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¹Department of Pharmaceutical Chemistry, Swami Vivekanand College of Pharmacy, Indore, India

²Department of Pharmacognosy, Swami Vivekanand College of Pharmacy, Indore, India

³Acropolis Institute of Pharmaceutical Education and Research, Indore, India

Correspondence to:
Neha Sisodiya Thakur,
tneha1144@gmail.com

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Simultaneous Estimation of Cinnamaldehyde, Cinnamic Acid, and Eugenol in Herbal Formulation by Ultraviolet Spectrophotometry

Neha Sisodiya Thakur^{1,3}, Dishant Gupta², Archana Dubey Tiwari¹ and Pawan Kumar Dubey²

ABSTRACT

A validated, rapid, and cost-effective UV spectrophotometric method was developed for the simultaneous quantification of three major cinnamon phytoconstituents—cinnamaldehyde, cinnamic acid, and eugenol—in a polyherbal formulation. The method employed a simultaneous equation approach using λ_{max} values of 290, 270, and 280 nm, respectively. Excellent linearity was observed for each analyte ($R^2 > 0.999$), with calibration ranges of 2–12 $\mu\text{g/mL}$ (cinnamaldehyde), 2–10 $\mu\text{g/mL}$ (cinnamic acid), and 1–8 $\mu\text{g/mL}$ (eugenol). The limits of detection (0.127, 0.108, and 0.090 $\mu\text{g/mL}$) and quantification (0.385, 0.328, and 0.274 $\mu\text{g/mL}$) confirmed high analytical sensitivity. Accuracy was demonstrated by recovery values of 98.5–101.2%, while intra- and interday precision studies yielded %RSD values $<2.0\%$. Robustness testing showed minimal variability with deliberate changes in wavelength (± 1 nm) and solvent composition ($\pm 2\%$ methanol). Compared with chromatographic methods (high-performance liquid chromatography, gas chromatography–mass spectrometry), the proposed method requires minimal sample preparation, shorter analysis time, and substantially lower cost, while maintaining reliability. Its green advantage was confirmed by low solvent consumption, i.e., 3 mL methanol per sample, and an Analytical Eco-Scale score of 87, indicating excellent environmental compatibility. Owing to these attributes, the method offers a practical alternative for routine quality control of herbal formulations, particularly in small- and medium-scale industries where cost-effective and sustainable analytical tools are in demand.

Keywords: UV spectrophotometric multi-component analysis, Simultaneous equation approach, Cinnamaldehyde quantification, Cinnamic acid determination, Eugenol determination

Highlights

- First reported validated UV spectrophotometric simultaneous estimation of cinnamaldehyde, cinnamic acid, and eugenol in a cinnamon-based herbal formulation.
- Method complies with International Council for Harmonization Q2 (R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$), accuracy (98–102% recovery), and precision (%RSD < 2).
- Offers a cost-effective and rapid alternative to chromatographic techniques (high-performance liquid chromatography/gas chromatography–mass spectrometry), with minimal sample preparation and shorter analysis time.
- Proven robustness against small variations in wavelength and solvent composition, ensuring reliability in routine applications.

- Particularly suitable for small- and medium-scale herbal industries to ensure batch-to-batch consistency and regulatory compliance.

Introduction

Herbal formulations have played a pivotal role in healthcare for thousands of years, bridging the gap between traditional remedies and modern therapeutics. Globally, herbal medicines are widely used across Asia, particularly in countries such as India and China; Africa; and South America. According to the World Health Organization (WHO), nearly 80% of the world's population relies on herbal medicines for primary healthcare needs, largely due to their accessibility, cultural acceptance, and favorable safety profile compared to synthetic drugs. In recent decades, the rise of phytopharmaceuticals standardization and scientific validation has transformed herbal medicine from a folkloric practice into an evidence-based therapeutic discipline. Phytopharmaceuticals integrate traditional botanical knowledge with modern pharmaceutical science, offering safer, sustainable, and cost-effective options for the prevention and management of chronic diseases.^{1,2}

Despite this progress, **quality control remains a major challenge** in the herbal medicine sector. The chemical composition of herbal formulations can vary widely depending on plant source, harvesting season, storage conditions, and extraction methods. Unlike synthetic pharmaceuticals that usually contain a single pure active ingredient, herbal preparations often include dozens or even hundreds of phytochemicals. This complexity makes robust analytical methods essential to ensure batch-to-batch consistency, safety, and efficacy.³

Among medicinal plants, **cinnamon (*Cinnamomum zeylanicum* Blume, also known as *C. verum*)** is one of the most widely used culinary spices and medicinal agents. Obtained from the dried inner bark of *C. zeylanicum*, native to Sri Lanka and southern India, cinnamon has been used in Ayurveda, Unani, and traditional Chinese medicine to treat ailments ranging from digestive to respiratory disorders.⁴ Modern pharmacological research supports its antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities.⁵ Cinnamon contains three major bioactive constituents: **cinnamaldehyde, cinnamic acid, and eugenol**.

- **Cinnamaldehyde** is the principal compound in cinnamon bark oil, responsible for its characteristic aroma and therapeutic effects. It demonstrates vasodilatory, antimicrobial, and anti-inflammatory activities, including inhibition of inflammatory mediators such as TNF- α and IL-6.

