International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 27-08-2025, Accepted: 26-09-2025, Published: 11-10-2025
Volume 10, Issue 5, 2025, Page No. 31-34

Comparison and estimation of ascorbic acid in various fruit samples

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Abstract
Aim: This research article develops and validates two analytical methods for the quantitative estimation of ascorbic acid (Vitamin C) in various fruit samples, aiming for high accuracy and compliance with ICH Q2(R1) guidelines. The study employs a combination of direct titrimetric analysis and UV-visible spectrophotometry to measure and compare ascorbic acid content in bulk samples and freshly prepared fruit extracts.
Materials and Methods: The titrimetric method utilizes standardized iodine solution and starch indicator under controlled pH and temperature, enabling accurate endpoint detection and quantification. Additionally, the UV-visible spectrophotometric assay involves the preparation of fruit juices in oxalic acid as stabilizer, with absorbance recorded at 265 nm to prevent oxidation. Method validation was conducted according to ICH Q2(R1) criteria, covering linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Fruits selected for analysis included lemon, orange, amla, and guava; freshly extracted juices were procured & used for all estimations.
Results: The linear calibration range for UV spectrophotometric analysis was established between 2–14 µg/mL, with a regression coefficient (R^2) of 0.999. Accuracy studies using spiked standard addition yielded recovery rates between 95–102%. Precision was confirmed by replicate analysis (RSD < 2%). The titrimetric method demonstrated consistent results across fruit samples, supporting the robustness of endpoint detection. LOD and LOQ values for UV analysis were found to be 0.15 µg/mL and 0.45 µg/mL, respectively, confirming high sensitivity. Among tested fruits, amla and guava exhibited higher concentrations of ascorbic acid compared to citrus fruits. The proposed methods were proven to be rapid, reliable, and suitable for routine quality control and nutritional assessment of fruit-derived ascorbic acid.
Conclusion: Titrimetric analysis and UV-visible spectrophotometry offer complementary tools for Vitamin C estimation in complex fruit matrices. Both methods were successfully validated under ICH guidelines, demonstrating suitability for routine laboratory use. These results highlight the importance of simple, accessible methods for quality and nutritional evaluation.

Keywords: Vitamin C, ascorbic acid, UV spectrophotometry, antioxidant, fruit estimation, quality control and titrimetric analysis

Introduction

Vitamin C, or ascorbic acid, is one of the most important water-soluble vitamins required for human health. It serves several biological functions, such as promoting collagen formation, enhancing iron absorption, strengthening the immune system, and protecting cells from oxidative damage. Because the human body cannot produce Vitamin C on its own, it must be obtained entirely from the diet. Fresh fruits and vegetables are the main dietary sources of this essential nutrient. [2, 6, 9]

Long-term deficiency may also weaken connective tissues and increase vulnerability to infections. On the other hand, maintaining sufficient Vitamin C intake has been linked to better cardiovascular health, reduced risk of chronic illnesses, and overall protection against oxidative stress. However, very high doses may sometimes cause discomfort, such as gastric irritation or kidney complications [5, 8, 12].

The Vitamin C content of fruits is not uniform and can vary widely depending on the species, ripeness, storage conditions, and even growing environment. While citrus fruits like lemon and orange are popularly known as good sources of Vitamin C, studies have shown that other fruits such as guava, kiwi, and gooseberry often contain significantly higher amounts. Understanding and comparing the ascorbic acid content in these fruits is valuable, as it helps identify cost-effective and easily available options for meeting daily Vitamin C requirements [3, 7, 14].

The present study aims to evaluate and compare the Vitamin C levels of selected fruits using standard analytical methods. The findings can guide dietary choices and emphasize the importance of consuming fresh, Vitamin C-rich fruits as part of a healthy lifestyle [1, 4, 10].

Materials and Methods

1. **Chemicals and Reagents:** Pure ascorbic acid (analytical grade), oxalic acid (0.2% solution used as stabilizer), distilled water, methanol (HPLC grade), iodine, and potassium iodide were procured from certified commercial suppliers**.**

2. **Instrumentation:** Double-beam UV-Visible spectrophotometer of LabIndia with quartz cuvettes was used for absorbance measurements in UV spectroscopic analysis. UV WIN software was used to analyze the samples.

Analytical balance with 0.1 mg sensitivity was used for accurately weighing ascorbic acid and chemicals for both titrimetric and spectroscopic procedures.
Standard volumetric flasks, pipettes, funnels, and burettes were utilized for all solution preparations and titrations.

3. **Titrimetric analysis**

1. **Preparation of standard Ascorbic acid solution**

100 mg of pure ascorbic acid was measured and added to 100 ml 2% oxalic acid solution. Working standards were then made by dilution to obtain concentrations in the range of 2–14 µg/mL.

