MEKONG UNIVERSITY SCIENTIFIC JOURNAL
https://tapchikhdhcl.edu.vn
https://vjol.info.vn/index.php/dhcl

DEVELOPMENT OF A PROCEDURE FOR QUANTITATIVE DETERMINATION OF VITAMIN C IN RAW MATERIALS USING THE UV-VIS SPECTROPHOTOMETRIC METHOD

Nguyen Chi Toan[1], Giang Thi Thu Hong[1]*
[1]*Tay Do University*
[*]*Email: gtthong@tdu.edu.vn*

Date of submission: 24/10/2025; Review Day: 08/11/2025; Date of article approval: 15/12/2025

ABSTRACT

This study aims to develop and validate a direct UV-Vis spectrophotometric method for the quantitative determination of vitamin C in raw materials, using a phosphate buffer system at pH 5.4 combined with sodium oxalate as a stabilizing agent. The analytical procedure was evaluated in terms of specificity, linearity, accuracy, and precision. The results showed that the standard solution, test solution, and spiked solution exhibited a clear absorption peak at λmax = 265.5 nm. In contrast, the blank showed no significant absorption at this wavelength, demonstrating reasonable specificity. In the concentration range of 8-12 ppm, the calibration curve exhibited excellent linearity with a correlation coefficient R^2 = 0.99994. The recovery values ranged from 98% to 102% with RSD < 2%, indicating acceptable accuracy and repeatability. The validated method was then applied to quantify nine raw vitamin C samples (M1-M9), whose assay results were within the acceptable limit of 95-110% of the label claim. These findings confirm that the proposed UV-Vis method is reliable, cost-effective, and suitable for routine quality control of vitamin C in raw materials.

Keywords: *Vitamin C, UV-Vis, quantification, raw material*

1. Introduction

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that plays a crucial role in numerous biological processes, including collagen synthesis, carnitine and catecholamine metabolism, as well as the absorption of non-heme iron. The human body cannot synthesize vitamin C, so it must be obtained through diet or dietary supplements. Vitamin C is abundant in citrus fruits and fresh green vegetables; however, it is highly labile and can easily degrade under heat, light, and improper storage conditions.

Prolonged vitamin C deficiency can lead to scurvy, with symptoms such as fatigue, bleeding gums, delayed wound healing, and subcutaneous hemorrhage. Conversely, insufficient supplementation or the use of low-quality raw materials can reduce the efficacy of vitamin C products. Therefore, monitoring vitamin C content in raw materials used for pharmaceuticals or dietary supplements is essential to ensure product quality and therapeutic effectiveness [2].

In hot and humid climates like Vietnam, vitamin C in raw materials is highly susceptible to degradation during transportation and storage, resulting in active ingredient content lower than the labeled value. Without proper control, this can affect

the quality of incoming raw material batches and increase the risk of inadequate content in the final products. Therefore, establishing a reliable quantitative procedure for vitamin C in raw materials is of critical importance for routine quality control [13].

Currently, several methods are used to determine vitamin C content, such as high-performance liquid chromatography (HPLC) and volumetric titration. HPLC offers high accuracy and sensitivity but requires the use of pure, sometimes toxic solvents and expensive equipment, which not all testing facilities possess. In contrast, volumetric titration is simple and low-cost but is manual, prone to errors, and highly dependent on the analyst's experience [4].

In this context, ultraviolet-visible (UV-Vis) spectrophotometry emerges as a suitable alternative for testing facilities with limited equipment and budget. This method offers rapid analysis, simple procedures, minimal use of hazardous solvents, and can achieve good accuracy when properly validated [11]. This study was conducted to develop and validate a direct UV-Vis spectrophotometric procedure for quantifying vitamin C in raw materials, providing a basis for routine testing in herbal and pharmaceutical raw material laboratories.

2. Research methods

The experimental work was conducted on nine raw vitamin C samples (labeled M1-M9), randomly collected from different suppliers. The samples were stored according to the manufacturer's instructions until analysis.

2.1. Validation Method

The analytical procedure was validated using the UV-Vis method following the guidelines in the 2013 Drug Registration Handbook issued by the Ministry of Health. The results were processed and analyzed using Excel statistical software [6].

