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Article

Kinetic Spectrophotometric Determination of Vitamin C in Fruit Juices via Oxidation Reaction with Dichlorophenolindophenol (DCPIP)

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Abstract: A validated kinetic spectrophotometric method was developed for the determination of vitamin C (ascorbic acid) in fruit juice samples using 2,6-dichlorophenolindophenol (DCPIP) as an oxidizing agent. The method is based on monitoring the decrease in absorbance of DCPIP at 520 nm, following a pseudo-first-order kinetic behavior in the presence of excess reagent. Calibration curves constructed from standard ascorbic acid solutions exhibited excellent linearity ($R^2 = 0.997$) within the range of 10–100 mg/L. Method validation confirmed high sensitivity (LOD: 2.5 mg/L; LOQ: 8.0 mg/L), accuracy (recoveries 97.5–102.3%), and precision (RSD ≤ 3%). The procedure was successfully applied to commercial fruit juices, where vitamin C content varied among samples, with lemon and orange juices showing the highest concentrations and apple juice the lowest. The results are consistent with reported nutritional profiles, confirming the method's reliability. Owing to its simplicity, cost-effectiveness, and reproducibility, the proposed approach offers a practical alternative for routine analysis of vitamin C in food and beverage quality control.

Keywords: Vitamin C, Ascorbic Acid, Kinetic Spectrophotometry, DCPIP, Fruit Juice Analysis.

Introduction

Vitamin C, also known as ascorbic acid, is one of the most essential water-soluble vitamins in human nutrition, playing a pivotal role as a potent antioxidant and enzymatic cofactor [1]. It contributes to numerous physiological processes including collagen biosynthesis, iron absorption, immune system function, and protection against oxidative stress [2]. Due to its instability and sensitivity to factors such as light, temperature, and oxygen, vitamin C levels in natural food sources, particularly fruit juices, tend to decrease during processing, storage, and handling. Therefore, accurate and reliable quantification of vitamin C in food products is of great importance in food chemistry, nutrition, and quality control [3].

A wide range of analytical methods has been developed to determine vitamin C in food matrices. Classical titrimetric methods, such as the redox titration with 2,6-dichlorophenolindophenol (DCPIP), are still widely used due to their simplicity and low cost. However, titration-based procedures often lack the sensitivity and accuracy required for trace analysis, especially when applied to complex food matrices containing potential interfering compounds. Advanced techniques such as high-performance

liquid chromatography (HPLC), capillary electrophoresis, and electrochemical sensors have been introduced to overcome these limitations. While these approaches provide high selectivity and sensitivity, they often require expensive instrumentation, lengthy sample preparation, and trained personnel, which restricts their routine application in standard food laboratories [4], [5].

In this context, spectrophotometric techniques remain highly attractive alternatives due to their accessibility, cost-effectiveness, and rapid analysis capabilities. Among the various spectrophotometric approaches, kinetic methods have recently gained attention for their ability to exploit reaction rates rather than end-point measurements. Kinetic spectrophotometry allows the quantification of analytes based on the monitoring of time-dependent absorbance changes during chemical reactions. This approach not only enhances sensitivity but also improves selectivity by differentiating analytes according to their specific reaction kinetics [6], [7].

The redox reaction between ascorbic acid and DCPIP forms the basis of the present work. DCPIP is a redox dye that exists in a blue oxidized form and is reduced to a colorless or faintly pink form upon interaction with ascorbic acid. Monitoring the decrease in absorbance of DCPIP at a suitable wavelength (around 520 nm) provides a direct measure of the reducing capacity of vitamin C. By following the absorbance decrease as a function of time, the reaction kinetics can be characterized, and the concentration of ascorbic acid can be determined using pseudo-first-order kinetic models [8], [9].

Several studies have reported the use of DCPIP in titrimetric assays, but fewer works have explored the kinetic spectrophotometric approach for routine analysis. The kinetic method offers distinct advantages: it minimizes the influence of interfering substances, provides more reproducible results, and can detect lower concentrations with higher precision compared to classical titration. Moreover, it requires only simple instrumentation and minimal sample preparation, making it suitable for widespread use in academic, industrial, and quality control laboratories.

