



Research Article

Validated UV-vis spectrophotometric method for quantification of 3,4-Methylenedioxychalcone in bulk compound form

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Received: 31-01-2026

Accepted: 21-09-2026

Published: 30-09-2026

Abstract

Background: This study reports the method development and validation of a UV-Vis spectrophotometric method for quantifying the research compound 3,4-methylenedioxychalcone. Chalcones, a class of flavonoid derivatives, are known for their broad pharmacological potential and reliable quantification is crucial for further pharmacological investigations, purity assessment and formulation development. However, no validated UV-Vis spectrophotometric method has been reported for the estimation of 3,4-methylenedioxychalcone. **Methods:** The chosen solvent was ethanol due to its stable baseline and reproducible absorbance profile. The compound exhibited a sharp absorption maximum at 353 nm. Calibration curves created over the range of 2–12 µg/mL followed Beer's law with excellent linearity ($R^2 = 0.9974$). Validation was performed in compliance with ICH Q2(R1) recommendations, covering linearity, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ). **Results:** Sensitivity studies established the LOD at 1.06 µg/mL and the LOQ at 3.23 µg/mL. The developed method demonstrated excellent linearity, precision and reproducibility, with all validation parameters meeting the acceptance criteria specified in ICH guidelines. The method was found to be reliable for quantitative estimation of 3,4-methylenedioxychalcone under the experimental conditions employed. **Conclusion:** The developed method is precise, economical and suitable for laboratory estimation of 3,4-methylenedioxychalcone during preclinical research. Furthermore, the validated method may serve as a useful analytical tool for purity assessment, stability studies and future formulation development involving this synthetic chalcone derivative.

Keywords: 3,4 Methylenedioxychalcone, UV-Vis spectrophotometry, Method validation, Phytoconstituent analysis, Chalcone derivative, Flavonoids

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Website:
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DOI: <https://doi.org/10.47552/ijam.v17i2.6997>

Introduction

Chalcones represent an important class of naturally occurring compounds that serve as open-chain precursors of flavonoids(1). They are widely distributed in plants and have been extensively investigated because of various pharmacological activities, like anti-inflammatory, anticancer, antimicrobial and antioxidant activities(2,3). The structural framework of chalcones, distinguished by an aromatic ring-conjugated α,β -unsaturated carbonyl system, imparts strong chromophoric features(4). The specific structure of 3,4-methylenedioxychalcone is shown in Figure 1. These conjugated systems allow chalcones to absorb

light in the ultraviolet and visible regions, making them suitable candidates for spectrophotometric analysis. Among the various chalcones, 3,4-methylenedioxychalcone is a synthetic chalcone that has attracted considerable interest due to its promising therapeutic potential. It has been investigated as a lead molecule in medicinal chemistry, with report highlighting its cytotoxic, chemopreventive and antioxidant properties. Accurate quantification of this compound is therefore essential for ensuring accurate quantitative estimation during preclinical pharmaceutical research, purity assessment, stability studies and formulation development. Reliable analytical methods are required to establish its purity, stability and concentration during preclinical pharmaceutical research and experimental formulation development.

Spectrophotometric techniques, particularly those based on UV-visible absorption, remain highly relevant in pharmaceutical analysis(5,6). Compared to chromatographic methods such as HPLC, UV-Vis spectrophotometry is simpler, faster and more

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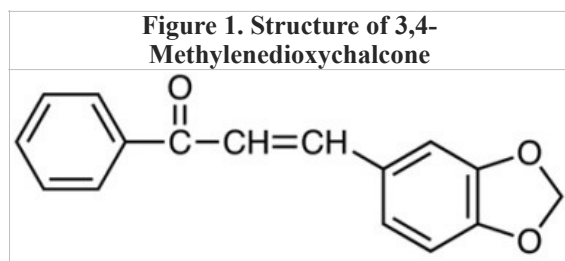
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economical(7). It requires minimal sample preparation and can be performed in laboratories with limited resources, enabling a broad spectrum of researchers and industries to access it(8). Furthermore, the present study focused on the quantitative determination of the pure compound rather than the analysis of complex biological samples or finished pharmaceutical formulations. Therefore, UV-Visible spectrophotometry was selected as an appropriate analytical technique. Compared with HPLC, this method offers a simple, rapid, cost-effective and readily accessible approach for laboratory based analytical applications during the early stages of pharmaceutical research. The technique is based on the absorption of ultraviolet and visible light by molecular chromophores, enabling reliable quantitative analysis of compounds with suitable absorption characteristics.

