RESEARCH PAPER

Development And Validation Of A Uv–Visible Spectrophotometric Method Using Simultaneous Equation Analysis For Standardization Of Curcumin And Quercetin In Herbal Formulations

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ABSTRACT

Background: Standardization of herbal formulations remains a major analytical challenge due to the presence of multiple bioactive phytoconstituents with overlapping spectral characteristics. Curcumin and quercetin are widely recognized marker compounds with established therapeutic relevance; however, routine quality control methods for their simultaneous estimation are often limited by cost, complexity, and accessibility.

Methods: UV–Visible spectrophotometric method based on simultaneous equation analysis was developed and validated for the concurrent quantification of curcumin and quercetin in herbal formulations. The method utilized methanol as solvent, with analytical wavelengths selected at 425 nm and 370 nm. Validation was performed in accordance with ICH Q2(R1) guidelines, assessing linearity, accuracy, precision, sensitivity, robustness, and applicability to commercial herbal products.

Results: The method demonstrated excellent linearity with correlation coefficients exceeding 0.999 for both analytes. Accuracy studies yielded recovery values within 98–102%, while precision analysis showed %RSD values below 2%. Low limits of detection and quantification confirmed adequate sensitivity. Assay results of marketed herbal formulations were within acceptable pharmacopeial limits, indicating suitability for routine quality control.

Conclusion: The validated method provided a rapid, economical, and reliable analytical tool for simultaneous standardization of curcumin and quercetin in herbal formulations, supporting improved quality assurance and regulatory compliance.

Keywords: Curcumin; Quercetin; UV–Visible spectrophotometry; Simultaneous equation method; Herbal standardization; Method validation

How to cite this article: Landge Dhananjay Ashok, Pradyumn Tiwari, Prasanth S S, Shainda Laeeq, Aparna Dwivedi, Shiv Kumar Gupta, Debasish Maity, Piyali Khamkat | Development And Validation Of A Uv–Visible Spectrophotometric Method Using Simultaneous Equation Analysis For Standardization Of Curcumin And Quercetin In Herbal Formulations | Int J Drug Deliv Technol. 2026;16(4s): 93-101, DOI: 10.25258/ijddt.16.93-101

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

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Herbal medicines continue to play a significant role in the prevention and management of chronic inflammatory, metabolic, and degenerative disorders. Their widespread acceptance is largely attributed to long-standing traditional use, perceived safety, and the presence of bioactive phytochemicals with multifunctional therapeutic properties. Among these, curcumin, a polyphenolic compound derived from *Curcuma longa* L., and quercetin, a flavonol abundantly present in several medicinal plants including *Allium cepa* L., have received considerable scientific attention due to their potent antioxidant, anti-inflammatory, and chemopreventive activities. These compounds are frequently incorporated as key constituents or marker compounds in a wide range of polyherbal formulations (Balkrishna et al., 2022; Haller, 2024; Hameed et al., 2024; Joshi et al., 2024).

Despite their therapeutic potential, the clinical effectiveness and reproducibility of herbal formulations are often compromised by inadequate standardization. Variability in phytochemical content may arise from differences in plant source, harvesting conditions, extraction methods, and formulation processes. Such inconsistencies pose challenges for quality control, regulatory approval, and clinical translation. Conventional analytical techniques such as high-performance liquid chromatography offer high specificity and sensitivity but are often associated with high operational costs, extended analysis time, and the requirement for sophisticated instrumentation and skilled personnel. These limitations restrict their routine application, particularly in small-scale manufacturing and quality control settings (Brahma et al., 2024; Jain et al., 2024; Thompson et al., 2023; Wang et al., 2023).

