

# An Eco-Friendly UV Spectrophotometric Method for the Determination of 5,7-Dihydroxyflavone in Bulk Drug

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### Article Citation:

Arif Salim Jamadar<sup>1</sup>, Ronak Dsouza<sup>2</sup>, Adarsh Anil Kamate<sup>3</sup>, Samarth Ugare<sup>4</sup>, Shailendra Sanjay Suryawanshi<sup>\*5</sup>, "An Eco-Friendly UV Spectrophotometric Method for the Determination of 5,7-Dihydroxyflavone in Bulk Drug", International Journal of Recent Trends in Multidisciplinary Research, July-August 2026, Vol 6(04), 396-403.



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**Abstract: Background:** 5,7-Dihydroxyflavone (Chrysin) is a naturally occurring flavonoid with diverse pharmacological activities, necessitating reliable analytical methods for its quantitative estimation. This study aimed to develop and validate a simple, rapid, and cost-effective UV spectrophotometric method for the determination of 5,7-Dihydroxyflavone. **Methods:** The method was developed using methanol as the solvent, and the absorption spectrum was recorded over the wavelength range of 200–400 nm. Quantification was performed at the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) of 313 nm. Calibration standards were prepared in the concentration range of 4–20  $\mu\text{g/mL}$ . The proposed method was validated according to the International Council for Harmonisation (ICH) Q2(R2) guideline for specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), and solution stability. **Results:** The method exhibited excellent linearity over the concentration range of 4–20  $\mu\text{g/mL}$  with a correlation coefficient ( $r^2$ ) of 0.9992. The LOD and LOQ were found to be 1.37  $\mu\text{g/mL}$  and 3.29  $\mu\text{g/mL}$ , respectively, indicating satisfactory sensitivity. Accuracy studies demonstrated recoveries within acceptable limits, while precision, robustness, and ruggedness showed %RSD values below 2%, confirming the reproducibility and reliability of the method. No interference from the solvent was observed at the selected analytical wavelength. **Conclusion:** The validated UV spectrophotometric method is simple, accurate, precise, sensitive, and economical, making it suitable for the routine quantitative estimation and quality control of 5,7-Dihydroxyflavone in pharmaceutical and research laboratories.

**Keywords:** 5,7-Dihydroxyflavone; UV spectrophotometry; Method development; Method validation; Herbal quality control; Analytical method.

## 1. Introduction

5,7-Dihydroxyflavone is a naturally occurring flavonoid abundantly present in several medicinal plants, honey, propolis, and passion flowers (*Passiflora* species). It has attracted considerable scientific attention because of its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, and neuroprotective properties. These biological effects have promoted the incorporation of 5,7-Dihydroxyflavone into herbal formulations, nutraceuticals, and pharmaceutical products intended for health promotion and disease management. Consequently, reliable analytical methods are

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required to ensure the quality, safety, efficacy, and consistency of 5,7-Dihydroxyflavone-containing products through accurate quantitative determination of the marker compound.<sup>1,2</sup> Among the available analytical techniques, UV-visible spectrophotometry remains one of the most widely employed approaches for routine quality control because of its simplicity, rapid analysis, cost-effectiveness, and minimal instrumentation requirements. Although chromatographic methods such as HPLC provide high sensitivity and selectivity, they often require expensive instrumentation, complex sample preparation, and greater solvent consumption. Therefore, a validated UV spectrophotometric method offers a practical alternative for routine laboratory analysis, particularly in academic and industrial quality control settings.<sup>3</sup> The present study aimed to develop and validate a simple, accurate, and sensitive UV spectrophotometric method for the quantitative estimation of 5,7-Dihydroxyflavone using methanol as the solvent and 313 nm as the analytical wavelength, following the recommendations of the International Council for Harmonisation (ICH) Q2(R2) guideline.<sup>4</sup>

## 2. Materials and Methods

### Procurement of Phytochemicals and Solvents

Analytical-grade 5,7-Dihydroxyflavone reference standard was procured from Yucca Enterprises, Mumbai, India. All solvents and reagents used for the study were of analytical reagent (AR) grade and were obtained from the Central Store of KLE College of Pharmacy, Belagavi. Methanol was selected as the analytical solvent owing to its excellent solubility for 5,7-Dihydroxyflavone and its ability to produce a stable UV absorption spectrum.

