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Research article

Validation of the UV-Vis Spectrophotometric Method for the Determination of Ascorbic Acid Content in Beverage Preparations Based on a Standard Vitamin C Calibration Curve

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ABSTRACT

Background: Vitamin C or ascorbic acid ($C_6H_8O_6$) is an essential water-soluble compound, widely used in functional beverages due to its antioxidant properties. This compound plays a role in neutralizing free radicals, strengthening the immune system, and helping the absorption of iron in the body. Packaged beverages containing vitamin C can experience degradation due to storage temperature (pH), production process, and the length of product circulation on the market. This impacts degradation of ascorbic acid and decreases the levels of vitamin C in beverage preparations, so accurate and validated analytical methods are needed to ensure the conformity of ascorbic acid levels with the label.
Methods: Determination of ascorbic acid levels using a UV-Vis spectrophotometer at maximum wavelength. A standard curve was prepared from a standard vitamin C solution with a concentration of 10–18 ppm, resulting in a regression equation as the basis for calculating sample levels. Method validation included linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) tests according to quantitative analysis guidelines.
Results: The standard curve equation is Y = 0.020x + 0.030Y with r2 = 0.995r indicating good linearity. The vitamin C content in the sample is 1.045 mg/140 mL or 103.5% of the label (1000 mg), still within the Pharmacopoeia limits (90–110%). The LOD and LOQ values are 0.429 ppm and 1.3 ppm, respectively. The precision results show a %RSD of 0.1260% and an accuracy (% recovery) of 103.5%.
Conclusion:The results showed that the UV-Vis spectrophotometry method had good linearity, accuracy, and precision. The vitamin C content in the product met standards. Validation parameters demonstrated that this method was consistent in analyzing vitamin C in beverage preparations.

I. Introduction

Indonesia, as a tropical country, boasts a rich biodiversity, including fresh fruits, which are a primary source of vitamin C. Vitamin C (ascorbic acid, $C_6H_8O_6$) is an essential, water-soluble compound that functions as a natural antioxidant. This compound helps protect cells from free radicals, boosts the immune system, and supports iron absorption.(Hagos et al., 2022)Ascorbic acid is available in two forms, namely L-ascorbate (active) and dehydroascorbate (oxidized), but is easily damaged by light, air and high temperatures (Meng et al., 2021). as a tropical country, is renowned for its remarkable biodiversity, which encompasses a vast array of fresh fruits that serve as a primary source of vitamin C. This essential nutrient, scientifically known as ascorbic acid ($C_6H_8O_6$), plays a pivotal role in human health. As a water-soluble compound, vitamin C is not only a powerful antioxidant but also a critical

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player in various physiological processes. It protects cells from oxidative damage caused by free radicals, bolsters the immune system, and enhances the absorption of iron from plant-based foods. For instance, the consumption of vitamin C-rich fruits such as guava, papaya, and oranges can significantly improve the body's ability to utilise iron, thereby helping to prevent anaemia, particularly in populations where iron deficiency is prevalent.

The structure of vitamin C allows it to exist in two forms: L-ascorbate, which is the active form, and dehydroascorbate, the oxidised form. However, its stability is a concern; ascorbic acid is easily compromised by exposure to light, air, and high temperatures. This susceptibility means that the nutritional quality of vitamin C can diminish rapidly if fruits are not stored correctly or if they are subjected to prolonged cooking processes. For example, while boiling vegetables can enhance their flavour, it can also lead to significant losses of vitamin C, emphasising the need for appropriate cooking methods that preserve this vital nutrient.

In addition to being naturally present in fresh fruits, vitamin C is frequently incorporated into functional beverages, making it more accessible for consumers seeking convenient ways to enhance their nutrient intake. These beverages often come in various formulations, including juices, smoothies, and fortified drinks. However, the stability of vitamin C in these products is influenced by several factors, including storage conditions, pH levels, the production process, and the overall shelf life of the product. For instance, beverages with lower pH levels (more acidic) tend to preserve vitamin C better than those with higher pH levels due to the reduced rate of oxidation. This understanding is crucial for manufacturers aiming to produce high-quality products that meet consumer expectations for both taste and nutritional value.

