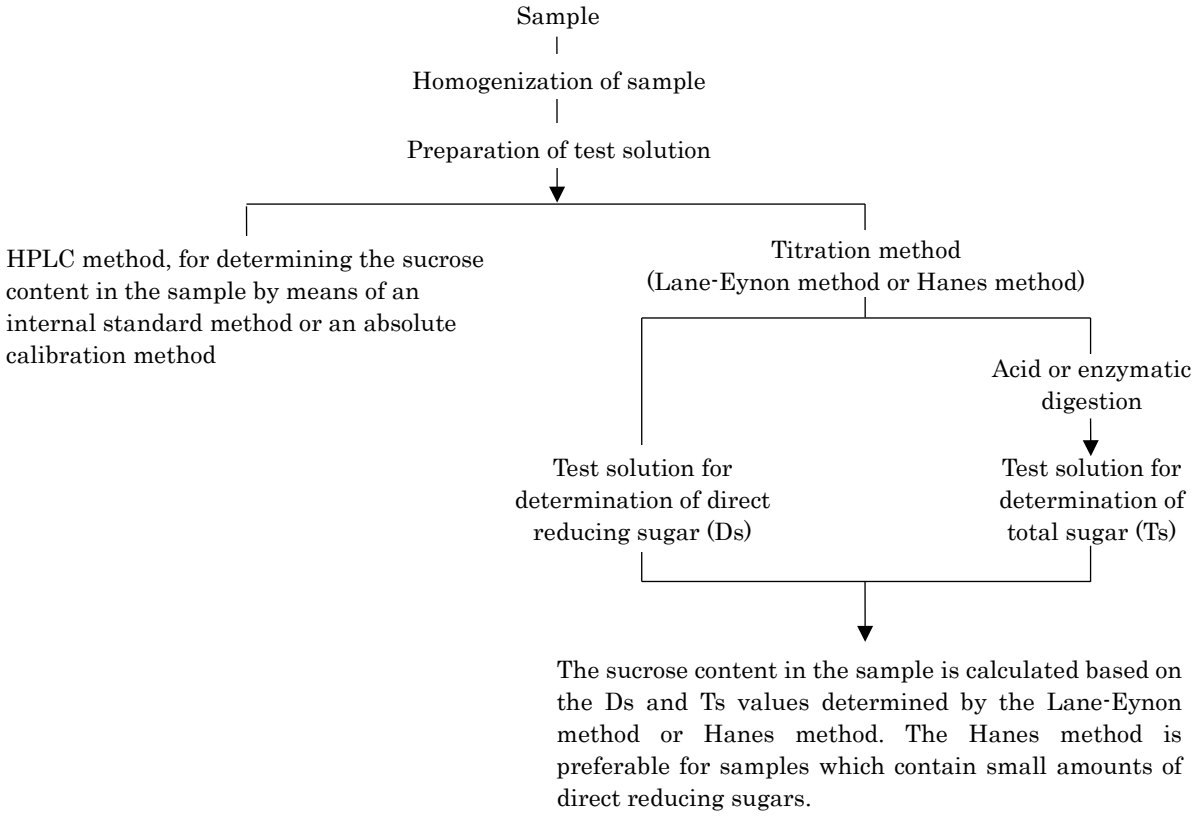

Fig. 1 Illustration of analysis flow

3. Apparatus

For the HPLC method

・ High performance liquid chromatograph with a Refractive Index Detector

Amino columns and gel columns, for sugar analysis, are available for the analysis. In either case, it is desirable that the resolution (*Rs*) values[1] between the sucrose peak and other ingredients' peaks and between the internal standard peak and other ingredients' peaks are 1.5 or higher. Do not use analytical conditions where the *Rs* value is less than 1. Analytical conditions[2] are as follows:

● Option 1 (with an amino column)

(a) Analytical column: Amino column for sugar analysis, 4.6 mmφ × 250 mm, or equivalent

(b) Column temperature: 30–40°C (constant)

(c) Mobile phase: Acetonitrile to Water = 75:25

(d) Flow rate: 0.5–1.0 mL/min (constant)

(e) Injection volume: 10–20 µL (constant)

● Option 2 (with a gel column)