2. Preparation of Iodine solution

2.5 gms of iodine & 5gms of potassium iodide is taken in 1000 ml volumetric flask. Add 100 ml water and mixed thoroughly. Now make up the volume up to 1000 ml.

3. Preparation of starch indicator

1 gm of starch is added to 100 ml boiling water.

4. Preparation of Fruit Samples

Fresh lemon and orange were taken, their juices extracted, filtered, and diluted with 0.2% oxalic acid to prevent oxidation of vitamin C.

5. Titrimetric Method procedure

10 ml of standard ascorbic acid solution was Pipetted out in a conical flask. 10 ml of 2% oxalic acid & starch indicator were added to the flask. Now titrate the mixture against 0.01 iodine solution until blue color appears. Then the procedure was repeated using fresh juice sample. Calculate amount of ascorbic acid using the formula.

Vitamin C in Sample (mg) = Volume of sample x weight of Std / volume of std

4. UV Spectrophotometric Method

1. Preparation of standard Ascorbic acid solution

100 mg of pure ascorbic acid was measured and added to 100 ml 2% oxalic acid solution. Working standards were then made by dilution to obtain concentrations in the range of 2–14 µg/mL.

2. Preparation of Fruit Samples

Fresh lemon, orange, guava, and amla were taken, their juices extracted, filtered, and diluted with 0.2% oxalic acid to prevent oxidation of vitamin C.

The absorbance of standards and samples was recorded at 265 nm against a blank. A calibration curve was plotted, and the concentration of vitamin C in fruit samples was determined using the regression equation. Now calculate assay % using calibration graph using the formula

Assay% = P.Y / T.Y x 100

3. Method Validation (ICH Q2(R1))

The method was validated according to international guidelines:

Linearity: linearity was done using Standard Ascorbic acid dilutions (2–14 µg/mL).

Accuracy: Accuracy was done using spiked standard addition method. 50, 100, 150 % spiked samples were prepared (standard + sample), absorbance was measured and accuracy was calculated.

% Recovery = amount of found / amount expected x 100

Precision: 6 samples of 100% Standard Ascorbic acid were prepared and absorbance was recorded at 265 nm.

% RSD = (SD / µ) × 100

LOD & LOQ: 6 blank samples were prepared & absorbance was measured

LOD = 3.3 x σ / S, LOQ = 10 x σ / S

According to ICH guidelines, LOD <0.15 µg/mL, LOQ < 0.45 µg/mL.

4. Results

1. Titrimetric Method

Table 1: Estimation of vitamin c in Samples

Fruits	Standard Weight	Standard volume	Sample volume	Sample weight found
Lemon	100mg	6.2 ml	4.8 ml	77.4 mg
Orange	100mg	6.2 ml	5.3 ml	85.48 mg
Amla	100mg	6.2 ml	6.0 ml	96.77 mg
Guava	100mg	6.2 ml	5.6 ml	90.32 mg