Ascorbic acid is not only obtained from fresh fruit but is also added to functional beverages for easy consumption. The stability of vitamin C in these products is influenced by storage factors, pH, the production process, and shelf life.(Putri et al., 2022)Analysis is performed to ensure the vitamin C content is as labeled and safe for consumption. UV-Vis spectrophotometry is a widely used method because ascorbic acid absorbs light at specific wavelengths. This method has the advantages of being practical, efficient, and economical.(I W. Sudiarta, and A. Suandi, 2021)Determining vitamin C levels is necessary to ensure product quality and the accuracy of label information. This process also serves as an important basis for quality control by agencies such as the BPOM.(I W. Sudiarta, A. Suandi, 2021)In this study, the UV-Vis spectrophotometry method was used and validated based on linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). Validation was conducted to ensure that the method was capable of producing accurate, consistent data and was applicable to the analysis of beverage products containing vitamin C.(Idris & Kasumawati, 2024).

Quality assurance in the production of vitamin C-rich beverages is paramount, and rigorous analysis is performed to ensure that the vitamin C content is accurately labelled and safe for consumption. One widely adopted method for this analysis is UV-Vis spectrophotometry, which exploits the unique light absorption properties of ascorbic acid at specific wavelengths. This analytical technique is favoured for its practicality, efficiency, and cost-effectiveness, making it an ideal choice for both large-scale manufacturers and smaller producers. Furthermore, the ability to quickly assess vitamin C levels helps maintain product integrity and consumer trust.

The determination of vitamin C levels is not merely a matter of compliance with regulatory standards; it is essential for ensuring product quality and the accuracy of label information. Regulatory agencies, such as the Indonesian National Agency of Drug and Food Control (BPOM), rely on these analyses to enforce safety protocols and protect public health. In this context, the UV-Vis spectrophotometry method is validated through a series of parameters including linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). Such validation processes are critical to confirm that the method can deliver reliable and reproducible data, which is particularly important when analysing beverage products that contain variable concentrations of vitamin C.

For instance, a recent study employing UV-Vis spectrophotometry demonstrated that the method could accurately quantify vitamin C levels in various commercial beverages, providing insights into the actual nutritional content compared to what is advertised on labels. This not only aids consumers in making informed dietary choices but also holds manufacturers accountable for the nutritional claims they make. The validation of this analytical method is essential, as it ensures that the results are both accurate and consistent across different batches of products, thereby establishing a reliable framework for quality

control. Significance of vitamin C in maintaining health cannot be overstated, particularly in a country like Indonesia where fresh fruits are abundant. The dual role of vitamin C as a natural antioxidant and a facilitator of iron absorption underscores its importance in the diet. However, the challenges associated with its stability in both fresh and processed forms necessitate careful consideration by both consumers and producers. The use of UV-Vis spectrophotometry as a reliable analytical method not only ensures that vitamin C levels are accurately measured but also reinforces the integrity of product labels, thereby promoting consumer safety and confidence. As research continues to evolve, the ongoing validation of analytical methods will play a crucial role in enhancing our understanding of vitamin C and its applications in food science, ultimately contributing to better health outcomes for the population.

II. METHODS

Tools and materials

The tools used in this study include stirring rods, aluminum foil, 500 mL beakers, 5 mL beakers, 250 mL Erlenmeyer flasks, 25 mL measuring cylinders, 100 mL measuring flasks, 50 mL measuring flasks, 10 mL measuring flasks, 1 mL measuring pipettes, 5 mL measuring pipettes, droppers, pushballs, test tubes, filter paper, cuvettes, UV spectrophotometers, analytical scales.
The ingredients used consist of standard Vitamin C, 1000 mg Vitamin C drink, distilled water

Work procedures

The standard curve was conducted by preparing a standard vitamin C solution. The sample was measured six times. Determination of ascorbic acid levels was carried out by preparing a standard ascorbic acid solution, validating the method, and determining the maximum wavelength of the ascorbic acid solution. The samples were analyzed to determine the vitamin C levels. The results of the method validation were linearity test parameters, LOD and LOQ, precision, and accuracy.

Determination of the Vitamin C Standard Curve

Preparation of Vitamin C Standard Solution
Weigh 20 mg of standard vitamin C with an analytical balance, put it into a 100 ml volumetric flask, add distilled water to a volume of 100 ml, shake until homogeneous to become a 100 ppm stock solution. Then pipette 10 ml of stock solution into a 50 ml volumetric flask, add distilled water to a volume of 50 ml, shake until homogeneous to become a 20 ppm stock solution.

Preparation of Vitamin C Series Solution

This was done by pipetting a 20 ppm vitamin C solution, ppm into a 10 mL volumetric flask, each containing 5 mL, 6 mL, 7 mL, 8 mL, and 9 mL. Aquadest was added up to the mark. Homogenize until concentrations of 10 ppm, 12 ppm, 14 ppm, 16 ppm, and 18 ppm were obtained.