Chalcones, with their conjugated double bonds and aromatic rings, exhibit strong $\pi \rightarrow \pi^*$ transitions that enable sensitive detection and quantification(9). Although several analytical methods have been described for various chalcone derivatives, no validated UV-Visible spectrophotometric method has been reported for the quantitative determination of 3,4-methylenedioxychalcone. The availability of such a method is important for compound characterization, purity evaluation, stability assessment, formulation development and preclinical pharmacological research. Accordingly, the present study was designed to develop and validate a simple, rapid, and cost-effective UV-Visible spectrophotometric method in accordance with ICH Q2(R1) guidelines. While UV-Visible spectrophotometry is well suited for the analysis of the pure research compound, HPLC may be considered in future investigations involving biological matrices or finished pharmaceutical formulations where greater analytical specificity is required. In the present study, ethanol was selected as the solvent system after preliminary screening, as it provided a stable baseline and reproducible absorbance values for 3,4-methylenedioxychalcone. A double-beam UV-visible spectrophotometer was employed to record absorbance values and the compound exhibited a distinct absorption maximum (λ_{max}) at 353 nm. Calibration curves constructed between 2 and 12 $\mu\text{g/ml}$ of concentration, obeyed Beer's law with excellent linearity.

Validation was carried out in accordance with following instructions from the International Conference on Harmonization (ICH Q2(R1), guidelines, encasing aspects like linearity, precision, robustness, ruggedness, LOD and LOQ. To the best of our literature survey, no validated UV-Visible spectrophotometric method specifically developed for quantitative estimation of 3,4-methylenedioxychalcone following ICH Q2(R1) guidelines has been reported. This study therefore provides a validated analytical method suitable for laboratory estimation of this research compound. This work's objective is to create a validated UV-Vis spectrophotometric method that is straightforward, reliable and economical, thereby offering a practical analytical tool for laboratory estimation of 3,4-methylenedioxychalcone during preclinical pharmaceutical research, purity assessment, stability studies and experimental formulation development.



Materials and Methods

Materials

The compound 3,4-methylenedioxychalcone was procured from Tokyo Chemical Industries, Japan. Every chemical and reagent utilized in the research had an analytical quality to ensure accuracy and reproducibility. Spectral measurements were carried out using a Shimadzu 1800 double beam UV-Vis (Japan) with 10 mm quartz cuvettes, which were selected for their affordability and ease of handling(10).

Preparation of standard solutions

A precisely weighed quantity of 10 mg of 3,4-methylenedioxychalcone was put into a 10 ml capacity volumetric flask and dissolved in ethanol. The solution was sonicated for 5 minutes in a bath sonicator to achieve complete solubilization, resulting in a 1000 $\mu\text{g/ml}$ primary stock solution.

A secondary stock solution was prepared, 1 ml of the primary is diluted to 10 ml with ethanol, followed by sonication for 5 minutes. This yielded 10 $\mu\text{g/ml}$ of solution.

Determination of maximum wavelength (λ_{max})

To determine the absorption maximum, Ethanol was added to 1 ml of the secondary stock solution to dilute it to 10 ml, producing a solution of 10 $\mu\text{g/ml}$. The range of the scan was 200–400 nm against ethanol as a blank. The compound showed a clear peak of absorption at 353, which was selected for all subsequent analyses.

Construction of calibration curve

Calibration standards were prepared at concentration from 2-12 $\mu\text{g/ml}$ by appropriate dilution of the secondary stock solution. Absorbance values were recorded at 353 nm and concentration was plotted on the x-axis and absorbance on the y-axis of a calibration curve. The curve was used to evaluate linearity and establish the regression equation.

Analytical method validation

As per the ICH, validation is defined as the documented process that provides a high level of assurance that an analytical procedure consistently produces results meeting predetermined requirements and quality standards(11). In this study, the developed UV-Vis spectrophotometric method was validated by assessing several critical parameters.

Linearity and range

Linearity was examined to confirm the direct relationship between analyte concentration and absorbance. Standard solutions of 3,4-methylenedioxychalcone were prepared between 2 and 12 $\mu\text{g/ml}$ using the primary stock solution. The calibration curve demonstrated outstanding linearity and correlation coefficients exceeding 0.99, thereby meeting the acceptance criteria outlined in ICH guidelines(12).

Precision

Precision was evaluated to identify the repeatability and reliability of the method. Both intraday and interday variations were studied(13). For intraday precision, samples at levels of concentration 4 $\mu\text{g/ml}$, 8 $\mu\text{g/ml}$ and 12 $\mu\text{g/ml}$ were analyzed in triplicate at different times of the day (morning, afternoon and evening). Interday precision was assessed by analyzing the same concentrations in triplicate on three consecutive days. Mean absorbance values, percent relative standard deviation (%RSD)

were calculated. In all cases, %RSD values were within the acceptable limit of 2%, confirming the reliability of the method.

Robustness

Robustness was determined by deliberately varying the detection wavelength. Samples at 4, 8 and 12 µg/ml were analyzed at three different wavelengths (351, 353 and 355 nm). The

%RSD values obtained were consistently below 2%, indicating that minor changes in wavelength did not significantly affect the accuracy of the results. This demonstrates that the method is stable under small variations in experimental conditions(14).