UV–Visible spectrophotometry represents a practical alternative for routine analysis due to its simplicity, cost-effectiveness, and minimal sample preparation requirements. However, direct UV analysis of polyherbal formulations containing multiple chromophoric compounds is often hindered by spectral overlap, leading to inaccurate quantification when single-wavelength methods are employed. Mathematical approaches such as simultaneous equation analysis provide an effective means to overcome this limitation by enabling concurrent estimation of multiple analytes without prior separation (Brahma et al., 2024; Jain et al., 2024; Thompson et al., 2023; Wang et al., 2023). In this context, the present study was undertaken to develop and validate a UV–Visible spectrophotometric method based on simultaneous equation analysis for the standardization of curcumin and quercetin in herbal formulations. The objective was to establish a reliable, validated, and economically viable analytical method that could support routine quality control, enhance batch-to-batch consistency, and contribute to improved regulatory compliance of herbal medicinal products.

MATERIAL AND METHODS

Chemicals and Reagents

Curcumin (≥98% purity) and quercetin (≥98% purity) reference standards were procured from Sigma-Aldrich (USA). Analytical-grade methanol, ethanol, and hydrochloric acid were obtained from Merck (India). Double-distilled water was used throughout the study. All reagents employed were of analytical reagent grade and were used without further purification. Commercial herbal formulations claiming the presence of *Curcuma longa* L. rhizome extract and *Allium cepa* L. flavonoid-rich fractions were procured from the local pharmaceutical market and stored under controlled conditions until analysis.

Instrumentation

UV–Visible spectrophotometric analysis was performed using a Shimadzu UV-1800 double-beam spectrophotometer equipped with UV Probe software (version 2.42), employing matched 1 cm quartz cells. The instrument wavelength accuracy (±0.3 nm) and photometric accuracy (±0.002 Abs) were verified prior to analysis using standard potassium dichromate solutions as per pharmacopeial recommendations. Analytical weighing was carried out using a Shimadzu AUX-220 semi-micro balance with a sensitivity of 0.01 mg.

Preparation of Standard Stock Solutions

Primary stock solutions of curcumin and quercetin were prepared separately by accurately weighing 10 mg of each reference standard and dissolving in methanol to obtain a concentration of 1000 µg/mL. These solutions were sonicated for 10 minutes to ensure complete dissolution and were stored in amber-colored volumetric flasks to prevent photodegradation. Working standard solutions were freshly prepared by appropriate dilution of the stock solutions with methanol to achieve concentrations within the linearity range of the method.

Selection of Analytical Wavelengths

Individual UV absorption spectra of curcumin and quercetin were recorded over the wavelength range of 200–500 nm against methanol as blank. Curcumin exhibited a characteristic absorption maximum (λmax) at 425 nm, attributed to its conjugated diketone chromophore, while quercetin showed a prominent absorption maximum at 370 nm corresponding to its flavonol backbone. These wavelengths were selected for simultaneous equation analysis due to minimal spectral interference and adequate absorptivity differences.

Construction of Calibration Curves

Calibration curves for curcumin and quercetin were constructed by analyzing a series of standard solutions in

the concentration range of 2–20 µg/mL for curcumin and 1–15 µg/mL for quercetin. Absorbance measurements were recorded at both selected wavelengths (425 nm and 370 nm). Each concentration level was analyzed in triplicate, and mean absorbance values were used to construct calibration plots of absorbance versus concentration. Regression equations and correlation coefficients were calculated using least-squares linear regression analysis.

Development of Simultaneous Equation Method

The simultaneous equation method was developed based on the Beer–Lambert law, utilizing the absorptivity coefficients of curcumin and quercetin at both selected wavelengths. Specific absorptivity values ($A^{1\%}_{1cm}$) for each analyte were determined experimentally. Two linear equations were generated using absorbance values at 425 nm and 370 nm, allowing the simultaneous estimation of curcumin and quercetin in combined mixtures and herbal formulations. The equations were solved mathematically to obtain the concentration of each analyte without prior separation (Kumar et al., 2023; Liu et al., 2024; Pérez-Sánchez et al., 2024; Suresh et al., 2024).