### Instrumentation

UV spectrophotometric analysis was performed using a Shimadzu UV-1800 UV-Visible spectrophotometer equipped with UV-Probe software and matched 1 cm quartz cells.

### Preparation of Standard Solutions

A stock solution of 5,7-Dihydroxyflavone (1000 µg/mL) was prepared by dissolving 10 mg of 5,7-Dihydroxyflavone in methanol and diluting to 10 mL. An intermediate solution (100 µg/mL) was obtained by appropriate dilution with methanol water (80:20, v/v). Working standard solutions in the concentration range of 4–20 µg/mL were prepared by serial dilution.<sup>5-10</sup>

### Method Development

A UV spectrophotometric method was developed for the quantitative estimation of 5,7-Dihydroxyflavone using methanol as the solvent. The absorption spectrum of the standard solution was scanned over 200–400 nm against methanol as the blank. The maximum absorbance ( $\lambda_{\text{max}}$ ) was observed at 313 nm, which was selected as the analytical wavelength for quantification. Calibration standards in the concentration range of 4–20 µg/mL were prepared by appropriate dilution of the stock solution, and their absorbance was measured at 313 nm to construct the calibration curve.<sup>11-16</sup>

### Method Validation

The developed method was validated according to the International Council for Harmonisation (ICH) Q2(R2) guideline to establish its suitability for routine quantitative analysis.<sup>17-20</sup> Validation parameters included specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), and solution stability.<sup>21-24</sup>

**Specificity:** Specificity was assessed by comparing the absorbance spectra of methanol (blank) and the standard solution at 313 nm. No interfering absorbance from the solvent was observed, confirming the specificity of the method.<sup>25-27</sup>

**Linearity:** Linearity was evaluated over the concentration range of 4–20 µg/mL using five calibration levels. The calibration curve was generated by plotting absorbance against concentration, and linear regression analysis was performed to determine the correlation coefficient, slope, and intercept.<sup>28-30</sup>

**Accuracy:** Accuracy was determined by the standard addition method at 80%, 100%, and 120% concentration levels. Each level was analysed in triplicate, and percentage recovery was calculated to assess the accuracy of the method.<sup>17,18</sup>

**Precision:** Repeatability (intra-day precision) was evaluated by analysing six replicates of a 12 µg/mL solution on the same day, whereas intermediate precision (inter-day precision) was assessed over three consecutive days. Precision was expressed as the percentage relative standard deviation (%RSD).<sup>19,20</sup>

**Robustness:** Robustness was examined by introducing small deliberate variations in analytical conditions, including wavelength ( $313 \pm 2$  nm) and solvent composition ( $\pm 2\%$  methanol). The influence of these changes on analytical performance was evaluated using %RSD.<sup>21</sup>

**Ruggedness:** Ruggedness was evaluated by performing the analysis using different analysts and instruments under identical experimental conditions. The consistency of the results confirmed the reproducibility of the method.<sup>22</sup>

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**Limit of Detection and Limit of Quantification:** The sensitivity of the method was determined using the standard deviation of the response ( $\sigma$ ) and the slope (S) of the calibration curve according to the following equations:

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

**Solution Stability:** The stability of the standard solution was evaluated by measuring absorbance at predetermined intervals under recommended storage conditions. The solution was considered stable when no significant change in absorbance was observed during the study period.<sup>25</sup>

### 3. Results and Discussion

#### Determination of $\lambda_{\text{max}}$ of 5,7-Dihydroxyflavone

A standard solution of 5,7-Dihydroxyflavone (4  $\mu\text{g/mL}$ ) was scanned over the wavelength range of 200–400 nm using a UV–Visible spectrophotometer. The absorption spectrum exhibited a distinct maximum absorbance ( $\lambda_{\text{max}}$ ) at 313 nm, which was selected as the analytical wavelength for quantitative estimation. The corresponding UV absorption spectrum is shown in Figure 1. The optimized analytical conditions established for the developed UV spectrophotometric method are summarized in Table 1.