Spectrophotometric Calibration

Turn on the UV-Vis Spectrophotometer, fill the cuvette with distilled water as a blank solution, insert the blank cuvette into the UV-Vis Spectrophotometer.

Making a standard curve for a standard solution

Measurements are carried out at a wavelength of 200-400 nm with distilled water as a blank and a series v of 14 ppm, put it into the cuvette in the order of 1.2 then press scan, after the maximum wavelength (lamda) is known, enter all the series and blank solutions into the tool, press (ppm) and the amount used, press Maximum wavelength (lamda). The results of the standard curve will appear on the monitor.

Determination of Ascorbic Acid Levels

Preparation of Ascorbic Acid Standard Solution
Sample preparation was carried out by taking 1 ml of the drink sample solution, putting it into a 100 ml measuring flask, adding distilled water to 100 ml. Take 15 ml and put it into a 50 ml measuring flask, adding distilled water to a volume of 50 ml, shaking it until it becomes homogeneous and becomes a 20 ppm Vitamin C standard solution.

Making a Series Solution (Standard Curve)
This was done by pipetting a 20 ppm vitamin C solution into 10 mL volumetric flasks, each containing 5 mL, 6 mL, 7 mL, 8 mL, and 9 mL. Add distilled water up to the mark. Homogenize until concentrations of 10 ppm, 12 ppm, 14 ppm, 16 ppm, and 18 ppm are obtained.

Spectrophotometric Calibration
Turn on the UV-Vis Spectrophotometer, then fill the cuvette with distilled water as a blank solution, insert the blank cuvette into the UV-Vis Spectrophotometer, press blank

Preparation of standard curve for standard solution
Measurements are carried out at a wavelength of 200-400 nm with distilled water as a blank and a series of 14 ppm vitamin C, put it into the cuvette in the order of 1.2 then press scan, after the maximum wavelength (lamda) is known, enter all the series and blank solutions into the tool, then press (ppm) and the amount used, press the maximum wavelength (lamda) press start, the standard curve results will appear on the monitor.

Determination of the maximum wavelength of vitamin C solution
Determination of the maximum wavelength of vitamin C solution is made by weighing 20 mg of standard vitamin C with an analytical balance, then put it into a 100 ml measuring flask. The vitamin C solution is added with aquadest solvent up to 100 ml, shaken until homogeneous to become a stock solution (100ppm). Pipette 10 ml of standard vitamin C solution from (stock solution) 100 ppm, put it in a 50 ml measuring flask, add aquadest to the limit mark so that a solution with a concentration of 20 ppm is obtained.

III. RESULT

Based on the research conducted, the results of the standard vitamin C standard curve measurements using the UV-Vis spectrophotometry method were found. The results obtained can be seen in Table 1.

Table 1: Results of Reading the Ascorbic Acid Standard Calibration Curve

Standard	Concentration (ppm)	Absorbance
1	10,0000	0.221
2	12,0000	0.272
3	14,0000	0.302
4	16,0000	0.340
5	18,0000	0.382

Accordingly, the data can be represented in the following linear regression equation

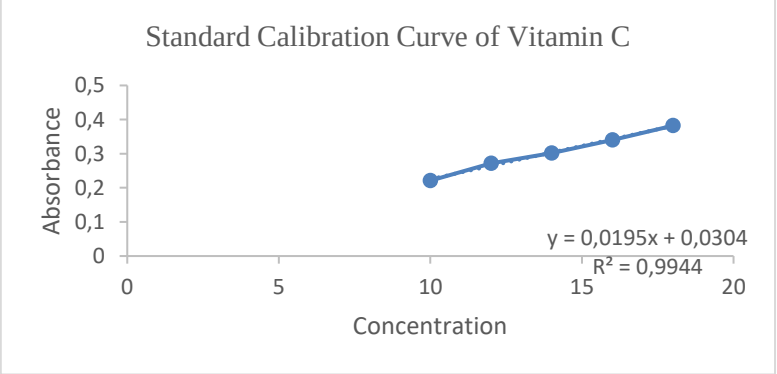

Figure 1. Linear regression curve graph of Standard Vitamin C

The absorbance measurement of standard vitamin C solutions at concentrations of 10, 12, 14, 16, and 18 ppm yielded values of 0.221; 0.272; 0.302; 0.340; and 0.382, respectively. The relationship between concentration and absorbance showed a very good linear correlation, with a regression equation of Y = 0.020x + 0.030 and a coefficient of determination (r^2) of 0.995. The results of the calibration curve showed a linear relationship between concentration and absorbance, because the correlation coefficient value (r) was between 0.99 and 1. This means that all measurement points were very close to a straight line, so this method can be considered quite accurate and consistent for use.(Idris & Kasumawati, 2024)