2.2. Experimental Procedure

A procedure for the determination of vitamin C using the UV-Vis spectrophotometric method was established, employing a phosphate buffer at pH 5.4 and sodium oxalate. The method was used to evaluate characteristics such as specificity, linearity, range, accuracy, and precision.

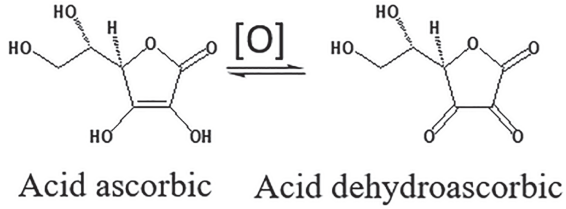


Figure 1. Reversible reaction of ascorbic acid

A phosphate buffer at pH 5.4 was used to prevent ascorbic acid from oxidizing to dehydroascorbic acid, as this maintains the equilibrium in the reverse direction and avoids errors in measurement. Adding sodium oxalate to the buffer system stabilizes the solution for at least 30 minutes at room temperature.

2.3. Preparation of Solutions

- Phosphate buffer solution (pH 5.4): Dissolve a mixture of 1.76 g disodium hydrogen phosphate (Na_2HPO_4) and 1.00 g potassium dihydrogen phosphate (KH_2PO_4) in water and dilute to 1000 mL [5].
- 0.0056 M Sodium oxalate solution: Accurately weigh approximately 0.75 g of sodium oxalate and dissolve it in 1000 mL of the phosphate buffer solution (solution A).
- Stock standard solution: Accurately weigh about 10 mg of standard vitamin C (purity 99.72%) into a 100 mL volumetric flask and dilute with solution A to the mark.
- Stock test solution: Accurately weigh a sample equivalent to 100 mg vitamin C, place it in a 100 mL volumetric flask, add 70 mL of solution A, sonicate for 5 minutes, then dilute to the mark with solution A and mix well. Discard the first 20 mL of the filtrate

and collect the remaining solution. Pipette 10 mL of this filtrate into a 100 mL volumetric flask and dilute to the mark with solution A to obtain a 100 ppm solution [6].

2.4. Investigation of Maximum Wavelength (λmax)

2.4.1. Preparation of 10 ppm standard vitamin C solution:

Pipette 10 mL of the stock standard solution into a 100 mL volumetric flask and dilute to the mark with solution A.

2.4.2. Preparation of 10 ppm test vitamin C solution:

Pipette 10 mL of the stock test solution into a 100 mL volumetric flask and dilute to the mark with solution A.

Scan the absorbance spectrum of the solutions in the range of 220-350 nm, using solution A as the blank.

2.4.3. Specificity Study:

Specificity was investigated to determine the maximum wavelength (λmax) and to check the stability of the absorption spectrum of vitamin C solutions.

- Standard solution: Pipette 10 mL of the stock standard solution into a 100 mL volumetric flask and dilute to the mark with solution A.
- Test solution: Pipette 10 mL of the stock test solution into a 100 mL volumetric flask and dilute to the mark with solution A.
- Blank solution: Solution A.
- Spiked solution: Pipette 5 mL of the stock standard solution and 5 mL of the stock test solution into a 100 mL volumetric flask and dilute to the mark with solution A.

Scan the absorption spectra of the standard, test, blank, and spiked solutions over the range of 220-350 nm to determine λmax.

2.4.4. Linearity Study:

Determine the regression equation y=ax+b and the correlation coefficient (R) between concentration and absorbance.

2.4.5. Accuracy Study:

Assess the method's accuracy by comparing the detected amount with the actual amount, expressed as percent recovery (%).

2.4.6. Repeatability Study:

Perform six replicate determinations on the same vitamin C raw material sample following the stock test solution procedure described in section 2.3.

For each determination, accurately weigh the vitamin C sample, prepare the stock test solution, pipette 10 mL into a 100 mL volumetric flask, dilute to the mark with solution A, and measure the absorbance.

From the absorbance AAA of each solution, calculate the corresponding concentration CCC using the calibration equation, and then apply the following formula to calculate the vitamin C content:

$$H(\%) = \frac{C \times 10000 \times m_{tb}}{m \times P \times 1000} \times 100$$

Where:

- **C**: Concentration of vitamin C calculated by substituting the absorbance AAA into the calibration equation.
- **mtb**: Average mass of raw material used to prepare the stock test solution (mg).
- **m**: Mass of raw material weighed for each determination (mg).
- **P**: Nominal vitamin C content of the raw material according to the manufacturer's specification (mg).

