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UV-visible spectrophotometry and titrimetric method for determining Reducing Sugars in different brands of honey and soft drinks

K Bhavya Sri *, Md. Saba Fatima, M Nandhini and M Sumakanth

Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India.

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Abstract

Two simple, easy, and rapid methods were developed for the determination of glucose reducing sugar in various soft drink samples and total reducing sugars in honey sample. A colorimetric method was developed to analyze the soft drink samples (non-diet and not dark colored) and titrimetric method was developed to determine the total reducing sugars in honey sample. The various soft drink samples obtained from market were analyzed for the glucose content in them using UV-Visible spectrophotometry and DNSA solution. The DNSA imparts color to the sucrose standard and samples and later the absorbance values were checked at 580 nm. The calibration curve of the standard sucrose solution was plotted and the concentration of the samples were determined using the calibration curve. The titrimetric method is based on the principle of Lane Eynon method for determination of reducing sugars and it involves two steps: standardization of invert sugar and titration of honey solution using Fehling's A and B solutions. There were no titrimetric methods available in literature for determination of total reducing sugars with use of simple reagents. Hence the proposed methods were developed which are rapid, and reliable.

Keywords: Glucose; Total reducing sugars; Honey; Soft drink samples; Colorimetric method; Titrimetric method

1. Introduction

Sugars are major sources of energy, for almost all living organisms. Plants produce sugars by photosynthesis process and convert them into different disaccharides such as sucrose, or convert them into starch for storage. The average human's healthy digestive system can only digest and eliminate two to four teaspoons of sugar daily, usually without noticeable side-effects [1]. An excess of sugar in the diet results in weight gain, thereby increasing the risk of diabetes, heart disease, and high blood pressure, apart from dental caries. It is therefore important to know what amount of sugar is present in food and beverages that are commonly consumed [2–7].

For the determination of reducing sugars like glucose, the sucrose present in the soft drink samples must be broken down into glucose. This is done by boiling it with hydrochloric acid which then reacts with DNSA which forms the basis for the proposed method developed. This method is based on the colour which forms when sugar is reduced from 3, 5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid as shown in Figure 1. In addition, the sugar in soft drinks is highly concentrated to be used for this method unless it is diluted. The red-brown colour of the product is detected at 580 nm.

Honey is the oldest sweet food known to man. Because 96% of its dry matter is made up of the simple sugars fructose, glucose, and sucrose, honey is regarded as refined sugar even though it is a natural sweetener. In comparison to table sugar's 48 calories per tablespoon, honey has the greatest per-tablespoon calorie intake of all the sugars at 65. Honey

* Corresponding author: K Bhavya Sri

is made by honey bees from honey dew as well as plant and floral nectar. Honey is vulnerable to adulteration by cane sugar, invert syrup, and high fructose glucose syrup because of its low output and high price. [8].