| Sr. No. | Parameter                                        | Specification                |
|---------|--------------------------------------------------|------------------------------|
| 1       | Method                                           | UV Spectrophotometric Method |
| 2       | Instrument                                       | UV–Visible Spectrophotometer |
| 3       | Model                                            | Shimadzu UV-1800             |
| 4       | Software                                         | UV-Probe                     |
| 5       | Analyte                                          | 5,7-Dihydroxyflavone         |
| 6       | Scanning Range                                   | 200–400 nm                   |
| 7       | Solvent                                          | Methanol                     |
| 8       | Analytical Wavelength ( $\lambda_{\text{max}}$ ) |                              |

Table 1. Optimized Parameters of the Developed UV Spectrophotometric Method

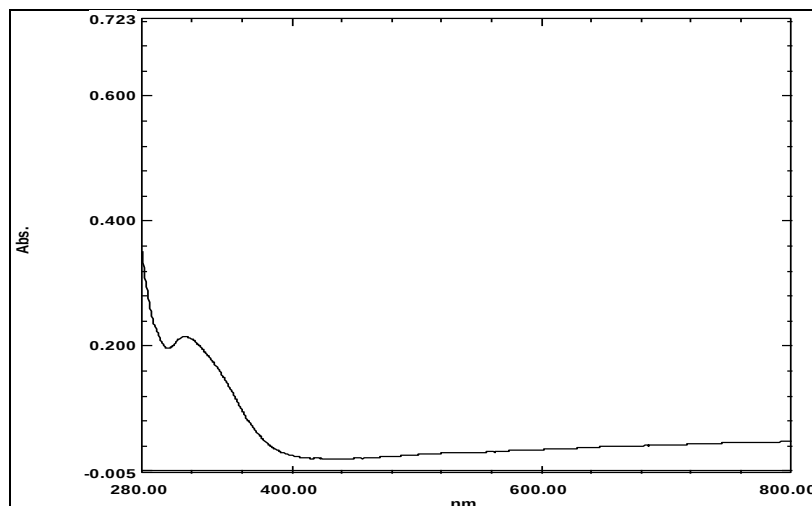


Figure 1: UV Spectrum of 5,7-Dihydroxyflavone showing  $\lambda_{\text{max}}$  at 313nm

#### Validation of the Developed UV Spectrophotometric Method

The developed method was validated according to the ICH Q2(R2) guideline to evaluate its analytical performance. The validation included specificity, linearity and range, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), and solution stability. The obtained results demonstrated that the proposed method is suitable for routine quantitative estimation of 5,7-Dihydroxyflavone.

#### Specificity

Specificity was assessed by recording the UV spectrum of the blank solvent over 200–400 nm. No measurable absorbance was observed at the analytical wavelength of 313 nm, indicating the absence of solvent interference. The standard solution exhibited a sharp absorption peak at 313 nm, confirming that the analytical response was solely due to 5,7-Dihydroxyflavone. The UV spectra of the blank solvent and standard solution are shown in Figure 2 and Figure 3, respectively.