Ruggedness

Ruggedness was assessed by performing the assay with different analysts while maintaining identical instrumental conditions. The absorbance values recorded for low, medium and high concentrations showed minimal variation, with %RSD values well within the acceptable range. These findings confirm that the method is reproducible and not dependent on the operator(15).

LOD and LOQ

The method's sensitivity was established by calculating the LOD and LOQ. The lowest analyte concentration that can be detected but may not be measured is represented by the LOD, whereas the LOQ corresponds to concentration that can be found via reasonable precision and accuracy. These parameters ensure that The technique can identify and measure 3,4-methylenedioxychalcone even at low levels(16,17).

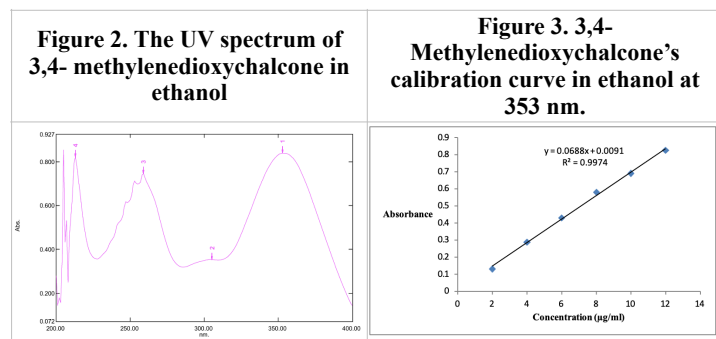
Result and Discussion

Initial The developed UV-Vis spectrophotometric method turned out to be straight forward, reliable and cost effective in order to quantitative estimate 3,4-methylenedioxychalcone. The experimental data demonstrated excellent precision, with RSD values consistently less than 2% for both intraday and interday studies. Such low variability highlights the reproducibility of the method and confirms its robustness under laboratory conditions.

The procedure's sensitivity was further established through the calculation of the LOQ and LOD. The LOQ was found to be 3.23 µg/ml, while the LOD was determined to be 1.06 µg/ml, indicating that the method is capable of detecting and quantifying even small amounts of the analyte with acceptable accuracy and precision.

Determination of Maximum Wavelength

To identify the absorption maximum, a solution of 10 µg/ml was examined across the UV range. The compound exhibited its highest absorbance at 353 nm, which was chosen as the analytical wavelength for every measurement that followed. This distinct λ_{max} ensured reliable detection and quantification of 3,4-methylenedioxychalcone as shown in figure 2.



Standard Calibration Curve

The Using a correlation coefficient of 0.9974, the curve of calibration for 3,4-methylenedioxychalcone showed outstanding linearity throughout the investigated concentration range, confirming adherence to Beer's law. The linear connection between absorption and concentration is illustrated in Figure 3.

Validation of analytical methods

The new UV-Vis spectrophotometric method was tested in compliance with the ICH Q2(R1) guidelines to validation to make sure it is accurate and reliable. The parameters assessed included precision, ruggedness, robustness, LOD and LOQ(18).

Linearity and Range

Linearity was evaluated to confirm the proportional relationship between analyte concentration and absorbance(19). Standard solutions of 3,4-methylenedioxychalcone were analyzed across between 2 to 12 µg/ml concentration. The calibration curve showed excellent linearity, with a correlation coefficient (R^2) of 0.9974 (Table 1). This strong correlation demonstrates that the method follows Beer's law within the specified range, ensuring accurate quantification across different concentration levels.

Table 1: Linearity table of 3,4-Methylenedioxychalcone

Sr. No.	Concentration	Absorbance (µg/ml)	$y = 0.0688x + 0.0091$ $R^2 = 0.9974$
1	2	0.132	
2	4	0.288	
3	6	0.431	
4	8	0.579	
5	10	0.689	
6	12	0.825	

Precision

Intraday and interday studies showed no significant variation, confirming the reliability of the method. Results for 4, 8 and 12 µg/ml samples are summarized in Table 2 and Table 3, with %RSD values consistently below 2%, demonstrating reproducibility and consistency of the proposed spectrophotometric procedure.