(a) Analytical column: Gel-filtration and ligand-exchange column for sugar analysis, 8.0 mmφ × 250–300 mm, or equivalent

(b) Column temperature: 80°C

(c) Mobile phase: Water

(d) Flow rate: 0.5–1.0 mL/min (constant)

(e) Injection volume: 10–20 µL (constant)

Note 1) Resolution (Rs) between peaks of two components is calculated from the following formula:

$$Rs = \frac{1.18 \times (t_2 - t_1)}{w_1 + w_2}$$

Where–

t_1, t_2: Retention times of two adjacent peaks

w_1, w_2: Peak widths at half-height, of two adjacent peaks

Note 2) Optimize the conditions of flow rate, column temperature, etc., depending on the analytical column used.

4. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

4.1. For the Lane-Eynon method and Hanes method

(1) Standard invert sugar solution

Accurately weigh 4.75 g of sucrose, transfer with 90 mL of water to a 500 mL volumetric flask, and dissolve. Add 5 mL of hydrochloric acid (specific gravity: 1.18) to the flask and store for three days at room temperature (20–30°C). Then, dilute the solution to volume with water and keep in a cool dark place. Transfer a 50 mL portion of the solution to a 200 mL volumetric flask, neutralize with 1 mol/L sodium hydroxide aqueous solution using phenolphthalein as an indicator, and dilute to volume with water. Use the solution, as a standard invert sugar solution for the standardization of Fehling's solution.

(2) 1% Methylene Blue solution

Dissolve 1 g of methylene blue in water to make 100 mL.

(3) Fehling's Solutions

・ Solution A

Dissolve 34.639 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water to make 500 mL exactly. Allow it to stand for two days and filter.

・ Solution B

Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 50 g of sodium hydroxide in water to make 500 mL exactly. Allow it to stand for two days and filter.

・ Standardization of Fehling's solution

Put 5.0 mL of Fehling's Solution A and 5 mL of Fehling's Solution B in a 200 mL Erlenmeyer flask containing a few glass beads and add from a 50 mL burette 19.5 mL of the standard invert sugar solution. Boil the solution on an electric stove (heater) for two minutes, add four drops of the methylene blue solution, and complete

titration within a total boiling time of three minutes by dropwise addition of the standard invert sugar solution—without preventing boiling—until the blue color disappears. Repeat titration twice and calculate the mean of three parallel titrations. [3]

Obtain the *factor* of the Fehling's solution from the following formula:

$$Factor^{(4)} = \frac{20.36}{A}$$

Where–

A: Volume (mL) of the standard invert sugar solution required

Note 3) Use the mean value of three parallel titrations as the volume of the standard invert sugar solution required; Duplicate titrations must agree to within 0.1 mL in the volume of the standard invert sugar solution required.

Note 4) Calculate the *factor* by rounding off fractions to the third decimal place; the *factor* must be within a range of 1± 0.02.

(4) Deproteinizing agent

・ Solution A

Dissolve 2 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in water to make 100 mL.

・ Solution B

Dissolve 1.8 g of barium hydroxide [$(Ba(OH)_2 \cdot 8H_2O)$] in water to make 100 mL.

(5) Invertase solution

Prepare by dissolving 40 mg of invertase (150 units/mg or equivalent) in 15 mL of a 0.1 mol/L acetic acid buffer (pH 4.6). Prepare the solution before use.

(6) 0.1M acetic acid buffer (pH 4.6)

Weigh 30 g of glacial acetic acid in a beaker, dissolve in water and transfer to a 500 mL volumetric flask. Dilute the solution to volume with water. Transfer a 100 mL portion of the solution in a beaker and adjust at a pH of 4.6 by gradually adding 5% sodium hydroxide aqueous solution. Transfer it to a 1,000 mL volumetric flask and dilute to volume with water.

(7) 1% soluble starch solution

Weigh 1 g of soluble starch in a beaker, disperse in about 30 mL of water and transfer to about 70 mL of boiling water while stirring continually. Heat the mixed solution until it becomes transparent, add 5 g of sodium chloride and dissolve. After cooling, store the prepared solution in a refrigerator.