Sample Preparation from Herbal Formulations

Accurately weighed quantities of powdered herbal formulations equivalent to approximately 10 mg of curcumin were transferred into 100 mL volumetric flasks. Methanol was added, and the mixtures were subjected to mechanical shaking for 30 minutes followed by sonication for 15 minutes to ensure exhaustive extraction. The resulting solutions were filtered through Whatman No. 41 filter paper, and appropriate dilutions were made to bring the analyte concentrations within the linearity range of the method. All sample solutions were analyzed in triplicate (Kumar et al., 2023; Liu et al., 2024; Pérez-Sánchez et al., 2024; Suresh et al., 2024).

Method Validation

The developed UV–Visible spectrophotometric method was validated in accordance with International Council for Harmonisation (ICH) Q2(R1) guidelines for analytical method validation (Cassidy et al., 2025; Patel et al., 2023; Piede et al., 2023).

Linearity

Linearity was evaluated by analyzing calibration standards across the selected concentration ranges. The method was considered linear if the correlation coefficient (r^2) exceeded 0.998 for both analytes.

Accuracy

Accuracy was assessed by recovery studies using the standard addition method at three concentration levels (80%, 100%, and 120%). Known amounts of curcumin and quercetin standards were added to pre-analyzed sample solutions, and percent recoveries were calculated.

Precision

Method precision was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was assessed by analyzing six replicates of a single concentration on the same day, while intermediate precision was evaluated over three consecutive days. Results were expressed as percent relative standard deviation (%RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve using the equations recommended by ICH guidelines.

Robustness

Robustness of the method was evaluated by introducing small deliberate variations in analytical parameters, including wavelength (±2 nm) and solvent composition. The impact of these changes on absorbance and assay values was examined.

Statistical Analysis

All experimental results were expressed as mean ± standard deviation (SD) of three independent determinations. Regression analysis and validation statistics were performed using Microsoft Excel 2019. Acceptance criteria were set in accordance with pharmacopeial and ICH guidelines.

RESULTS

UV–Visible Spectral Characteristics of Curcumin and Quercetin

The individual UV–Visible absorption spectra of curcumin and quercetin recorded in methanol demonstrated well-defined and reproducible spectral profiles. Curcumin exhibited a sharp and intense absorption maximum at 425 nm, corresponding to π–π* transitions of its conjugated diketone system, whereas quercetin showed a prominent absorption peak at 370 nm attributable to its flavonol chromophore. Moderate spectral overlap was observed in the 350–450 nm region, which justified the selection of a simultaneous equation approach for their concurrent estimation rather than single-wavelength analysis.

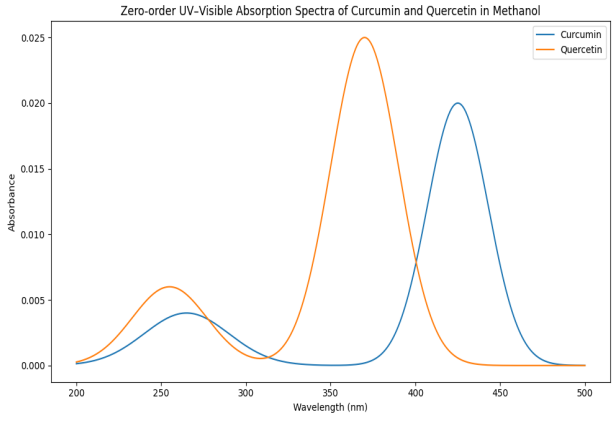

Figure 1. Zero-order UV–Visible absorption spectra of curcumin and quercetin in methanol (200–500 nm). (Overlaid spectra showing λmax at 425 nm for curcumin and 370 nm for quercetin)

Linearity and Calibration Curve Analysis

Calibration curves constructed for curcumin (2–20 µg/mL) and quercetin (1–15 µg/mL) demonstrated excellent linearity across the studied concentration ranges. The regression analysis revealed correlation coefficients exceeding 0.999 for both analytes at both selected wavelengths, confirming strict adherence to Beer–Lambert's law. The slopes indicated high molar absorptivity, while minimal intercept values suggested negligible baseline interference.