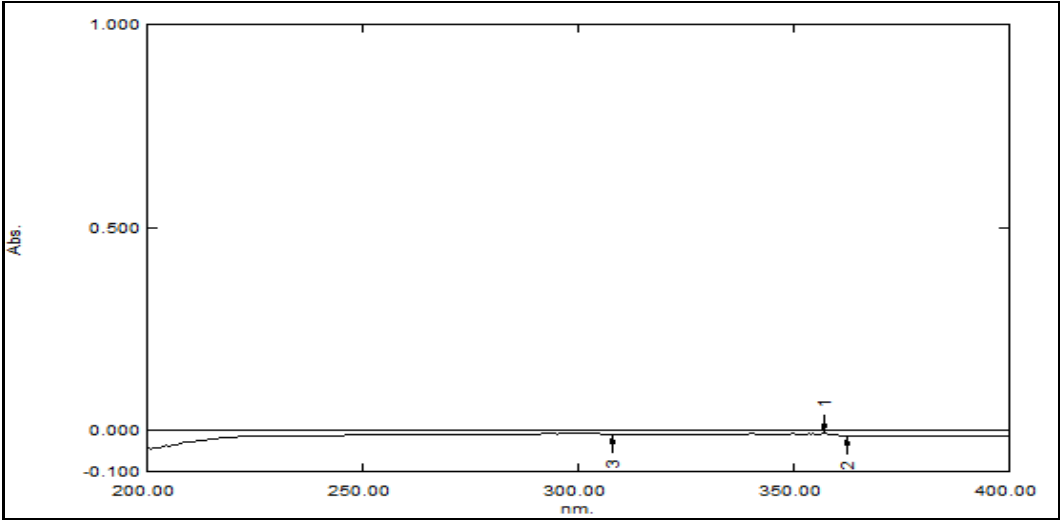


Figure 2: UV Spectrum of Methanol Water

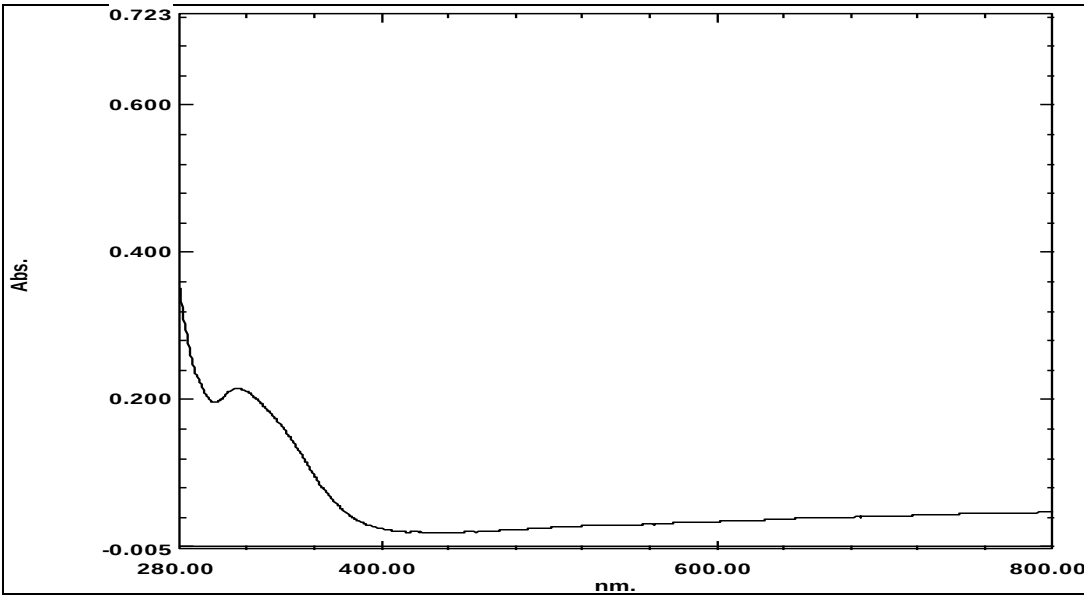


Figure 3: UV Spectrum of 5,7-Dihydroxyflavone

Linearity and Range

Linearity was evaluated over the concentration range of 4–20 µg/mL. A calibration curve was constructed by plotting absorbance against concentration. The method demonstrated excellent linearity with a regression coefficient ( $r^2 = 0.9992$ ), indicating a strong linear relationship between concentration and absorbance. The linearity data are summarized in Table 2, while the overlay spectra and calibration curve are presented in Figure 4 and Figure 5, respectively.

| Concentration (µg/mL)             | Absorbance       |
|-----------------------------------|------------------|
| 4                                 | 0.214            |
| 8                                 | 0.422            |
| 12                                | 0.659            |
| 16                                | 0.823            |
| 20                                | 1.095            |
| Regression equation               | $y = 0.054x + c$ |
| Slope                             | 0.054            |
| Correlation coefficient ( $r^2$ ) | 0.9992           |