(8) Standard invert sugar solution (for the Harness method)

Accurately weigh 380 mg of sucrose, dissolve in water and transfer to a 200 mL volumetric flask. Dilute it to volume with water. Pipet a 50 mL portion of the sucrose solution into a 100 mL volumetric flask, add 5 mL of 25% hydrochloric acid and place the flask in a hot water bath at a constant temperature of exactly 65±1°C for 20 minutes for hydrolysis. After the hydrolysis, immediately cool the flask under running water, neutralize the hydrolysate solution with 4 mol/L sodium hydroxide aqueous solution using phenolphthalein as an indicator, and dilute to volume with water.

(9) Hanes reagents

・ Reagent A

Dissolve 8.25 g of potassium ferricyanide [$K_3 Fe(CN)_6$] and 10.6 g of sodium carbonate (anhydrous) in water, transfer to a 1,000 mL volumetric flask and dilute to volume with water.

After leaving to stand for 2–3 days in a dark cool place, filter the solution through filter paper. Store the filtrate in a brown bottle. If precipitates are observed in the bottle during the storage, filter the solution before use.

・ Reagent B

Dissolve 12.5 g of potassium iodine, 25 g of zinc sulfate and 125 g of sodium chloride in water, transfer to a 500 mL volumetric flask and dilute to volume with water. Store the solution in a brown bottle. If precipitates are observed in the bottle during the storage, filter the solution before use.

・ Reagent C

Dissolve 5 mL of glacial acetic acid in water to make 100 mL.

(10) 0.1 mol/L sodium thiosulfate solution

Dissolve 24.8 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in about 500 mL of water, add 2 g of sodium carbonate (anhydrous) and 10 mL of isoamyl alcohol and dissolve. Transfer the solution to a 1,000 mL volumetric flask and dilute to volume with water. Dilute the prepared solution with water ten-fold before use.

(11) Standardization of the 0.1 mol/L sodium thiosulfate solution prepared

Prepare 0.1 mol/L potassium iodate solution by dissolving 3.567 g of dried KIO_3 (which has been previously dried at 120 to 140°C for two hours and cooled in a desiccator) in water to make exactly 1 L. Pipet a 25 mL portion of the potassium iodate solution into a 300 mL Erlenmeyer flask with a stopper. Add 50 mL of water, 2 g of potassium iodide and 5 mL of 3 mol/L sulfuric acid, plug the flask tightly and leave to stand in a dark place for ten minutes.

Then, dilute the solution with 100 mL of water and titrate-free iodine in the solution with a 0.1 mol/L sodium thiosulfate solution to be standardized, while shaking constantly, until the yellow color has almost disappeared. Add 3–4 drops of the 1% soluble starch solution and continue the titration by adding the thiosulfate solution slowly until the purplish blue color just disappears.

Perform a blank test separately. Use the difference between the volumes of the thiosulfate required to titrate the blank and the standard solution, as the titer (M mL). Calculate the *factor* (*F*) of the sodium thiosulfate solution used from the following equation.

$$F = \frac{25}{\mathrm{M}}$$

Round off fractions to the third decimal place.

4.2. For the HPLC method

(1) Standard sucrose stock solution

Accurately weigh about 1 g of sucrose in a 100 mL volumetric flask, dissolve in water and dilute to volume with water.

(2) Internal standard stock solution [5]

Accurately weigh about 10 g of sorbitol in a 100 mL volumetric flask, dissolve in water and dilute to volume with water.

(3) Preparation of standard solutions for absolute calibration method [6]

Prepare standard solutions (3–5 points) using the standard sucrose stock solution prepared in (1) above, depending on the sucrose content in the test sample.

(4) Preparation of standard solutions for internal standard method [6]

Prepare standard solutions (3–5 points) using the standard sucrose stock solution prepared in (1) above together with a constant volume of the internal standard stock solution prepared in (2) above, depending on the sucrose content in the test sample.

(5) Water – HPLC grade (or equivalent)

(6) Acetonitrile – HPLC grade (for the amino column condition)

Note 5) As alternative internal standard substances, glycerin, diethylene glycol, etc. will be available.

Note 6) When analysis samples require a deproteinization treatment, standard solutions to be used for making a calibration curve also have to be treated under the same deproteining conditions.