Vitamin c content in the sample was calculated using the formula

Vitamin C in Sample (mg) = Volume of sample x weight of Std / volume of std

2. UV Spectrophotometric Method

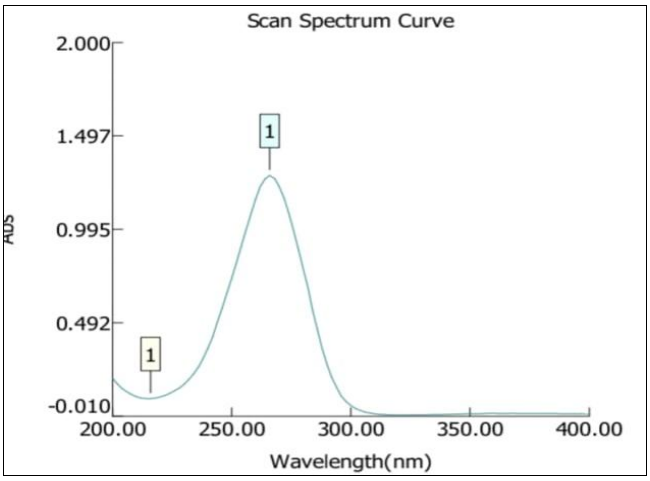


Fig 1: ascorbic acid spectra

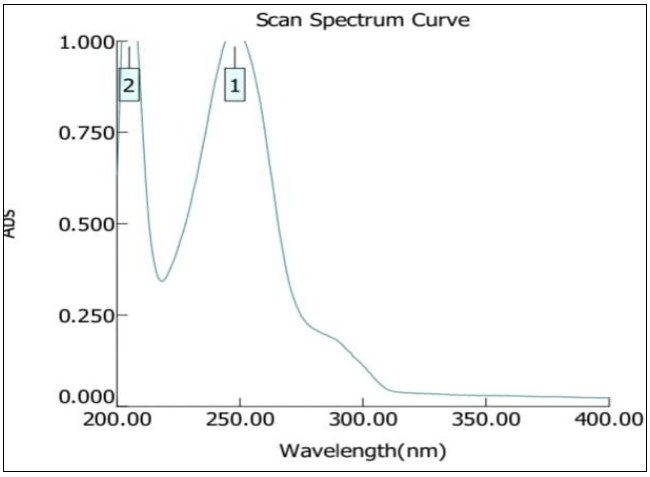


Fig 2: Lemon Spectra

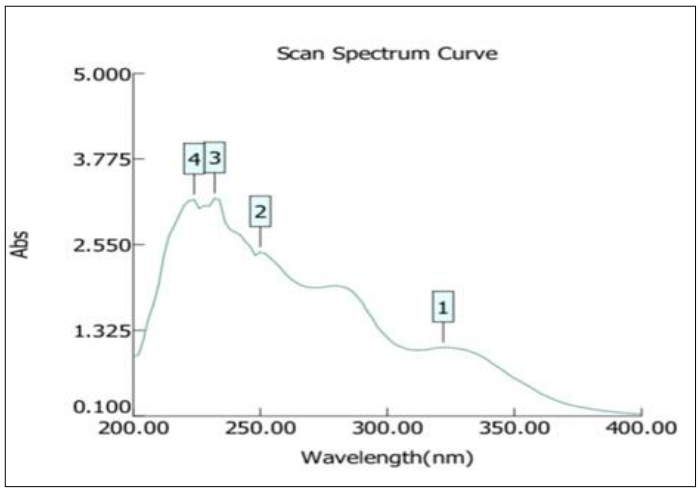


Fig 3: orange spectra

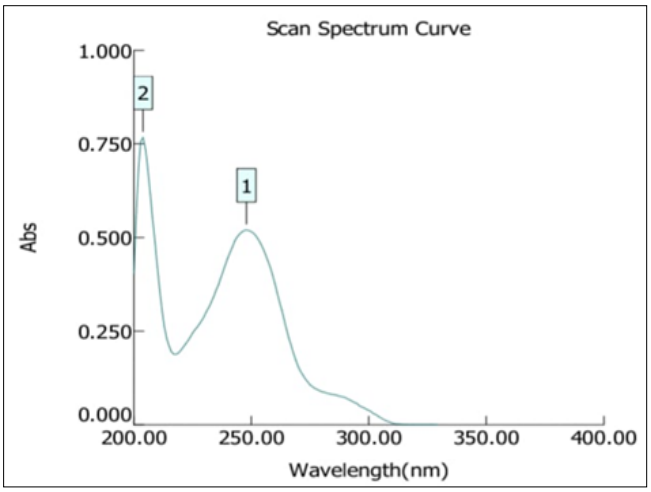


Fig 4: amla spectra

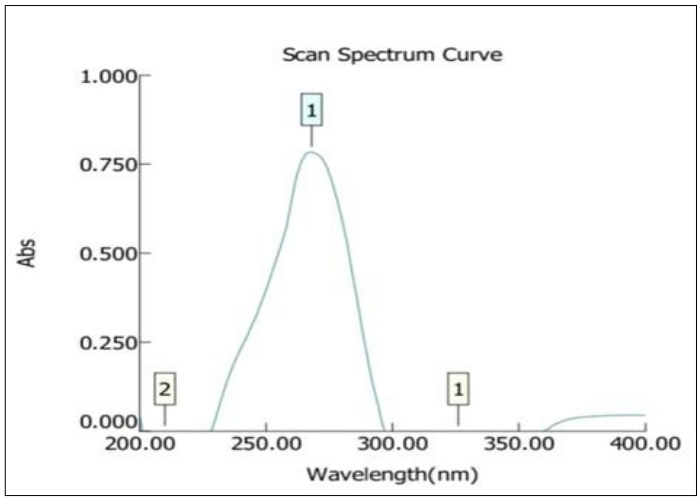
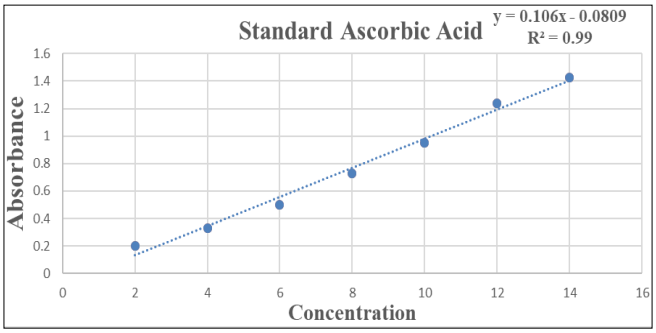