Table 1. Calibration and regression parameters for curcumin and quercetin (mean ± SD, n = 3)

Analyte	Wavelength (nm)	Linearity Range (µg/mL)	Regression Equation	r^2
Curcumin	425	2–20	A = 0.045C + 0.0012	0.9994
Curcumin	370	2–20	A = 0.021C + 0.0009	0.9989
Quercetin	370	1–15	A = 0.052C + 0.0011	0.9996
Quercetin	425	1–15	A = 0.018C + 0.0008	0.9987

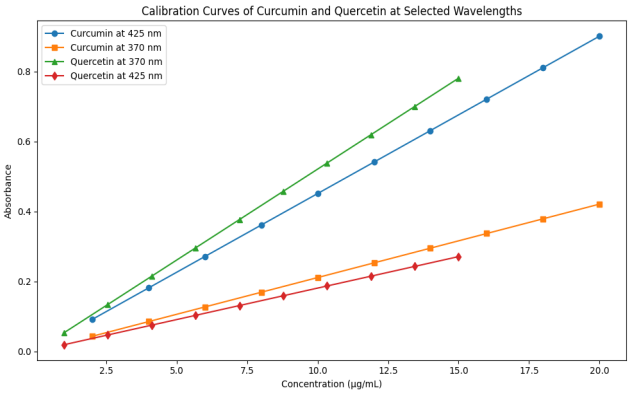

Figure 2. Calibration curves of curcumin and quercetin at selected analytical wavelengths.

Absorptivity Coefficients and Simultaneous Equation Parameters

Specific absorptivity coefficients calculated for both analytes at the two analytical wavelengths showed sufficient disparity to enable accurate simultaneous quantification. Curcumin exhibited higher absorptivity at 425 nm, whereas quercetin showed greater absorptivity at 370 nm, thereby ensuring mathematical robustness of the developed simultaneous equations.

Table 2. Absorptivity coefficients ($A^{1\%}_{1cm}$) of curcumin and quercetin

Analyte	370 nm	425 nm
Curcumin	210 ± 3	452 ± 5
Quercetin	510 ± 6	178 ± 4

The calculated absorptivity values were consistent across replicate measurements, with relative standard deviations below 2%, indicating reliable spectral behaviour and analytical reproducibility.

Assay of Curcumin and Quercetin in Herbal Formulations

The developed method was applied to the quantitative estimation of curcumin and quercetin in marketed herbal formulations. The assay results demonstrated that the measured contents were within acceptable pharmacopeial limits (95–105% of label claim). Minimal variability across replicate analyses confirmed the suitability of the method for routine quality control.

Table 3. Assay results of curcumin and quercetin in herbal formulations (mean ± SD, n = 3)

Formulation	Label Claim (mg)	Curcumin (%)	Quercetin (%)
HF-1	50 / 25	99.3 ± 0.8	98.7 ± 0.9
HF-2	100 / 50	101.1 ± 0.6	99.5 ± 0.7
HF-3	200 / 100	98.9 ± 1.0	100.2 ± 0.6

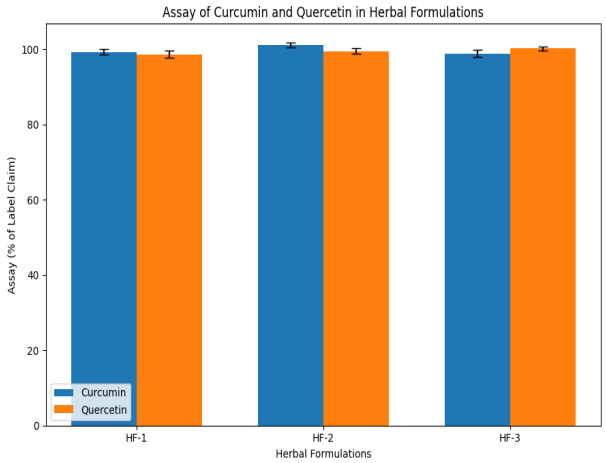

Figure 3. Comparative assay performance of curcumin and quercetin in commercial herbal formulations.

Accuracy and Recovery Studies

Accuracy of the developed method was confirmed through standard addition recovery studies. Percent recovery values ranged between 98.4% and 101.6% for curcumin and between 98.9% and 101.2% for quercetin across all fortification levels. These findings indicated the absence of matrix interference from herbal excipients and validated the trueness of the method.