5. Sample preparation

Prepare analysis samples appropriately depending on their conditions. For solid materials, grind them using a grinder or a mixer. For pasty or wet materials, homogenize them in mortars. In any case, collect a relatively large amount of the sample randomly and homogenize it by grinding and/or mixing to uniformity.

6. Procedure

6.1. Determination of sucrose content by the Lane-Eynon method

6.1.1. Sampling quantities

The collection quantities of samples necessary for the analysis are to be decided by making reference to the following table, noting that the concentrations of reducing sugars, expressed as invert sugar, in the test solutions become 150–250 mg/100mL.

Kinds of materials	Direct reducing sugar (%)	Sucrose (%)	Sample amount (g) to be needed	
			For direct reducing sugar analysis (in 6.1.2.1)	For total sugar analysis (in 6.1.2.2)
(Sugar confectionary)				
Chewing gum	5–20	40–60	8–3	5–4
Candy	5–30	60–90	8–2	3–2
(Cocoa preparations)				
Chocolate	5–15	30–70	8–4	6–3
Preparations of cocoa	0–20	80–90	90–3	3–2
(Bakery products)				
Biscuits, cookies	0.5–10	5–40	90–5	40–5
(Bean paste)				
Boiled red beans	0–2	40–65	90–20	5–3

6.1.2. Preparation of test solution

6.1.2.1. Test solution for determination of direct reducing sugar

Accurately weigh a certain amount of the sample homogenized in 5., remove fat[7] if a large amount of it is contained, and dissolve in water. Transfer the solution with about 200 mL of water to a 250 mL volumetric flask, and shake well (about one hour) for extraction.[8]

Add 20 mL each of deproteinizing solutions A and B into the flask if necessary, and shake well to mix. Dilute the solution to volume with water and mix well again. After leaving to stand for 30 minutes, filter the solution through filter paper. Use the filtrate as a test solution.

Note 7) For fat-rich samples, remove the fat with the following treatment:

Accurately weigh a certain amount of the sample homogenized in 5., and transfer with about 200 mL of water to a 250 mL volumetric flask. Add 20 mL of hexane into the flask and shake gently for about 10 minutes to extract the fat. After leaving to stand for a while, remove the hexane layer with a pipette. Add 20 mL of hexane into the flask and repeat the procedure above for removal of fat.

Note 8) For samples containing large quantities of water insoluble matters, such as biscuits and boiled red beans, prepare their test solutions using the following filtration method (or centrifugation method):

Accurately weigh a certain amount of the sample homogenized in 5., and transfer it with about 150 mL of water to a baker. Leave it for about one hour, while stirring occasionally. Transfer the content to a 250 mL volumetric flask through filtration using filter paper (JIS P 3801 No.2 grade or equivalent). Wash residue on the filter paper with water carefully and add the washings to the filtrate above. Deproteinize the obtained solution if necessary and dilute to volume with water. If a large amount of water is required to wash residue after filtration, a dilution to 500 mL could be allowed (although it would depend on the amount of the sample collected).

6.1.2.2. Test solution for determination of total sugar

A) Acid digestion method [9]

Accurately weigh a certain amount of the sample homogenized in 5., remove fat[7] if a large amount of it is contained, and dissolve in water. Transfer the solution with about 200 mL water to a

250 mL volumetric flask, and shake well (about one hour) for extraction.[8]

Add 20 mL each of deproteinizing solutions A and B if necessary, and shake well to mix. Dilute the solution to volume with water and mix well again. After leaving to stand for 30 minutes, filter the solution through filter paper.

Put a 50 mL portion of the filtrate into a 100 mL Erlenmeyer flask using a whole pipet, add 2.5 mL of 25% hydrochloric acid and place the flask in a water bath at a constant temperature of exactly 65±1°C for 20 minutes for hydrolysis of sucrose in the solution. After the hydrolysis, immediately cool the flask under running water. Neutralize the hydrolysate solution with 4 mol/L sodium hydroxide aqueous solution using phenolphthalein as an indicator, transfer to a 250 mL volumetric flask and dilute to volume with water. Use the resulting solution as test solution. [10]

Note 9) Provided that acid hydrolysis is carried out under the exact conditions described here, sucrose is selectively hydrolyzed even if disaccharides other than sucrose, e.g. maltose, lactose, etc., are contained. When raffinose coexists, both the acid digestion method and enzymatic digestion method hydrolyze it. However, since raffinose is rarely added into confectionary and when it is the amount is small, it would have almost no impact on this analysis in practice.