Table 2: Results of Standard Curve Reading of Ascorbic Acid Drink Samples

Sample	ABS
Sample 1	0.474
Sample 2	0.475
Sample 3	0.474
Sample 4	0.473
Sample 5	0.474
Sample 6	0.473

Percentage Level

$$\% \text{ Content} = \frac{Spektro\ Result\ mg}{Sample\ Weight} = \frac{1{,}045\ mg}{1000\ mg} \text{ x } 100\% = 103.5\%$$

Analysis of the vitamin C content in the beverage preparation showed a result of 1,045 mg in 140 mL, or equivalent to 103.5% of the amount listed on the label (1000 mg) (Temova Rakuša & Roškar, 2024). Many drinks contain higher levels of vitamin C than the label, up to 110%, in order to maintain stability during distribution and storage.

Table 3: Validation Results of the Ascorbic Acid Drink Sample Method

Blank (Replication)	Y	y′	(y Y')	(y – y') 2
1	0,000	0.002	0.002	0.000004
2	0,000	0.002	0.002	0.000004
3	-0.001	0.002	0.003	0.000009
4	0,000	0.002	0.003	0.000009
5	-0.001	0.002	0.002	0.000004
6	-0.001	0.002	0.002	0.000004
			Average	0.000034

LOD (Limit of Detection)

$$\text{LOD} = \frac{3{,}3\ X\ SD}{a} = \frac{3{,}3\ x\ 0{,}0026}{0{,}020} = 0.429 \text{ ppm}$$

The limit of detection (LOD) is the lowest concentration of a substance (analyte) that can still be detected by the instrument, although at this level it cannot be calculated quantitatively. (Riyanto, 2016)In this study, the LOD value obtained was 0.429 ppm, indicating that this method is capable of detecting vitamin C in samples if the concentration is ≥ 0.429 ppm.

LOQ (Limit of Quantity)

$$\text{LOD} = \frac{10\ X\ SD}{a} = \frac{10\ x\ 0{,}0026}{0{,}020} = 1.3$$

Limit of Quantitation (LOQ) of a substance in a sample that can still be measured accurately(Idris & Kasumawati, 2024)In this study, the LOQ value obtained was 1.3 ppm, which indicates that the

UV-Vis spectrophotometry method can be used to measure the vitamin C content in samples if the concentration is at least 1.3 ppm..

Table 4: Precision Results of Ascorbic Acid Drink Samples

Blank (Replication)	**X1**	**X´**	**(X1– X')**	**(X1 – X') 2**
1	22.2	22, 1916	0.0084	0.00007056
2	22.25	22, 1916	0.0584	0.00341
3	22.2	22, 1916	0.0084	0.00007056
4	22.15	22, 1916	-0.0416	0.00017
5	22.2	22, 1916	0.0084	0.00007056
6	22.15	22, 1916	-0.0416	0.00017
			Average	0.00396168

% RSD = $\frac{SD}{mean} = \frac{0,0281}{22,1916} x\ 100\%$ = 0,1260%

Precision describes how consistent the results of an analysis are when repeated under the same conditions. Precision is expressed as %RSD (Relative Standard Deviation), which indicates the degree of dispersion of data relative to the average value.(Idris & Kasumawati, 2024)In this study, the %RSD value obtained was 0.1260%, still within the acceptable precision limit, namely %RSD ≤ 2%. The method used in this analysis has a very good level of precision and consistent results.

ACCURACY

Recovery = $\frac{Spektro\ Result}{Data\ Etiket} = \frac{1035\ mg}{1000\ mg}$ = x 100% = 103.5%

Accuracy measures the ratio of measurement results to their true values. In quantitative analysis, accuracy is expressed as the percentage recovery (% recovery) of the analyzed substance. In this study, the % recovery value was 103.5%, which is still within the acceptable range of 98–105%, indicating that the method used has good accuracy.(González et al., 2010)

IV. DISCUSSION

The determination of ascorbic acid levels in beverage products is a critical process that serves to ensure that the information presented on product labels accurately reflects the actual content within the beverages. This alignment is not merely a regulatory necessity; it is fundamental to consumer trust and safety. Ascorbic acid, commonly known as vitamin C, is an essential nutrient that plays a myriad of roles in human health, including its function as an antioxidant, its involvement in collagen synthesis, and its contribution to immune function. Given these benefits, consumers are increasingly reliant on the labels of beverage products to provide truthful information regarding vitamin C content. Therefore, the accuracy of such measurements is paramount.