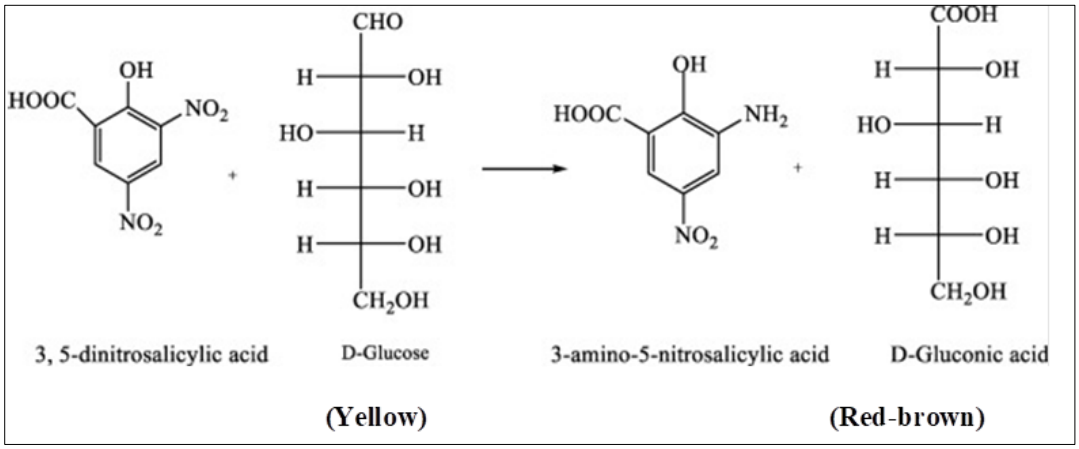

Figure 1 Reaction of 3,5-dinitrosalicylic acid and glucose

To assess the quality of honey, the total reducing sugars and sucrose content is commonly determined. Reducing sugars are those, which have free sugar groups (e.g., glucose, fructose etc.) and they can be estimated by Lane and Eynon method, directly by titrating the solution of the sample with Fehling's solution.Non-reducing sugars (e.g., maltose, lactose, sucrose etc.) do not contain free sugar groups and cannot reduce Fehling's solution. Hence, non-reducing sugars must be hydrolysed to monosaccharides by heating with acid before titration. Reducing sugars are acted upon by the alkali of the Fehling's solution to form enediols. These enediols are very unstable and reactive and they reduce Cu^{2}+ ions to Cu+ ions. These Cu+ ions combine with hydroxyl groups to form cuprous hydroxide, which on heating gives red precipitate of cuprous oxide. To get a sharp end point, methylene blue is added which is reduced to a colourless compound restoring the red colour of the solution. Sodium potassium tartarate keeps the Cu^{2}+ ions in the solution, thus ensuring a continuous supply of Cu^{2}+ ions for reduction.

2. Material and methods

2.1. Determination of reducing sugar (glucose) in soft drinks samples

2.1.1. Reagents required

Sucrose stock solution (1000 mg/dL), Soft drinks (non-diet, not dark-coloured), 6 M HCl solution, 2.5 M NaOH solution, 0.05 M 3,5-dinitrosalicylic acid (DNSA) solution, distilled water.

2.1.2. Apparatus required

Volumetric flasks, Test tubes, pipette, beaker.

2.1.3. Instrument

UV-Visible Spectrophotometer.

2.1.4. Procedure

Five diluted sucrose stock solutions as standards were prepared of concentrations 200, 400, 600, 800 and 1000 µg/mL. The diluted soft drinks were taken as samples. 2 mL of each sucrose standard and samples were pipetted out into different test tubes. 2 mL of Distilled water was pipetted out into a separate test tube taken as the blank solution. To each test tube, 2 mL of 6 M HCl solution was added and placed in boiling water for 10 minutes. Later, to it 8 mL of2.5 M NaOH solution and 2 mL of 0.05 M DNSA solution were added and the test tubes were covered with parafilm and shaken well to mix. The test tubes were placed in boiling water for 5 minutes followed by ice water for 10 minutes. The absorbance of the five standards were measured at 580 nm usingUV-Visible spectrophotometer. The samples were measured for the absorbance values at the same wavelength and the concentrations were calculated from the calibration curve of standard dilutions.

2.2. Determination of total reducing sugars in Honey

2.2.1. Materials Required

Burette, Pipette, Conical flask, Beaker,

2.2.2. Reagents required

Sample of honey, Invert sugar, Distilled water, Fehling's A and Fehling's B solutions, Methylene blue indicator.

2.2.3. Preparation of Standard invert sugar solution

Weigh accurately 0.985 g of sucrose and dissolve in 500 ml of water. Add 2 ml of concentrated H_2SO_4. Boil gently for 30 minutes and keep aside for 24 hours. Neutralize this with Na_2CO_3 and make the final volume to 1000 ml. 30 ml of this solution contains 0.05 g of invert sugar.

2.2.4. Procedure

There are two steps in this method.

Step 1: Standardization of copper sulphate solution.

5 ml of Fehling's Solution A and Fehling's Solution B was pipetted out in conical flask of 250 ml capacity. This mixture is heated to boiling on an asbestos gauge and standard invert sugar solution was added from the burette, which will reduce the Fehling's solution. To this 1 ml methylene blue indicator was added. The titration was carried out and completed within 3 minutes. The change in blue to reddish brown colour due to cuprous oxide formation is taken as the end point. From the volume of the invert sugar solution used, the strength of $CuSO_4$ is calculated by multiplying the titrated value with 0.001 (mg/ml of the standard invert sugar solution). This is known as Fehling factor. The titration was done in triplicate.

Step 2: Titration of the sample honey solution.

1 gm of honey was accurately weighed and placed in 250 ml of volumetric flask and diluted with about 150 ml of water. The contents of glass were thoroughly mixed and volume was made up to 250 ml. In another conical flask, 5 ml of Fehling's solution A and 5 ml of Fehling's solution B were added and heated to boiling with 20 ml of water. From burette honey solution was added and boiled as the titration is carried out simultaneously, using methylene blue indicator. The titration was carried out within 3 minutes until the blue colour changed to red. The reducing sugar were calculated using the calculation given next.