Table 2. Linearity Data of 5,7-Dihydroxyflavone

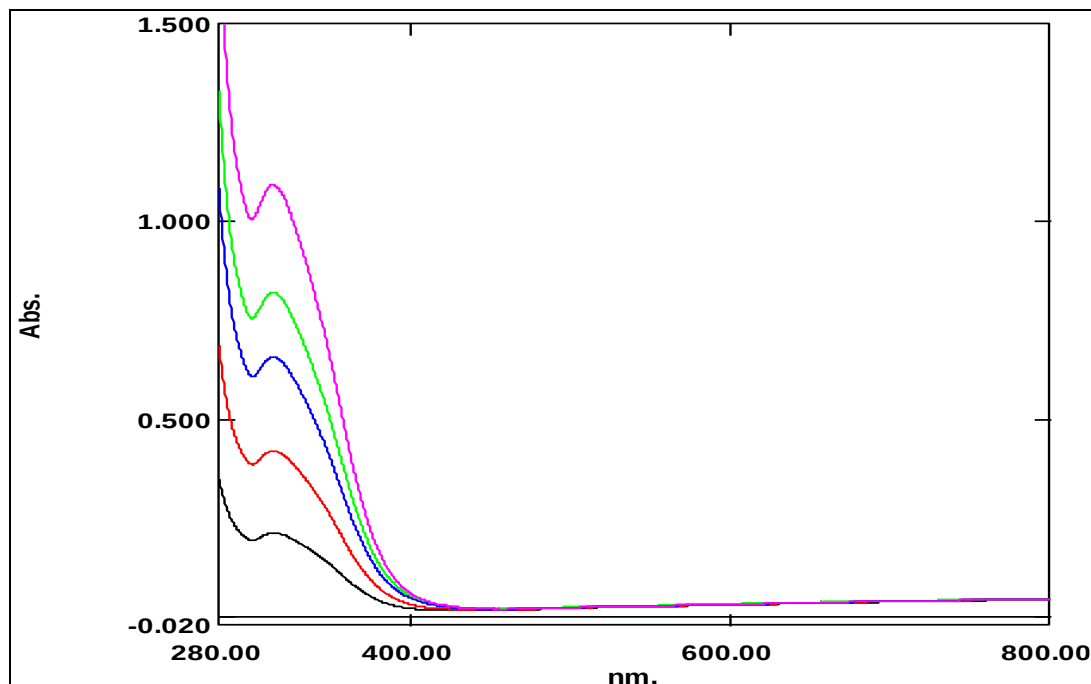


Figure 4: Overlay UV Spectrum of 5,7-Dihydroxyflavone

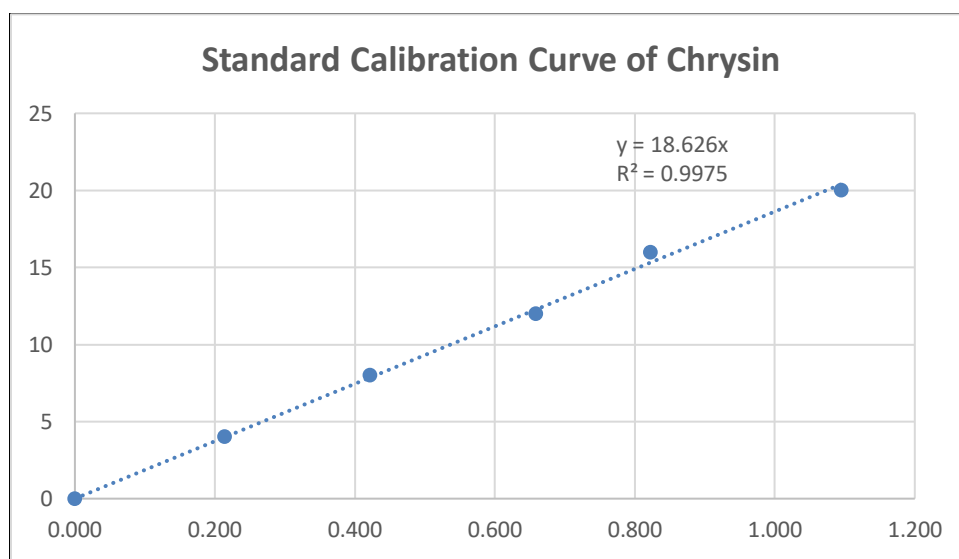


Figure 5: Standard Calibration Curve of 5,7-Dihydroxyflavone

### Accuracy

The accuracy of the developed method was evaluated by the standard addition technique at 80%, 100%, and 120% concentration levels. The percentage recovery values were found to be within the acceptable limits of 98–102%, with low %RSD values, demonstrating excellent accuracy and the absence of matrix interference. The recovery results are summarized in Table 3.

| Recovery Level (%) | Amount Added (µg/mL) | Amount Recovered (µg/mL) | % Recovery | % RSD |
|--------------------|----------------------|--------------------------|------------|-------|
| 80                 | 8                    | 7.95 ± 0.08              | 99.38      | 1.01  |
| 100                | 10                   | 10.02 ± 0.07             | 100.20     | 0.72  |
| 120                | 12                   | 11.96 ± 0.09             | 99.67      | 0.78  |

Table 3. Accuracy (Recovery) Results

### Precision

#### Repeatability (Intra-day Precision)

Repeatability was assessed by analysing six replicate measurements of a 12 µg/mL standard solution under identical

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conditions. The method exhibited excellent repeatability with %RSD below 2%, indicating satisfactory precision.

### Intermediate Precision (Inter-day Precision)

Intermediate precision was evaluated on three consecutive days. The obtained %RSD values were also below 2%, confirming consistent analytical performance over time. The precision results are summarized in Table 4.

| Replicate     | Concentration ( $\mu\text{g/mL}$ ) | Absorbance          |
|---------------|------------------------------------|---------------------|
| 1             | 12                                 | 0.658               |
| 2             | 12                                 | 0.661               |
| 3             | 12                                 | 0.660               |