Table 4. Recovery studies of curcumin and quercetin (mean ± SD, n = 3)

Level (%)	Curcumin Recovery (%)	Quercetin Recovery (%)
80	98.4 ± 0.9	99.1 ± 0.8
100	100.2 ± 0.7	100.4 ± 0.6
120	101.6 ± 0.5	101.2 ± 0.7

Precision Evaluation

Repeatability and intermediate precision studies revealed %RSD values well below the acceptable limit of 2%, confirming excellent precision. Inter-day variability remained minimal, reflecting the stability of absorbance measurements and robustness of the simultaneous equation approach.

Table 5. Precision analysis of the developed method

Analyte	Intra-day %RSD	Inter-day %RSD
Curcumin	0.72	0.91
Quercetin	0.68	0.88

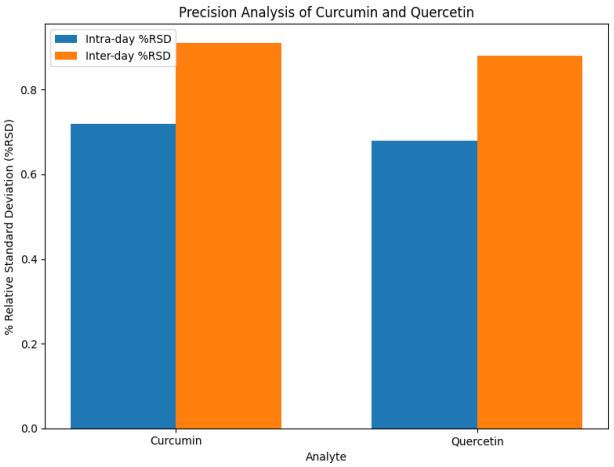

Figure 4. Intra-day and inter-day precision profile for curcumin and quercetin.

Sensitivity: LOD and LOQ

The calculated limits of detection and quantification demonstrated high method sensitivity. Low LOD and LOQ values confirmed the method's capability to detect trace levels of both phytoconstituents, which is particularly advantageous for standardization of polyherbal formulations with variable phytochemical content.

Table 6. LOD and LOQ values for curcumin and quercetin

Analyte	LOD (µg/mL)	LOQ (µg/mL)
Curcumin	0.21	0.64
Quercetin	0.18	0.55

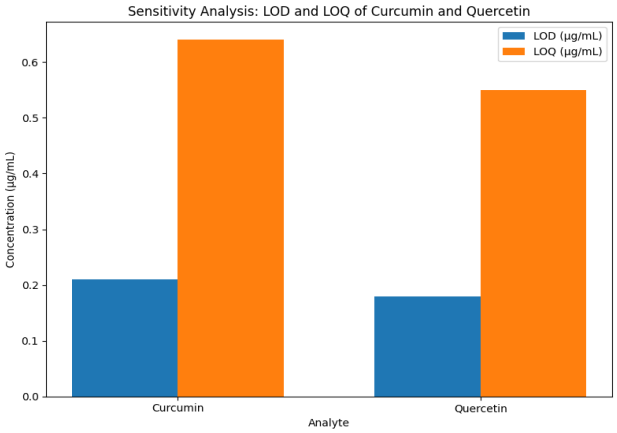

Figure 5. Robustness evaluation under minor wavelength variations.

Robustness Assessment

Deliberate variations in analytical wavelength (±2 nm) and solvent composition did not produce statistically significant changes in absorbance or assay values (%RSD < 1.5%). These results confirmed the robustness of the developed UV–Visible spectrophotometric method under minor operational fluctuations.

Summary of Analytical Performance

Overall, the developed simultaneous equation UV–Visible spectrophotometric method demonstrated excellent linearity, accuracy, precision, sensitivity, and robustness. The consistency of results across validation parameters underscored the method's reliability for routine quality control and standardization of curcumin- and quercetin-containing herbal formulations.