Note 10) For sucrose-rich samples, their test solutions for total sugar analysis can be prepared by diluting their test solutions for direct reducing sugar analysis to be prepared in 6.1.2.1.

B) Enzymatic digestion method

Accurately weigh a certain amount of the sample homogenized in 5., remove fat[7] if a large amount of it is contained, and dissolve in water. Transfer the solution with about 200 mL of water to a 250 mL volumetric flask, and shake well (about one hour) for extraction.

Add 2–3 mL of the invertase solution into the sample solution and mix. Place the flask in a water bath at a constant temperature of 37±1°C for 30 minutes for enzymatic reaction. Add 20 mL each of deproteinizing solutions A and B and shake well. Dilute the solution to volume with water and shake again. After leaving to stand for about 30 minutes, filter the solution through a filter paper.

Transfer a 50 mL portion of the filtrate into a 250 mL volumetric flask using a whole pipet and dilute to volume with water. Use the resulting solution as test solution.

6.1.3. Titration and calculation of sucrose content

6.1.3.1. Titration of direct reducing sugar

Put 5.0 mL of Fehling's Solution A and 5 mL of Fehling's Solution B into a 200 mL Erlenmeyer flask containing a few glass beads and add from a 50 mL burette 15 mL of the sugar solution prepared in 6.1.2.1. Boil the solution on an electric stove (heater) for 2 minutes and titrate in the same manner as in 4.1. (3) "Standardization of Fehling's Solution" (preliminary titration).

Subsequently, add from a 50 mL burette to another 200 mL Erlenmeyer flask the sugar solution within 1 mL of the anticipated end point from the result of the preliminary titration above and titrate in the same manner as in 4.1. (3). Multiply the titer by the *factor* of the Fehling's solution and find the concentration of invert sugar "Ds" (mg/100mL) in the sugar solution by making reference to the appended table "Lane-Eynon Table for invert sugar, without sucrose.".[11, 12]

Calculate from the following formula the content of direct reducing sugar in the sample. Round off fractions to the second decimal place.

$$\%, \text{direct reducing sugar} = \frac{\text{Ds}}{\text{S}} \times 0.25$$

Where–

S: Weight (g) of sample collected in 6.1.2.1.

Note 11) Refer to the appended "Lane-Eynon Table for invert sugar, without sucrose" for determination.

Note 12) Direct reducing sugar is deemed to be mainly composed of invert sugar and therefore expressed as invert sugar.

6.1.3.2. Titration of total sugar

Titrate with the sugar solution prepared in 6.1.2.2. in the same manner as in 6.1.3.1., obtain the concentration of invert sugar "Ts" (mg/100mL) in the sugar solution,[11] and calculate from the following formula the total sugar content of the sample. Round off fractions to the second decimal place.

$$\%,\text{total sugar} = \frac{\text{Ts} \times 2.5 \times 5 \times 100}{\text{S} \times 1000} = \frac{\text{Ts}}{\text{S}} \times 1.25$$

Where– S: Weight (g) of sample collected in 6.1.2.2.

6.1.4. Calculation of Sucrose Content

Using the contents of direct reducing sugar and total sugar determined in 6.1.3.1 and 6.1.3.2., calculate the sucrose content in the sample from the following formula. Round off fractions to the second decimal place.

%, sucrose = {total sugar (%) - direct reducing sugar (%)} × 0.95

6.2. Determination of sucrose by Hanes method

6.2.1. Amount of sample needed

The collection quantities of samples necessary for the analysis are generally 5 to 10 g for sucrose concentrations of less than 20%, 2 g for sucrose concentrations of 20% to 50%, and about 1 g for sucrose concentrations of more than 50%. As for individual cases for sampling, refer to the table in 6.1.1.

6.2.2. Preparation of sample solution

Accurately weigh a certain amount of the sample homogenized as described in 5., remove fat[7] if a large quantity of fat is contained, and dissolve in water. Transfer the solution with about 150 mL of water to a 200 mL volumetric flask and shake well (about an hour) for extraction. Dilute it to volume with water. If insoluble substances present, filter the solution through a filter paper. Use the filtrate for preparation of test solutions in 6.2.2.1. and 6.2.2.2.