2.4.7. Flowchart of the Quantitative Procedure

The quantitative procedure was applied according to the following flowchart:

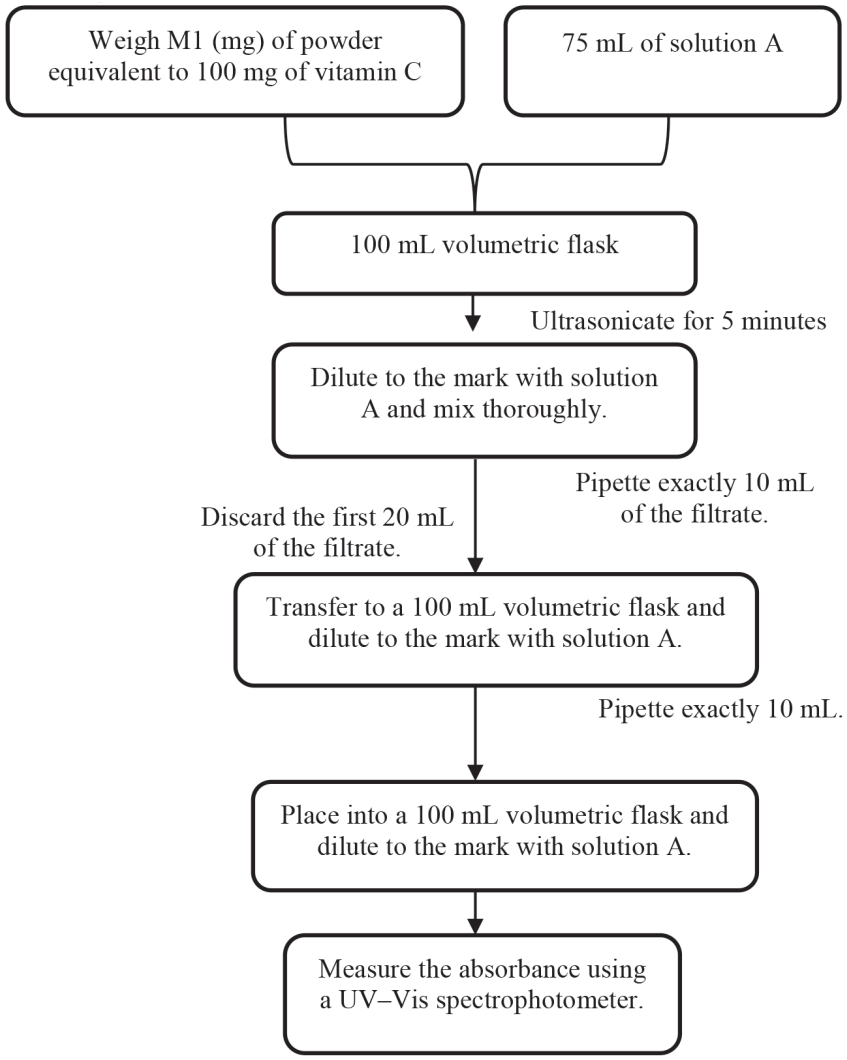


Figure 2. Detailed schematic of the procedure

2.5. Evaluation Criteria

The validation of the vitamin C quantitative procedure was conducted following the ASEAN Common Technical Dossier (ACTD) and the ASEAN Guideline for Validation of Analytical Procedures. The evaluation criteria included specificity, linearity, accuracy (with recovery within the acceptable range), and repeatability/precision (RSD ≤ 2% for the determined vitamin C content) [6], [9].