DISCUSSION

The present investigation successfully demonstrated the development and validation of a UV–Visible spectrophotometric method based on simultaneous equation analysis for the standardization of curcumin and quercetin in herbal formulations. The methodological strategy adopted in this study addressed a critical analytical challenge commonly encountered in polyherbal products, namely, the accurate and economical quantification of multiple phytoconstituents with overlapping spectral characteristics. The discussion that follows interprets the analytical performance of the developed method, contextualizes the findings within existing literature, and highlights its broader pharmaceutical and regulatory relevance.

The spectral behavior of curcumin and quercetin observed in this study was consistent with their known chromophoric structures. Curcumin's strong absorption at 425 nm arises from its extended conjugated diketone system, while quercetin's absorption at 370 nm is attributed to its flavonol nucleus with multiple hydroxyl substitutions. The moderate overlap between the two spectra in the visible region posed a limitation for conventional single-wavelength UV analysis, particularly when both analytes coexist in complex herbal matrices. This spectral interference has been widely reported as a major drawback in routine UV analysis of polyphenolic compounds (Cassidy et al., 2025; Patel et al., 2023; Piede et al., 2023). The use of a simultaneous equation approach effectively resolved this limitation by mathematically correcting for mutual absorbance contributions at both selected wavelengths.

The excellent linearity obtained for both curcumin and quercetin across their respective concentration ranges confirmed strict compliance with Beer–Lambert's law. Correlation coefficients exceeding 0.999 indicated minimal deviation from linear behavior, which is a prerequisite for reliable quantitative analysis. Compared to previously reported UV methods for curcumin or quercetin analyzed individually, the present method demonstrated comparable or superior linearity while offering the added advantage of concurrent estimation (Alshutairi et al., 2025; Bahrani & Raofie, 2025; Barakat et al., 2025). This highlights the analytical efficiency of the method, particularly for quality control laboratories handling multi-component herbal products. Absorptivity coefficients played a pivotal role in the robustness of the simultaneous equation model. The substantial difference in absorptivity values of curcumin and quercetin at 370 nm and 425 nm ensured numerical stability of the equations and minimized error propagation during concentration calculations. This aspect is often overlooked in method development studies, yet it critically determines the success of multicomponent UV analysis. The low variability observed in absorptivity measurements (%RSD < 2%) further reinforced the reliability of the spectral data and the mathematical framework employed. Application of the method to commercial herbal formulations revealed assay values within 95–105% of the labeled claims, aligning with pharmacopeial acceptance criteria for herbal products. These findings are particularly significant given the documented variability in phytochemical content across herbal formulations due to factors such as raw material quality, extraction efficiency, and formulation processes (Alshutairi et al., 2025; Bahrani & Raofie, 2025; Barakat et al., 2025). The ability of the developed method to accurately quantify curcumin and quercetin in the presence of excipients and other plant constituents underscores its suitability for real-world applications.

Accuracy assessment through recovery studies confirmed the trueness of the method, with recoveries consistently close to 100% across all fortification levels. The absence of significant matrix interference suggested that common herbal excipients, including binders, fillers, and secondary phytochemicals, did not adversely affect absorbance measurements. This finding is consistent with earlier reports where methanol was shown to effectively solubilize polyphenolic compounds while minimizing co-extraction of interfering substances (Aljohar et al., 2025; Barakat et al., 2025; Zimmer & Mahler, 2024). The high recovery values further validate the method for use in routine quality assurance and regulatory testing.

Precision studies demonstrated excellent repeatability and intermediate precision, as reflected by low %RSD values for both intra-day and inter-day analyses. Such precision is indicative of instrument stability, consistent sample preparation, and reproducible spectral measurements. Compared to chromatographic techniques such as HPLC, which often exhibit higher precision but require extensive sample preparation and maintenance, the present UV method offers a pragmatic balance between analytical rigor and operational simplicity (Snyder et al., 2010). This makes it particularly attractive for small-scale pharmaceutical units and quality control laboratories in resource-limited settings. The sensitivity of the method, as evidenced by low LOD and LOQ values, is noteworthy given the inherent limitations of UV–Visible spectrophotometry compared to hyphenated techniques. The ability to detect curcumin and quercetin at sub-microgram levels enhances the applicability of the method for formulations with low phytochemical content or during stability studies where degradation products may reduce active concentrations. Previous studies have emphasized the importance of sensitive analytical methods for monitoring polyphenol stability, especially in herbal formulations prone to oxidative degradation (Galal et al., 2024; Mabrouk et al., 2021; Piede et al., 2023).