| 4             | 12                                 | 0.657               |
| 5             | 12                                 | 0.662               |
| 6             | 12                                 | 0.659               |
| Mean $\pm$ SD |                                    | $0.6595 \pm 0.0019$ |
| %RSD          |                                    | 0.29                |

Table 4A. Repeatability (Intra-day Precision) Study of the Developed UV Spectrophotometric Method

| Day   | Mean Absorbance $\pm$ SD | %RSD |
|-------|--------------------------|------|
| Day 1 | $0.659 \pm 0.002$        | 0.30 |
| Day 2 | $0.661 \pm 0.003$        | 0.45 |
| Day 3 | $0.658 \pm 0.002$        | 0.34 |

Table 4B. Intermediate Precision (Inter-day Precision) Study of the Developed UV Spectrophotometric Method

### Robustness

Robustness was investigated by introducing small deliberate variations in analytical wavelength ( $313 \pm 2$  nm) and solvent composition. These changes did not significantly affect the analytical response, and the %RSD values remained below 2%, confirming the robustness of the developed method. The robustness results are presented in Table 5.

| Parameter Varied      | Condition       | Mean Absorbance $\pm$ SD | %RSD |
|-----------------------|-----------------|--------------------------|------|
| Analytical wavelength | 311 nm          | $0.656 \pm 0.003$        | 0.46 |
|                       | 313 nm          | $0.659 \pm 0.002$        | 0.30 |
|                       | 315 nm          | $0.655 \pm 0.003$        | 0.49 |
| Solvent composition   | Methanol (98%)  | $0.658 \pm 0.002$        | 0.35 |
|                       | Methanol (100%) | $0.659 \pm 0.002$        | 0.30 |
|                       | Methanol (102%) | $0.660 \pm 0.003$        | 0.42 |

Table 5. Robustness Study of the Developed Method

### Ruggedness

Ruggedness was evaluated by performing the analysis using different analysts and instruments under identical experimental conditions. Comparable assay results with %RSD below 2% demonstrated excellent reproducibility of the method. The ruggedness data are summarized in Table 6.