3. Results and Discussion

The research results are presented as follows:

3.1. Maximum Wavelength (λmax)

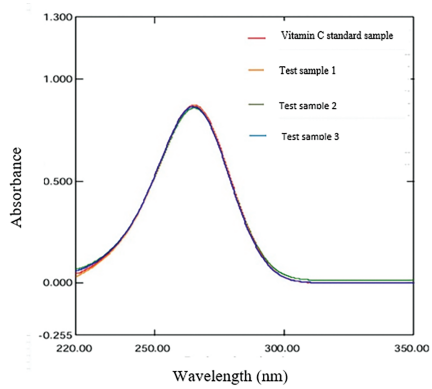


Figure 3. Absorption spectra of the vitamin C standard and test solutions

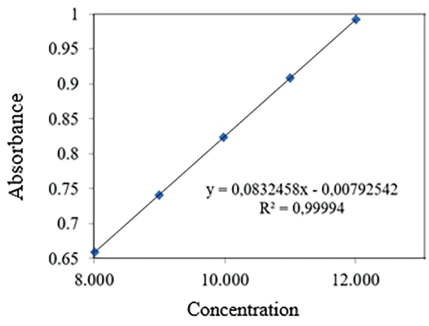
Based on the presented absorption spectra, it is evident that all solutions exhibited a clear absorption peak at λmax = 265.5 nm, indicating that vitamin C strongly absorbs

and remains stable at this wavelength. Moreover, at λ = 265.5 nm, the absorbance of vitamin C is minimally affected by other components in the sample, which helps reduce analytical errors. These results are consistent with previous studies [10], confirming that selecting 265.5 nm as the measurement wavelength is appropriate to ensure accurate determination of vitamin C.

3.2. Specificity

The absorption spectra of the vitamin C standard solution, test solution, and placebo solution (containing no vitamin C) all showed a clear peak at λmax = 265.5 nm, demonstrating good specificity of the method and consistent with previous studies [11], [13], [14].

Figure 4. Calibration curve of the vitamin C solution



3.3. Linearity

Within the concentration range of 8-12 ppm, the graph followed the Lambert-Beer law and showed high linearity between vitamin C concentration and the absorbance of the solution, with the regression equation y=0.0832458, x−0.00792542 and a correlation coefficient R2=0.99994. This demonstrates that the UV-Vis spectrophotometric method has very high linearity and can accurately quantify vitamin C in raw material test samples.

These results are fully consistent with the study by [11], which confirmed that the UV-Vis spectrophotometric method can be effectively applied for determining vitamin C in raw material samples without accuracy issues. Furthermore, research by [12] also indicated that the UV-Vis method exhibits high linearity when determining vitamin C content in test samples, ensuring method stability and accuracy.

The UV-Vis method not only provides accurate results but also offers low cost and simpler equipment compared to other methods such as high-performance liquid chromatography (HPLC). This makes the UV-Vis method an ideal choice when cost efficiency and reduced reliance on expensive equipment are required, while still ensuring accurate quality control of vitamin C in test samples, particularly in raw materials.

3.4. Repeatability

From the absorbance measurements, calculations and descriptive statistics were performed using Microsoft Excel, yielding the following table:

Table 1. Repeatability results for the quantitative determination of vitamin C in raw materials

Measurement	Sample weight (mg)	Concentration (ppm)	Absorbance at λ 265.5 nm	Content (%)
1	126.4	10.474	0.864	104.775
2		10.462	0.863	104.655
3		10.486	0.865	104.895
4		10.438	0.861	104.414
5		10.438	0.861	104.414
6		10.414	0.859	104.175
Average content (%):				104.5547
Standard deviation (SD):				0.2676
Relative standard deviation (RSD, %):				0.256

The results indicate that the absorbance at the maximum wavelength (265.5 nm) remained nearly constant across repeated measurements, and the calculated vitamin C content varied very little between measurements. The RSD value was below 2%, demonstrating that the method has good repeatability for quantifying vitamin C in raw materials without the need for hazardous solvents.

3.5. Accuracy

Table 2. Recovery of vitamin C samples

Solution	Amount in test sample (ppm)	Amount of standard added (ppm)	Measured absorbance at λmax = 265.5 nm	Recovered amount (ppm)	Recovery (%)
90%	5.225	4.5	0.754	9.153	99.220
			0.755	9.165	99.350
			0.754	9.153	99.220
100%		5	0.840	10.186	99.610
			0.839	10.173	99.490
			0.840	10.186	99.610
110%		5.5	0.915	11.197	99.830
			0.915	11.197	99.830
			0.914	11.185	99.763
Average recovery (%)					99.547
Relative standard deviation (RSD, %)					0.242

The recovery study (accuracy) of vitamin C at 90%, 100%, and 110% levels showed an average recovery of 99.547% with RSD = 0.244% (< 2%). These values fall within the acceptable range for accuracy (recovery 98-102% or 95-105%) and precision (RSD ≤ 2%) according to ACTD guidelines [6], and are consistent with similar reports in the literature [10]. This confirms that the method meets validation requirements for the quantitative determination of vitamin C.