6.2.2.1. Test solution for determination of direct reducing sugar

Put a 20 mL portion of the sample solution prepared in 6.2.2. into a 100 mL volumetric flask using a whole pipet. Add 5 mL each of deproteinizing solutions A and B into the flask using whole pipets if needed, and shake thoroughly. Dilute it to volume with water and mix well again. After leaving to stand for about 30 minutes, filter the solution through filter paper. Use the filtrate as a test solution for determination of direct reducing sugar.

6.2.2.2. Test solution for determination of total sugar

Put 20 mL of the sample solution prepared in 6.2.2. into a 100 mL volumetric flask using a whole pipet, add 3 mL of the invertase solution and mix. Place the flask in a water bath at a constant temperature of 37 ±1°C for 30 minutes for enzymatic reaction.

Add 5 mL each of deproteinizing solutions A and B to the flask using whole pipets, shake thoroughly and dilute to volume with water. Shake the flask again. After leaving to stand for about 30 minutes, filter the solution through filter paper. Use the filtrate as test solution for the determination of total sugar. The acid digestion method described in 6.1.2.2. (A) can be applied instead of this enzymatic digestion method.

6.2.3. Titration

Put 0.5, 1.0, 2.0 and 3.0 mL of the standard invert sugar solution (for the Harness method) prepared in 4.1. (8) into four respective large test tubes (2.5–3 cm in diameter) using whole pipets. Dilute each of the solutions to 5 mL accurately by adding 4.5, 4.0, 3.0 and 2.0 mL of water using whole pipets. Use the solutions to construct a calibration curve.

Put 5 mL each of the two test solutions, namely the test solution for the determination of direct reducing sugar and the test solution for the determination of total sugar, into two separate large test tubes using whole pipets.[13] Separately, prepare a blank by adding 5 mL of water[14] in another large test tube.

Add 5.0 mL of Hanes reagent A into each of the

large test tubes using a whole pipet. After mixing well, heat the solutions by placing the test tubes in a boiling water bath exactly for 15 minutes. After the heating, cool them immediately under running water. Then, for each of the test tubes, add 5 mL of Hanes reagent B, mix well and after adding 3 mL of Hanes reagent C and mixing, immediately titrate with 0.01 mol/L sodium thiosulfate solution (to be prepared by diluting 0.1 mol/L sodium thiosulfate solution 10-fold at the time of use) with constant and vigorous shaking. When the solution turns pale yellow-white, add 3 or 4 drops of the 1% soluble starch solution, continue the titration carefully until the purple color just disappears and measure the consumption of the 0.01 mol/L sodium thiosulfate solution (titer A (mL)). Perform a blank test under the same conditions and measure the consumption of the 0.01 mol/L sodium thiosulfate solution (titer B (mL)).

Note 13) For test solutions of high sucrose concentrations, it is allowed to reduce their collection volumes to less than 5 mL. In that case, the final volumes of their test solutions must be adjusted to 5 mL with water. Test solutions can also be prepared by diluting the test solutions for the Lane-Eynon method (prepared in 6.1.2.) appropriately.

Note 14) In order to prevent the difference in the concentrations of deproteinizing solutions A and B between test solutions and a blank solution having an negative impact on the titration results, use filtrate, obtained by treating water in the same manner as in the preparation of test solutions for the determination of direct reducing sugar, for preparation of blank.

6.2.4. Preparation of calibration curve

Subtract the titer for the standard invert sugar solution for calibration curve "A" (mL) from the titer for the blank "B" (mL) and multiply the obtained value "C" (mL) by the *factor* of the titrant (use the *factor* of the 0.1 mol/L sodium thiosulfate solution). This provides corrected titer "T" (mL). Prepare a calibration curve by plotting corrected titers "T" (mL) against the amounts of invert sugar in the standard invert sugar solutions.

$$B - A = C$$

$$C \times F = T$$

Where–

A: Titer (ml) of 0.01 mol/L sodium thiosulfate solution, for standard invert sugar solution (for calibration curve)

B: Titer (ml) of 0.01 mol/L sodium thiosulfate solution, for blank

C: Difference (ml) between the two titers above

F: *Factor* of 0.01 mol/L sodium thiosulfate solution

T: Corrected titer (ml)

6.2.5. Calculation of sucrose content

Subtract the titers of the test solution for determination of total sugar and the test solution for determination of direct reducing sugar from the titer of the blank, respectively. Multiply the obtained values by the *factor* of the 0.01 mol/L sodium thiosulfate solution, respectively. Using these values, obtain the quantities of invert sugar in 5 mL of the test solutions from the calibration curve prepared in 6.2.4.