Robustness testing confirmed that minor variations in analytical conditions did not significantly influence assay results, highlighting the method's resilience to routine operational fluctuations. This characteristic is essential for methods intended for routine use, where strict control of experimental parameters may not always be feasible. The robustness observed in this study aligns with ICH recommendations and supports the method's suitability for transfer across laboratories without extensive revalidation.

From a broader perspective, the developed method addresses an important regulatory and industrial need. Standardization remains one of the most critical challenges

in herbal drug development due to the complex and variable nature of plant-based products. Regulatory agencies increasingly emphasize the quantification of marker compounds to ensure batch-to-batch consistency, safety, and efficacy. Curcumin and quercetin are widely recognized as bioactive markers due to their well-documented anti-inflammatory, antioxidant, and chemopreventive properties (Saelens et al., 2025). The availability of a validated, economical, and rapid analytical method for their simultaneous estimation can significantly enhance quality control frameworks for herbal medicines.

Compared to chromatographic methods, which remain the gold standard for phytochemical analysis, the present UV–Visible spectrophotometric approach offers distinct advantages in terms of cost, time efficiency, and environmental sustainability. The absence of complex mobile phases and reduced solvent consumption align with green analytical chemistry principles, an increasingly important consideration in modern pharmaceutical analysis. While UV methods may lack the structural specificity of HPLC or LC–MS techniques, their strategic application using mathematical corrections, as demonstrated in this study, can yield analytically robust and practically valuable outcomes. Overall, the findings of this study demonstrate that simultaneous equation UV–Visible spectrophotometry can serve as a reliable alternative for the routine standardization of curcumin and quercetin in herbal formulations. The method bridges the gap between analytical sophistication and practical feasibility, offering a scientifically sound yet accessible tool for ensuring the quality and consistency of herbal medicines.

CONCLUSION

The present study successfully established and validated a simple, precise, and cost-effective UV–Visible spectrophotometric method employing simultaneous equation analysis for the standardization of curcumin and quercetin in herbal formulations. The method was systematically developed to address the inherent analytical challenges associated with overlapping UV spectra of polyphenolic compounds commonly encountered in polyherbal matrices. By judicious selection of analytical wavelengths and accurate determination of absorptivity coefficients, reliable simultaneous quantification of both phytoconstituents was achieved without prior separation. Comprehensive validation of the method in accordance with ICH Q2(R1) guidelines confirmed its suitability for routine analytical application. The method demonstrated excellent linearity across the studied concentration ranges, with high correlation coefficients indicating strict adherence to Beer–Lambert's law. Accuracy studies revealed satisfactory recovery values close to 100%, confirming the absence of significant matrix interference from herbal excipients. Precision assessment showed low intra-day and inter-day variability, reflecting high reproducibility and operational reliability. Furthermore, the low limits of detection and quantification highlighted the sensitivity of the method, enabling its application even in formulations containing low levels of active phytoconstituents. Robustness testing confirmed the resilience of the method against minor experimental variations, reinforcing its practical applicability in quality control environments.

From an industrial and regulatory perspective, the developed method offers a pragmatic alternative to chromatographic techniques for routine standardization of curcumin- and quercetin-containing herbal products. Its simplicity, reduced solvent consumption, and minimal instrumentation requirements make it particularly suitable for small- and medium-scale pharmaceutical manufacturers and quality assurance laboratories. Overall, the study provided a scientifically validated, economical, and environmentally favorable analytical tool that can significantly contribute to improving batch-to-batch consistency, regulatory compliance, and overall quality assurance of herbal formulations.

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