3.6. Determination of Vitamin C Content in Raw Materials

The vitamin C content was determined as follows:

Table 3. Vitamin C content in raw material samples

Sample	Average absorbance	Average content (%)	Conclusion
M1	0.8650	104.89	Pass
M2	0.8583	104.05	Pass
M3	0.8250	100.08	Pass
M4	0.8360	103.15	Pass
M5	0.8507	103.25	Pass
M6	0.8557	103.70	Pass
M7	0.8456	102.54	Pass
M8	0.8390	101.77	Pass
M9	0.8007	97.11	Pass

The results in Table 3 show that the average vitamin C content in the nine raw material samples (M1-M9) ranged from 97.11% to 104.89%, all within the acceptable limit of 95-110% of the labeled content. This indicates that the tested vitamin C raw materials meet quality requirements, and the proposed UV-Vis spectrophotometric procedure can be reliably applied for the quantitative determination of vitamin C in raw materials.

4. Conclusion

A UV-Vis spectrophotometric method for the determination of vitamin C in raw materials was developed using a phosphate buffer at pH 5.4 and sodium oxalate as a stabilizer. The validation results met all requirements, with linearity in the range of 8-12 ppm, an average recovery of 99.546%, and RSD of 0.244%. The method is simple, cost-effective, and suitable for routine quality control testing. Ministry of Health (2022). *Vietnamese Pharmacopoeia V.* Hanoi Medical Publishing House, Hanoi, pp. 12-14, Appendix 98.

REFERENCES

Ministry of Health (2015). *National Drug Formulary.* Hanoi Medical Publishing House, Hanoi, pp. 121-123.

Ministry of Health (2011). Dang Van Hoa, Vinh Dinh. *Drug Testing.* Education Publishing House, Hanoi, pp. 135-180.

Ministry of Health (2007). Le Quan Nghiem, Huynh Van Hoa. *Pharmaceutical Formulation, Volume 2.* Education Publishing House, Hanoi, pp. 170, 174, 199, 268, 279.

Ministry of Health (2013). Decision of the Director of the Drug Administration on the issuance of the *Drug Registration Handbook*, Appendix 8.

Ministry of Health (2014). Decision No. 44/2014/TT-BYT dated November 25, 2014, *ASEAN Common Technical Dossier (ACTD) and technical guidelines.*

Phan Dinh Chau (2011). *Medicinal Chemistry and Synthetic Techniques*. Bach Khoa Publishing House, Hanoi.

Tran Thi Thu Hang (2016). *Pharmacology.* Phuong Dong Publishing House, pp. 632-633.

ASEAN guideline for validation of analytical procedures (2008), pp. 1-17.

de Silva, D. S. P. C. N., Nonis, P. C. M., & Jayasundara, U. K. (2025). Method development and validation of vitamin C formulations using zeroth-order UV-visible spectrophotometry. *European Journal of Applied Sciences,* 13(1), 473-485. https://doi.org/10.14738/aivp.1301.18346

Muralikrishna, U., & Murty, J. A. (1989). Spectrophotometric determination of ascorbic acid in pharmaceutical preparations and fruit juices. *Analyst,* 114(3), 407-408. https://doi.org/10.1039/an9891400407

Verma, K. K., Jain, A., Verma, A., & Chaurasia, A. (1991). Spectrophotometric determination of ascorbic acid in pharmaceuticals by background correction and flow injection. *Analyst,* 116(6), 641-645. https://doi.org/10.1039/AN9911600641

Salkić, M., & Selimović, A. (2015). Spectrophotometric determination of L-ascorbic acid in pharmaceuticals based on its oxidation by potassium peroxymonosulfate and hydrogen peroxide. *Croatica Chemica Acta,* 88(1), 73-79. https://doi.org/10.5562/cca2551

Shrivas, K., Agrawal, K., & Patel, D. K. (2005). A spectrophotometric determination of ascorbic acid in fruits, beverages, and pharmaceuticals. *Journal of the Chinese Chemical Society,* 52(3), 503-506. https://doi.org/10.1002/jccs.200500072