Calculate the sucrose content in the test sample from the following equations with the total sugar and direct reducing sugar contents determined. Round off fractions to the first decimal place.

$$\%, \text{total sugar (Ts)} = \frac{A \times V}{S} \times 100$$

$$\%, \text{direct reducing sugar (Ds)} = \frac{A' \times V'}{S'} \times 100$$

$$\%, \text{sucrose} = (Ts - Ds) \times 0.95$$

Where–

A: Amount (mg) of invert sugar in 5 mL of test solution for determination of total sugar

A': Amount (mg) of invert sugar in 5 mL of test solution for determination of direct reducing sugar

V: Dilution ratio of test solution for determination of total sugar

V': Dilution ratio of test solution for determination of direct reducing sugar

S, S': Weight (mg) of sample collected

6.3. Determination of sucrose content by the High Performance Liquid Chromatography (HPLC) method

Select either the absolute calibration method or internal standard method, depending on samples to be analyzed.

6.3.1. Preparation of Sample [15]

(A) For the absolute calibration method

Accurately weigh a certain amount of the sample homogenized in 5. into a 100 mL volumetric flask so that the sucrose content becomes 0.1–1%. Add a small amount of water into the flask, remove fat[7] if a large amount of it is contained, and shake well to mix. Add 10 ml each of deproteinizing solutions A and B into the flask and dilute to volume with water. Filter the solution though a filter paper and a membrane filter. Use the filtrate as test solution for the HPLC analysis.

Note 15) When samples do not require any deproteinization treatment, it is allowed to skip that treatment. The amounts of deproteinization solutions added depend on samples.

(B) For the internal standard method

Accurately weigh a certain amount of the sample prepared in 5. into a 100 mL volumetric flask so that the sucrose content becomes 0.1–1%. Add a small amount of water into the flask, remove fat[7] if a large amount of it is contained, and shake well to mix. Add 10 ml each of deproteinizing solutions A and B into the flask, add a constant amount of the internal standard stock solution using a whole pipette and dilute to volume with water. Filter the solution through filter paper and a membrane filter (plus an ion-exchange filter if needed). Use the filtrate as test solution for the HPLC analysis.

6.3.2. Determination of sucrose content

(A) The absolute calibration method

Inject the standard solutions prepared in 4.2.(3) into a HPLC instrument which has been previously set up under the conditions of 3. Construct a calibration curve for sucrose using the peak areas of sucrose in the obtained chromatograms.

Subsequently, measure the test solution prepared in 6.3.1.(A) under the same operation as that for the standard solutions and calculate the amount of sucrose in the sample solution using the calibration curve with the peak area of sucrose in the obtained chromatogram. Using the amount of sucrose determined, calculate the sucrose content (%) in the sample from the following formula:

$$\%, \text{sucrose} = \frac{W}{S \times 1000} \times 100$$

Where–

W: Amount (mg) of sucrose contained in test solution

S: Weight (g) of sample collected

(B) Internal standard method

Inject the standard solutions prepared in 4.2.(4) into a HPLC instrument which has been previously set up under the conditions of 3. From the obtained chromatograms, construct a calibration curve for sucrose by plotting the ratio (Wx/Ws) of the weight of sucrose (Wx) to the weight of the internal standard (Ws) against the ratio (Ax/As) of the peak area of sucrose (Ax) to the peak area of the internal standard (As).