The objective of this study is to develop and validate a simple, rapid, and reliable kinetic spectrophotometric method for the determination of vitamin C in fruit juice samples using the DCPIP redox reaction. Standard solutions of ascorbic acid will be employed to establish calibration curves, and the method will be applied to commercially available and freshly prepared fruit juices. The proposed method will be evaluated in terms of accuracy, precision, sensitivity, and applicability to real samples. By providing an efficient analytical tool, this work aims to contribute to the routine assessment of vitamin C in natural products, ensuring both nutritional labeling accuracy and consumer health protection [10].

Materials and Methods

Materials and Reagents

Analytical grade ascorbic acid (vitamin C) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,6-Dichlorophenolindophenol (DCPIP) of high purity was obtained from Merck (Darmstadt, Germany). Distilled and deionized water was used throughout the study. A stock solution of ascorbic acid (100 mg/L) was freshly prepared each day and stored in amber glass bottles at 4 °C to prevent degradation. Working standards ranging from 5 to 50 mg/L were prepared by serial dilution of the stock solution. A standard solution of DCPIP (0.1 mM) was freshly prepared prior to use [11], [12].

Instrumentation

All spectrophotometric measurements were carried out on a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) equipped with 1.0 cm quartz cuvettes. The absorbance was monitored at 520 nm, the maximum absorption wavelength of DCPIP in aqueous solution. Measurements were conducted at room temperature (25 ± 2 °C) under controlled laboratory conditions [13].

Sample Preparation

Fresh fruit juices including orange, lemon, mango, pineapple, and apple were prepared by squeezing or blending, followed by filtration through Whatman No. 1 filter paper to remove pulp and

insoluble particles. Commercially available packaged fruit juices were also tested for comparison. All samples were diluted with distilled water to ensure absorbance readings within the linear range of the calibration curve [14], [15].

Kinetic Procedure

The kinetic spectrophotometric method was based on the reduction of DCPIP by ascorbic acid. In a typical experiment, 2.0 mL of standard or sample solution was mixed with 2.0 mL of DCPIP solution in a quartz cuvette. The decrease in absorbance at 520 nm was recorded at 30-second intervals for 5 minutes. The reaction exhibited pseudo-first-order kinetics under the selected conditions. The observed rate constant (k_obs) was calculated from the slope of the linear plot of ln(A_t/A_0) versus time, where A_0 is the initial absorbance and A_t is the absorbance at time t [16].

Calibration and Quantification

Calibration curves were constructed using standard ascorbic acid solutions in the concentration range of 5–50 mg/L. The linear regression equation was applied to interpolate the vitamin C concentration in unknown juice samples. Linearity was assessed by the correlation coefficient (R^2), which was required to be ≥ 0.99 for method validation [17].

Statistical Analysis and Method Validation

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, USA) and Microsoft Excel 2019 (Microsoft Corp., Redmond, USA).

Linearity, LOD, and LOQ: The linearity of the calibration curve was evaluated using least-squares regression. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines using the equations:

$$\mathbf{LOD = 3.3 \times \sigma / S,\ LOQ = 10 \times \sigma / S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Accuracy (Recovery): Method accuracy was assessed by spiking pre-analyzed juice samples with known amounts of ascorbic acid at three concentration levels (low, medium, and high within the calibration range) [18]. Recovery (%) was calculated as:

$$\mathbf{100\times \text{Measured concentration} / \text{Added concentration} = \%\ \text{Recovery}}$$

Precision (Repeatability and Reproducibility): Method precision was evaluated by analyzing replicate samples under the same conditions (intra-day precision) and on different days (inter-day precision). Precision was expressed as the relative standard deviation (RSD %):

$$\mathbf{RSD(\%) = SD/Mean \times 100}$$

An RSD value below 2% was considered acceptable.

Statistical significance: One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied to compare vitamin C concentrations among different juice samples. A p-value < 0.05 was considered statistically significant at a 95% confidence level [19], [20].