In this study, the UV-Vis spectrophotometry method was employed for the determination of ascorbic acid levels, chosen for its numerous advantages. One of the primary benefits of this analytical technique is its simplicity. The procedure can be executed with relatively straightforward equipment and does not require extensive training, making it accessible for routine laboratory use. Additionally, the rapid analysis time associated with UV-Vis spectrophotometry allows for a high throughput of samples, which is particularly beneficial in commercial settings where efficiency is key. The operational costs are also relatively low compared to other analytical methods, thus making it an economical choice for laboratories aiming to maintain quality control without incurring prohibitive expenses.

To quantify ascorbic acid levels accurately, a standard curve was constructed using a standard vitamin C solution with concentrations ranging from 10 to 18 ppm. This range was carefully selected to encompass the expected levels of ascorbic acid in various beverage products. The results from the

absorbance measurements displayed a strong linear relationship, characterised by a regression equation of Y = 0.020x + 0.030 and a coefficient of determination (r^2) of 0.995. This high r^2 value is significant as it meets the linearity criteria established by the Indonesian Pharmacopoeia, which stipulates that a minimum r^2 of 0.990 is necessary for reliable results. Such a robust linearity indicates that the method is capable of accurately reflecting changes in concentration, a crucial factor for quality assurance in food and beverage testing.

The analysis of beverage samples revealed an ascorbic acid level of 1.045 mg per 140 mL, which corresponds to approximately 103.5% of the value stated on the label (1000 mg). This finding highlights an important aspect of product formulation in the beverage industry: the actual vitamin C levels in market products can vary significantly from what is claimed on labels. The excess levels of ascorbic acid observed can often be attributed to the intentional addition of active substances beyond the stated amount. This practice is typically employed as a precautionary measure to counteract the degradation of ascorbic acid during storage and distribution, given its known instability in response to factors such as temperature, light, and oxidation.

Research has shown that ascorbic acid can degrade rapidly under adverse conditions, leading to significant losses in nutritional value (Hidayati, 2024). Therefore, manufacturers may opt to fortify their products with higher levels of vitamin C to ensure that consumers receive the intended benefits, even after accounting for potential losses. This strategy, however, raises questions regarding label accuracy and consumer expectations, as excessive fortification can mislead consumers into believing they are receiving a higher intake of nutrients than they actually are. To demonstrating strong linearity, the validation of the UV-Vis spectrophotometry method also revealed excellent precision and accuracy. The %RSD value of 0.126% is significantly below the maximum allowable limit of 2%, indicating a high level of consistency in the measurements. Precision is vital in analytical chemistry, as it ensures that repeated measurements yield similar results, thereby enhancing the reliability of the data obtained. Furthermore, the %recovery value of 103.5% suggests that the method is capable of accurately quantifying ascorbic acid levels in various samples. This level of recovery is indicative of a well-optimised analytical procedure that can effectively account for any losses or variances during the analysis.

With validation results that satisfy all required parameters, the UV-Vis spectrophotometry method is established as a proven and effective means of analysing ascorbic acid levels in beverages. This method not only guarantees accuracy and precision but is also particularly well-suited for routine testing as part of a comprehensive quality assurance system for vitamin-enriched food or beverage products. The implications of this study extend beyond mere compliance with regulations; they also highlight the importance of transparency and accountability in the food and beverage industry.

Determination of ascorbic acid levels in beverage products using UV-Vis spectrophotometry is a vital component of quality control that ensures consumer safety and trust. The method's simplicity, rapidity, and cost-effectiveness make it an ideal choice for routine analysis, while its strong linearity, precision, and accuracy reinforce its reliability. As the beverage industry continues to evolve, the need for rigorous testing and validation of nutritional claims will only grow, underscoring the significance of adopting robust analytical techniques. Ultimately, ensuring that consumers receive products that meet their expectations is not just a regulatory obligation; it is a cornerstone of ethical business practices in the food and beverage sector.

V. CONCLUSION

This study shows that the UV-Vis spectrophotometry method can be used to determine the ascorbic acid content in beverage preparations with results that meet the Pharmacopoeia requirements, namely 103.5% of the content stated on the label. Method validation produced good parameters, including linearity (r^2 = 0.995), LOD (0.429 ppm), LOQ (1.3 ppm), precision (%RSD = 0.1260%), and accuracy (%recovery = 103.5%). This method can be used as a reference in testing vitamin C levels to ensure the quality and conformity of functional food products to the listed labels.

VI. ACKNOWLEDGMENTS
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VII. CONFLICTS OF INTEREST
The authors declare that this study is free from any conflicts of interest

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