Subsequently, measure the test solution prepared in 6.3.1.(B) and calculate the weight ratio (Wx/Ws) of sucrose to the internal standard using the calibration curve with the obtained peak area ratio of sucrose to the internal standard. Using the obtained weight ratio, calculate the sucrose content (%) in the sample from the following formula:

$$\%, \text{sucrose} = \frac{(Wx/Ws) \times Ms}{S \times 1000} \times 100$$

Where–

Wx/Ws: Weight ratio of sucrose to internal standard

Ms: Amount (mg) of internal standard contained in 100 mL of test solution

S: Weight (g) of sample collected

8. References

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Appendix 1

Lane-Eynon Table
(Invert sugar, without sucrose)

mL sugar solution required	Invert sugar (without sucrose) mg/100ml
15	336
16	316
17	298
18	282
19	267
20	254.5
21	242.9
22	231.8
23	222.2
24	213.3
25	204.8
26	197.4
27	190.4
28	183.7
29	177.6
30	171.7
31	166.3
32	161.2
33	156.6
34	152.2
35	147.9
36	143.9
37	140.2
38	136.6
39	133.3
40	130.1
41	127.1
42	124.2
43	121.4
44	118.7
45	116.1
46	113.7
47	111.4
48	109.2
49	107.1
50	105.1

Appendix 2

The following chromatograms are examples from analyzing a confectionary sample using the internal standard method with an amino column or a gel column.

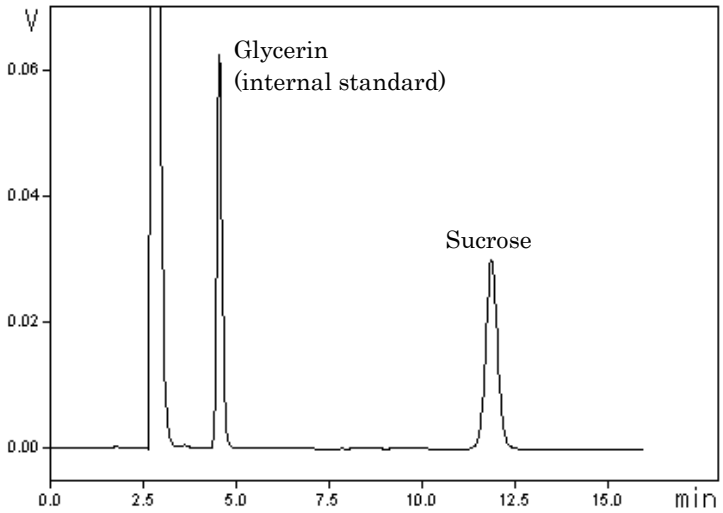

Fig. 1 HPLC chromatogram using an amino column

Analytical column: Asahipak NH2P-50 AE 4.6 mmφ × 250 mm
Column temperature: 40 ºC
Mobile phase: Acetonitrile to Water (75:25)
Flow rate: 1.0 mL/min
Detector: RI detector
Injection volume: 10 µL

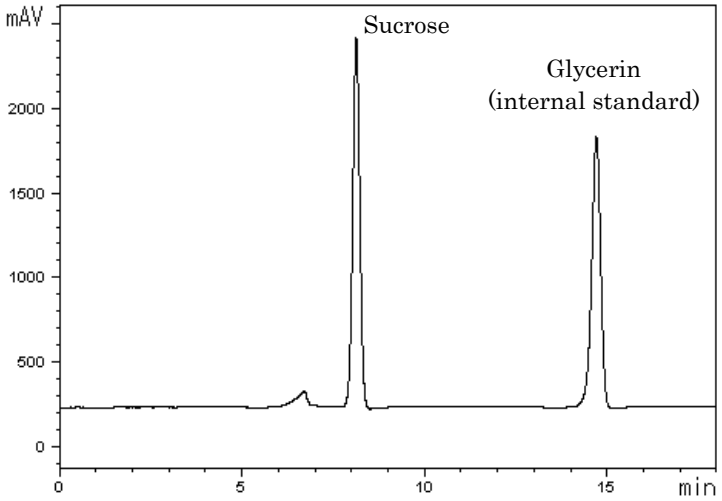

Fig. 2 HPLC chromatogram using a gel column

Analytical column: MCI GEL CK08EC 8.0 mmφ × 300 mm
Column temperature: 80 ºC
Mobile phase: Water
Flow rate: 0.8 mL/min
Detector: RI detector
Injection volume: 10 µL