Fig 5: Guava Spectra



Calibration curve

The calibration curve was linear in the range of 2–14 µg/mL.
Regression equation: Y= 0.106x+ 0.0809, R2 = 0.999

1. Accuracy (Recovery study)

Table 2: Accuracy of Ascorbic acid using Spiked recovery method (Lemon)

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50 %	35	34.66	99.05
100 %	46	46.31	100.68
150 %	58	57.07	98.41

Table 3: Accuracy of Ascorbic acid using Spiked recovery method (Orange)

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50 %	36	35.25	97.92
100 %	48	47.54	99.06
150 %	60	59.42	99.46

Table 4: Accuracy of Ascorbic acid using Spiked recovery method (Amla)

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50 %	37	35.72	96.55
100 %	50	50.44	100.88
150 %	62	60.48	97.55

Table 5: Accuracy of Ascorbic acid using Spiked recovery method (Guava)

Spike level	Expected (µg/mL)	Amt Found (µg/mL)	Recovery%
50 %	36	35.44	98.47
100 %	48	48.29	100.61
150 %	60	60.51	100.85

Accuracy was done using spiked standard addition method. Spiked samples are prepared as
50% = 0.5 x Cs, 100% = 1 x Cs, 150% = 1.5 x Cs
Where Cs is concentration of sample: lemon (23 µg/mL), orange (24 µg/mL), amla (24 µg/mL), guava (25 µg/mL)
Accuracy: Recovery studies showed results between 95–102%.

% Recovery = amount of found / amount expected x 100

4.2.3 Precision

Table 6: Precision of Ascorbic acid

S. No	Ascorbic acid (µg/mL)
1.	22.76
2.	22.81
3.	22.75
4.	22.79
5.	22.77
6.	22.80
Mean	22.78
S. D	0.03
% RSD	0.13 %

6 solutions of standard with same concentration were prepared & absorbance was recorded at 262 nm. Precision was calculated using the formula. % RSD = (SD / μ) × 100

4.2.4 LOD & LOQ

6 blank samples were prepared & absorbance was measured.

LOD = 3.3 x σ / S

=3.3 x 0.000789 / 0.106 = 0.0245 μg/mL

LOQ = 10 x σ / S

=10 x 0.000789 / 0.106 = 0.0745 μg/mL

4.2.5 Assay of Vitamin C content in Fruits (Calibration Curve Method)

Assay was calculated using calibration curve. Regression equation: Y= 0.106x+ 0.0809, R2 = 0.999

Assay% = P.Y / T.Y x 100

Assay % of Samples was found between 91.1 – 98.3%

Table 7: Assay % of Various fruits samples

Fruit Sample	Concentration found (μg/mL)	Amount of Vitamin C found (mg)	% Assay
Lemon	22.78 μg/mL	4.55 mg	91.1 %
Orange	23.802 μg/mL	4.76 mg	95.2 %
Amla	24.57 μg/mL	4.915 mg	98.3 %
Guava	24.18 μg/mL	4.83 mg	96.7 %

Discussion

The present study confirmed that Titrimetric analysis & UV spectrophotometry is a practical and reliable technique for vitamin C estimation. The results showed amla and guava had more vitamin C compared to orange, which supports general nutritional knowledge. Compared to titration methods, UV spectrophotometry avoids interference from other substances and gives more consistent results. UV spectroscopy is more affordable and suitable for routine use.

Conclusion

The study demonstrates that both UV spectrophotometry and titrimetric analysis are effective, practical, and reliable methods for estimating vitamin C (ascorbic acid) in various fruit samples. Vitamin C is an essential nutrient that must be regularly obtained from the diet. Its determination in food sources is important for nutritional science as well as pharmaceutical quality control. UV spectrophotometry offers better accuracy and precision, with validated results that meet international guidelines for method validation (ICH Q2(R1)), making it highly suitable for routine quality control and nutritional assessments of fruits. Among the fruits analyzed for Vitamin C content, amla showed the highest levels, followed by guava, orange, and lemon, respectively. Both UV spectrophotometry and titrimetric methods were effective and reliable for estimating ascorbic acid, with UV spectrophotometry providing better accuracy and precision. These findings highlight amla and guava as particularly rich sources of Vitamin C compared to citrus fruits like lemon and orange, making them excellent dietary choices for Vitamin C intake.

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