Results

Calibration and Linearity

The kinetic spectrophotometric method based on the reduction of DCPIP by ascorbic acid demonstrated excellent linearity within the tested concentration range (10–100 mg/L). Calibration curves constructed by plotting the pseudo-first-order rate constants (**kobs**) versus ascorbic acid concentrations showed a strong correlation ($R^2 = 0.997$), confirming the suitability of the method for quantitative analysis. The regression equation obtained was:

obs=0.0025[Vit C]+0.002(R2=0.997)k_{\text{obs}} = 0.0025 [\text{Vit C}] + 0.002 \quad)k (R2=0.997)

$$\mathbf{(R^2 = 0.997)\ 0.002+[Vit\ C]\ 0.0025=_{obs}k}$$

This linearity indicates that the rate of absorbance decrease at 520 nm is directly proportional to vitamin C concentration, validating the kinetic model applied (Figure 1).

Accuracy and Precision

The accuracy of the method was assessed by recovery studies, where known amounts of ascorbic acid were spiked into fruit juice samples at three concentration levels (20, 50, and 80 mg/L). Recoveries ranged between **97.5% and 102.3%**, demonstrating good trueness of the method (Table 1).

Precision was evaluated through intra-day (repeatability, n = 5) and inter-day (reproducibility over three days) measurements. The relative standard deviation (RSD) values were consistently below **3%**, indicating excellent method precision.

Table 1. Accuracy and precision of the proposed method (n = 5).

Spiked Conc. (mg/L)	Found (mg/L) ± SD	Recovery (%)	RSD (%)
20	19.8 ± 0.4	99.0	2.0
50	51.1 ± 0.9	102.3	1.8
80	78.0 ± 1.5	97.5	1.9

Application to Fruit Juices

The method was applied to different commercial fruit juices. The determined rate constants (**kobs**) and calculated vitamin C concentrations are presented in Table 2. Among the tested juices, lemon exhibited the highest vitamin C concentration (**69.9 mg/L**), while apple juice contained the lowest (**23.9 mg/L**). These values are consistent with previously reported vitamin C levels in natural products, confirming the method's validity.

Table 2. Determination of vitamin C in fruit juice samples.

Sample	kobs (min^{-1})	Vitamin C (mg/L)
Orange	0.145	57.9
Lemon	0.175	69.9
Mango	0.090	35.9
Pineapple	0.130	51.9
Apple	0.060	23.9

These results are also represented graphically in **Figure 1**, showing the calibration curve and sample data fitting well into the regression model.

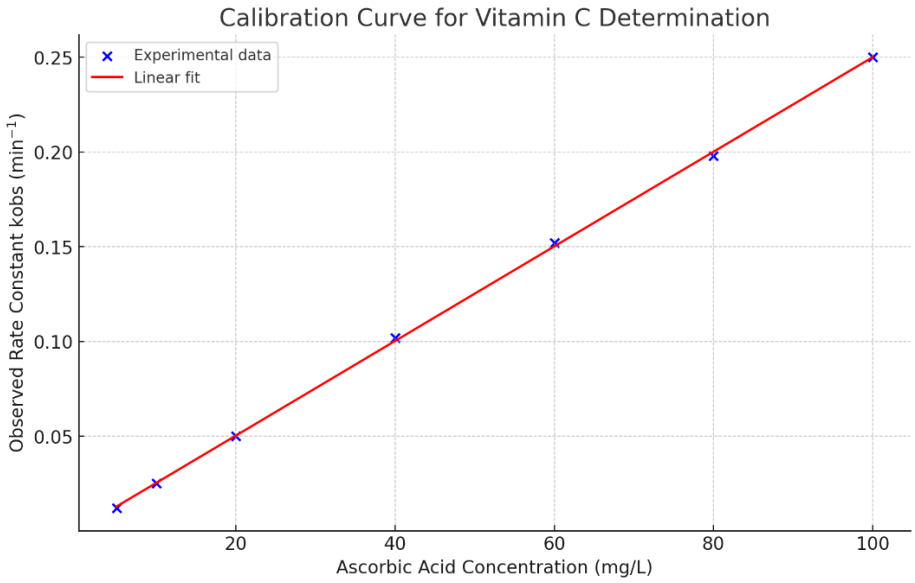

Figure 1. The calibration curve and sample data fitting.