| Analyst/Instrument               | Mean Absorbance $\pm$ SD | %RSD |
|----------------------------------|--------------------------|------|
| Analyst I                        | $0.659 \pm 0.002$        | 0.30 |
| Analyst II                       | $0.661 \pm 0.003$        | 0.45 |
| Instrument I (Shimadzu UV-1800)  | $0.659 \pm 0.002$        | 0.30 |
| Instrument II (Shimadzu UV-1800) | $0.660 \pm 0.003$        | 0.41 |

Table 6. Ruggedness Study of the Developed Method

### Limit of Detection and Limit of Quantification

The calculated LOD and LOQ were  $1.37 \mu\text{g/mL}$  and  $3.29 \mu\text{g/mL}$ , respectively, demonstrating that the developed method possesses adequate sensitivity for routine quantitative determination of 5,7-Dihydroxyflavone. The calculated sensitivity parameters are summarized in Table 7.

| Parameter                         | Result |
|-----------------------------------|--------|
| Slope                             | 0.054  |
| Correlation coefficient ( $r^2$ ) | 0.9992 |
| LOD ( $\mu\text{g/mL}$ )          | 1.37   |
| LOQ ( $\mu\text{g/mL}$ )          | 3.29   |

Table 7. Sensitivity Parameters

## Solution Stability

The stability of the standard solution was evaluated by measuring absorbance at predetermined time intervals under recommended storage conditions. No significant change in absorbance was observed throughout the study period, indicating that the prepared standard solution remained stable during the analytical procedure. The stability results are summarized in Table 8.

| Time    | Absorbance | % Assay | % Change |
|---------|------------|---------|----------|
| Initial | 0.659      | 100.00  | —        |
| 6 h     | 0.658      | 99.85   | 0.15     |
| 12 h    | 0.657      | 99.70   | 0.30     |
| 24 h    | 0.656      | 99.54   | 0.46     |
| 48 h    | 0.654      | 99.24   | 0.76     |

Table 8. Solution Stability of Standard 5,7-Dihydroxyflavone Solution

## 4. Conclusion

A simple, rapid, and validated UV spectrophotometric method was successfully developed for the quantitative estimation of 5,7-Dihydroxyflavone using methanol as the solvent and an analytical wavelength of 313 nm. The method demonstrated excellent linearity (4–20 µg/mL;  $r^2 = 0.9992$ ), satisfactory sensitivity (LOD: 1.37 µg/mL; LOQ: 3.29 µg/mL), and acceptable accuracy, precision, robustness, and ruggedness in accordance with ICH Q2(R2) guidelines. The absence of solvent interference confirmed its specificity. Owing to its simplicity, reliability, and cost-effectiveness, the proposed method is well suited for routine quality control, quantitative analysis, and future pharmaceutical and phytochemical investigations involving 5,7-Dihydroxyflavone.

## Use of Artificial Intelligence

No artificial intelligence (AI) or machine learning tools were used for the generation, interpretation, or analysis of the experimental data presented in this study. The manuscript was prepared, critically reviewed, and finalized entirely by the authors, who take full responsibility for the accuracy, originality, and integrity of the work.

## Declarations

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and materials:** All data generated and analyzed during this study are included in this article. Additional information may be obtained from the corresponding author upon reasonable request.

**Competing interests:** The authors declare that they have no competing financial or non-financial interests.

**Funding:** The authors received no external financial support for this research.

## Authors' Contributions

Shailendra Sanjay Suryawanshi conceived the study, designed the analytical methodology, supervised the research, interpreted the results, and finalized the manuscript. Samarth Ugare carried out the experimental work, collected and compiled the analytical data, and prepared the initial manuscript draft. Ronak Dsouza and Adarsh Kamate assisted in experimental investigations, literature review, and data compilation. Arif Salim Jamadar contributed to laboratory work, data verification, and manuscript revision. All authors read and approved the final version of the manuscript.

## Acknowledgements

The authors sincerely thank Dr. Sunil S. Jalalpure, Principal, KLE College of Pharmacy, Belagavi, for his continuous encouragement and institutional support throughout this work.

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