Table 2: Intraday Precision

Concentration (µg/ml)	Absorbance		
	Morning	Afternoon	Evening
4	0.289	0.298	0.290
4	0.289	0.298	0.291
4	0.288	0.299	0.290
%RSD	0.200	0.194	0.199
8	0.600	0.601	0.596
8	0.600	0.602	0.593
8	0.601	0.602	0.594
%RSD	0.096	0.096	0.257
12	0.814	0.818	0.810
12	0.811	0.819	0.808
12	0.813	0.818	0.807
%RSD	0.188	0.071	0.189

Table 3: Interday Precision

Concentration (µg/ml)	Absorbance		
	Day 1	Day 2	Day 3
4	0.273	0.279	0.285
4	0.275	0.278	0.286
4	0.273	0.279	0.287
%RSD	0.422	0.207	0.350
8	0.591	0.587	0.595
8	0.590	0.585	0.594
8	0.594	0.586	0.595
%RSD	0.352	0.171	0.097
12	0.806	0.799	0.808
12	0.805	0.800	0.808
12	0.804	0.801	0.807
%RSD	0.124	0.125	0.071

Ruggedness

Ruggedness was assessed by conducting the test in the same circumstances on various days with multiple analysts(20). Despite variations in personnel and timing, the results remained consistent, confirming the method's reliability and reproducibility is shown in table 4.

Table 4. Ruggedness

Concentration (µg/ml)	Analyst 1	Analyst 2
4	0.283	0.288
4	0.283	0.289
4	0.285	0.289
Mean	0.284	0.289
SD	0.001	0.001
%RSD	0.407	0.200
8	0.597	0.606
8	0.595	0.605
8	0.596	0.605
Mean	0.596	0.605
SD	0.001	0.001
%RSD	0.168	0.095
12	0.814	0.81
12	0.812	0.811
12	0.811	0.812
Mean	0.812	0.811
SD	0.002	0.001
%RSD	0.188	0.123

Robustness

Robustness was evaluated by performing the assay at different wavelengths (351, 353 and 355 nm). Across all tested concentrations, the %RSD values remained below 2%, confirming that minor variations in wavelength did not affect the accuracy of the method. These findings, summarized in Table 5, demonstrate the stability and reliability of the procedure under slight experimental changes.

Table 5. Robustness

Concentration (µg/ml)	Wavelength 351	Wavelength 353	Wavelength 355
4	0.280	0.282	0.284
4	0.279	0.282	0.284
4	0.279	0.281	0.283
Mean	0.279	0.282	0.284
SD	0.001	0.001	0.001
%RSD	0.207	0.205	0.204
8	0.608	0.614	0.618
8	0.609	0.615	0.617
8	0.610	0.616	0.619
Mean	0.609	0.615	0.618
SD	0.001	0.001	0.001
%RSD	0.164	0.163	0.162
12	0.827	0.835	0.841
12	0.829	0.835	0.839
12	0.829	0.834	0.839
Mean	0.828	0.835	0.840
SD	0.001	0.001	0.001
%RSD	0.139	0.069	0.138

LOD AND LOQ

LOD and LOQ values were determined to be 1.06 µg/ml and 3.23 µg/ml, respectively as demonstrated in Table 6.

Table 6: Optical characteristics

Parameters	Result
λ_{max}	353nm
Linearity range	2-12 µg/ml
Correlation coefficient (R ²)	0.9974
Regression equation	0.0688x + 0.0091
Slope	0.0688
Intercept	0.0091
LOD	1.06 µg/ml
LOQ	3.23 µg/ml

3,4-Methylenedioxychalcone at 353 nm was selected as the target phytoconstituent and the analytical method was developed in compliance with ICH requirements for linearity, precision, robustness, ruggedness and sensitivity parameters (LOD and LOQ). Quantitative evaluation was carried out at three concentration 4 µg/ml, 8 µg/ml and 12 µg/ml. The compound exhibited compliance with Beer's law across the tested range between 2 to 12 µg/ml, with triplicate measurements confirming accuracy. The equation $y = 0.0688x + 0.0091$ was generated by linear regression analysis and R² of 0.9974 (Figure 1), demonstrating excellent linearity.

Precision was assessed using %RSD values derived from replicate assays at the three concentration levels, all of which were below 1.5%, confirming the consistency of the method. Sensitivity was established with a LOD of 1.06 µg/ml and a LOQ of 3.23 µg/ml, indicating the method's ability to reliably identify and quantify the analyte at even low quantities. Overall, the validated procedure provides a simple, precise and reproducible approach for quantitative estimation of the research compound 3,4-methylenedioxychalcone during preclinical pharmaceutical research, purity assessment and formulation development.

Conclusion

The proposed UV-Vis spectrophotometric method offers a simple, rapid and cost effective method for the analysis of 3,4-methylenedioxychalcone. Validation studies confirmed excellent accuracy, precision and linearity, with all parameters meeting the acceptance criteria outlined in ICH guidelines. The method therefore offers a reliable and reproducible tool for laboratory estimation of 3,4-methylenedioxychalcone during preclinical pharmaceutical research. It may also be used for purity assessment, stability studies and experimental formulation development involving this synthetic chalcone derivative.

Conflict of Interest

The authors declare no conflict of interests for this manuscript.

Acknowledgement

The authors are grateful to KLE College of Pharmacy, Belagavi for kindly providing the facilities needed to conduct this study

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