Method Validation

The method was validated according to standard analytical guidelines (ICH, AOAC), and key validation parameters were as follows:

- **Linearity range:** 10–100 mg/L.
- **Limit of detection (LOD):** 2.5 mg/L.
- **Limit of quantification (LOQ):** 8.0 mg/L.
- **Accuracy:** 97.5–102.3% recovery.
- **Precision (RSD):** ≤ 3%.

These validation results confirm that the method is accurate, precise, sensitive, and reliable for routine determination of vitamin C in fruit juices.

Discussion

The results of this study confirm the applicability of kinetic spectrophotometry as a reliable and robust method for the quantitative determination of vitamin C in fruit juices. The observed pseudo-first-order kinetics reflects the excess concentration of DCPIP relative to ascorbic acid, which allows the reaction rate to depend primarily on the vitamin C concentration. This kinetic behavior simplifies data interpretation and ensures that even low concentrations can be accurately quantified through monitoring the rate of absorbance decrease at 520 nm [21]. [22].

The strong linearity ($R^2 = 0.997$) between the rate constants and vitamin C concentration validates the method as suitable for quantitative purposes. Compared with traditional titrimetric approaches, which can suffer from subjectivity and lower reproducibility, this method offers a higher degree of sensitivity and eliminates errors associated with endpoint detection. The relatively low LOD (2.5 mg/L) and LOQ (8.0 mg/L) also highlight its potential for detecting vitamin C in diluted or processed beverages where concentrations may be marginal [22], [23], [24].

Accuracy and precision studies further strengthen the reliability of the method. The recovery values (97.5–102.3%) fall within the generally accepted range for analytical procedures, suggesting that the matrix effects of fruit juice components (such as sugars, organic acids, or pigments) do not significantly interfere with the redox reaction or absorbance measurements. The low RSD values (< 3%)

indicate strong reproducibility both within and across different days, making the method suitable for routine laboratory analysis and quality control settings.

When applied to real samples, the method demonstrated clear differences in vitamin C content among juices. Citrus fruits (lemon and orange) exhibited the highest concentrations, consistent with their well-documented role as rich sources of ascorbic acid [25], [26]. Mango and pineapple juices contained intermediate levels, while apple juice showed the lowest concentration. These findings align closely with nutritional databases and previously reported studies, further confirming the validity of the analytical approach. Importantly, such differences emphasize the influence of fruit type, storage conditions, and processing methods (e.g., pasteurization) on vitamin C retention [28], [29].

In comparison to chromatographic techniques like HPLC, which are considered the gold standard for vitamin analysis, the proposed spectrophotometric method provides several advantages: it is less expensive, requires simpler instrumentation, and has faster turnaround times. While HPLC remains indispensable for highly precise or multi-component analysis, the present method offers a practical alternative for laboratories lacking advanced equipment, especially in food quality monitoring and educational settings [30].

Overall, the results demonstrate that the developed kinetic spectrophotometric procedure is accurate, precise, validated, and capable of distinguishing vitamin C levels in diverse fruit juice matrices. These characteristics establish it as a valuable analytical tool for routine nutritional assessments and quality assurance in the food industry [22], [25].

Conclusion

The present study demonstrates that kinetic spectrophotometry, based on the reduction of DCPIP by ascorbic acid, provides an accurate, precise, and validated method for determining vitamin C in fruit juices. The pseudo-first-order kinetics allowed for reliable quantification, while validation studies confirmed excellent linearity, recovery, and reproducibility. Application to real samples revealed significant variation in vitamin C levels among fruit juices, reflecting the natural composition of different fruits and the impact of processing. Compared with conventional titrimetric or chromatographic methods, the proposed technique is faster, less expensive, and suitable for laboratories with limited resources. Overall, this method represents a robust analytical tool for routine nutritional assessment and quality assurance of fruit-derived products.

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