Calculations:

$$\text{Reducing sugar } = \frac{250 \times 100 \times S}{H \times M}$$

where, S = Fehling's factor (as obtained from standardization procedure of $CuSO_4$ undertaken in step 1 of the procedure),

Strength of $CuSO_4$ solution /Fehling factor (S) = Titre value of standard invert sugar solution × 0.001

H = Volume of honey solution required (burette reading), M = Mass of honey

3. Results and discussion

The concentrations of sugar in the unknown samples were acquired using the calibration curve shown in Figure 1.

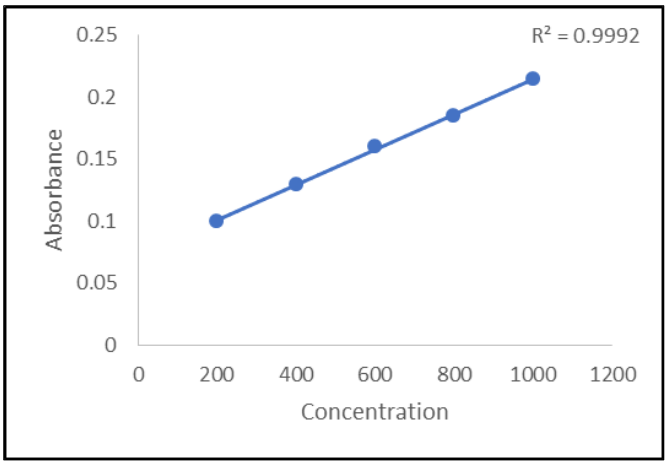

Figure 2 The Calibration curve of Sucrose standards

Table 1 Calibration data of Sucrose standards

Name	Concentration(µg/mL)	AU (580nm)
Standard 1	200	0.0999
Standard 2	400	0.1295
Standard 3	600	0.1601
Standard 4	800	0.1854
Standard 5	1000	0.2149

Table 2 Concentrations of sugar in soft drink samples

Name	AU (580nm)	Concentration(µg/mL)
7up Soft drink sample 1	2.79	18997.11739
Sprite Soft drink sample 2	3.601	24665.74788
Mountain dewsoft drink sample 3	3.147	21492.43315

The original concentrations of sugar in the soft drinks were calculated by the following equation:

$$\text{Original concentration} = \text{Diluted sample concentration x Dilution factor}$$

Various methods available to determine the amount of sugar which includes density [9], refractometric [10] and infrared spectroscopy [11-13] methods. These methods are performed after standardization. The density and refractometric methods were used to obtain the total amount of sugar in samples [14]. Other than these other popular methods include use of HPLC [15], alkaline copper in sulphate solution [16].Table 3 gives the data of the triplicate titration carried out for honey sample.

The titration was carried out in triplicate and average volume of the honey solution required to reduce the cupric ions (cu^{2}+) was calculated to be 38.5 ml. The invert sugar solution required to reduce Cupric ions was found to be 38 ml. The titrant volume and Fehling's factor (S) calculated by substituting invert sugar solution volume was substituted in the formula mentioned and value was calculated to be 24.67. The grade of the honey which it falls under was found to be a Grade 2 category. Generally, the reducing sugars present in the honey must be not less than 65% according to the Indian Standards. The method proposed can be used to calculate the reducing sugars present in the honey. There are reported methods on assessing the quality of the honey using HPLC, ICP and the presence of drug additives was measured using

GC-MS in Saudi samples [17], there is another reported method which determined the ratio of carbohydrates to some type of reducing and non-reducing sugars in honey fed to honey bees [18]. The other method was a case study on the characterization of honey based on physicochemical parameters and chemometrics analysis [19].Hence this method was developed which can serve as a routine quality control test for the determination of reducing sugars in honey sample which is easy, rapid, simple, and cost effective without use of sophisticated instruments.

Table 3 Triplicate titration values

Sr. No.	Initial volume of burette containing Honey solution	Final volume of burette containing Honey solution	Volume of honey solution required to reduce CuSO4 solution
1.	50 ml	11 ml	39 ml
2.	50 ml	11.5 ml	38.5 ml
3.	50 ml	12 ml	38 ml

4. Conclusion

The determination of sugar in soft drinks was performed using theUV-Visible spectrophotometer using DNSA solution. The method proposed is easy, rapid and gives reliable results.Hence this method can be used as a method to check amount ofglucose and sucrose in soft drink samples. Honey can be analyzed for its quality by using the proposed method. The grade of honey which it falls under can be identified by performing this method. Honey is obtained by various sources and nowadays various commercial brands of the honey are available in the market. Hence this test serves as a quality control test to identify any substandard or fake product by performing the simple, reliable, and rapid method developed.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest declared.

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