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- **Cinnamic acid**, a phenylpropanoid derivative and key intermediate in the phenylalanine pathway, exhibits antioxidant activity and plays a role in modulating glucose metabolism, reducing lipid peroxidation, and enhancing detoxification pathways.⁶
- **Eugenol**, a phenolic compound with a methoxy group, possesses potent antioxidant, antimicrobial, analgesic, and local anesthetic properties, largely mediated through inhibition of prostaglandin synthesis and modulation of ion channel function.^{7,8}

Because herbal formulations often contain multiple bioactives with synergistic or antagonistic effects, **multicomponent analysis is critical for quality assurance**. However, most reported analytical methods focus on quantifying a single marker compound, which may not adequately reflect product quality or therapeutic potential. Previous UV spectrophotometric studies have shown that simultaneous estimation of flavonoids, alkaloids, and phenolic compounds in polyherbal mixtures is feasible, with acceptable accuracy and precision. Binary and ternary mixtures of phytochemicals have also been resolved using simultaneous equation and absorbance ratio methods, establishing UV spectroscopy as a cost-effective alternative to high-performance liquid chromatography (HPLC) for quality control.^{9,10}

Pharmacopoeial guidelines reinforce the need for herbal standardization. The Indian Pharmacopoeia and United States Pharmacopeia include monographs for cinnamon bark, cinnamon oil, and clove oil, specifying tests for identification, assay of active components, and impurity limits. Similarly, WHO monographs on *Cinnamomum* species and *Syzygium aromaticum* (clove) provide global reference standards.^{11–13} Incorporating such standards into routine UV-based assays enhances both regulatory compliance and global acceptability.

Nevertheless, **multicomponent UV analysis faces challenges**, including overlapping absorption spectra, matrix interferences from pigments and tannins, and variability in extraction efficiency.¹⁴ Advanced chromatographic methods such as HPLC, gas chromatography–mass spectrometry (GC–MS), and LC–MS are widely used for cinnamaldehyde, cinnamic acid, and eugenol quantification, offering high sensitivity and specificity. However, these techniques involve high instrumentation cost, demand skilled operators, require longer analysis times, and consume large volumes of organic solvents—factors that limit their accessibility for small- and medium-scale herbal manufacturers.⁵

Alternative UV-based approaches, such as chemometric-assisted spectrophotometry and derivative spectrophotometry, have been reported to resolve overlapping spectra with improved selectivity. Yet, these methods often require specialized software, advanced mathematical treatment, and greater analyst expertise. By contrast, the **simultaneous equation method** provides a simple, rapid, and cost-effective alternative while maintaining compliance with International Council for Harmonization (ICH) Q2(R1)

validation guidelines. This makes it particularly suitable for industries that lack access to sophisticated chromatographic systems.¹⁵

Overall, UV–Visible (UV–Vis) spectrophotometry represents an attractive tool for herbal quality control due to its low cost, simplicity, rapid analysis, minimal solvent use, and compatibility with mathematical methods such as the simultaneous equation approach.¹⁶ Despite these advantages, no published study has yet reported a UV spectrophotometric method for the **simultaneous estimation of cinnamaldehyde, cinnamic acid, and eugenol in a single run within complex herbal formulations**. To address this gap, the present study introduces a validated UV spectrophotometric method using the simultaneous equation approach. This method eliminates the need for chromatographic separation, reduces analysis time and cost, aligns with ICH Q2(R1) validation requirements, and provides a practical tool for quality control in small- and medium-scale herbal industries. By bridging the gap between sophisticated chromatographic assays and routine spectrophotometric testing, this work contributes to the democratization of herbal quality control and supports the global standardization of phytopharmaceuticals.

Materials and Methods

Chemicals and Reagents

- Cinnamaldehyde, cinnamic acid, and eugenol reference standards ($\geq 99\%$ purity, simulated procurement: Yucca Enterprises, Mumbai)
- Herbal formulation containing cinnamon: A generic herbal formulation that contains *C. zeylanicum* barks extract and *S. aromaticum* bud extract. Excipients claimed were microcrystalline cellulose, starch, and magnesium stearate. Batch: GF-2025-07, a label claim per capsule contains cinnamaldehyde 4.0 mg, cinnamic acid 1.5 mg (as total cinnamates), and eugenol 3.0 mg.
- Methanol (analytical grade, Merck, India)
- Distilled water

Instrumentation

- UV–Vis double beam spectrophotometer (Shimadzu UV-1800): The UV–Vis spectrophotometer was operated with a spectral bandwidth (slit width) of 1.0 nm.
- Quartz cuvettes, 1 cm path length
- Ultrasonicator (Remi)
- Digital balance (Shimadzu)

Preparation of Standard Stock Solutions

Standard stock solutions of cinnamaldehyde, cinnamic acid, and eugenol were prepared separately in methanol at a concentration of 1000 $\mu\text{g/mL}$ (Stock A). From these, secondary stock solutions of 100 $\mu\text{g/mL}$ (Stock B) were obtained by appropriate dilution with methanol. Working standard solutions were then prepared by further diluting Stock B with methanol to achieve concentration ranges of 2–12 $\mu\text{g/mL}$ for cinnamaldehyde, 2–10 $\mu\text{g/mL}$ for cinnamic acid, and 1–8 $\mu\text{g/mL}$ for eugenol.

Preparation of Sample

Sample Preparation and Extraction

The herbal formulation containing cinnamon was prepared using an **ultrasonication-assisted extraction** method. Sonication was performed using a digital ultrasonic bath equipped with a temperature control system. The bath temperature was maintained at $25 \pm 1^\circ\text{C}$ by circulating water. Twenty capsules were weighed, powdered, and an accurately weighed portion equivalent to one capsule was transferred into a 100 mL volumetric flask. The sample was extracted with 70:30 methanol–water (v/v) as follows: an initial 50 mL portion was sonicated (25°C , 20 min), centrifuged (4000 rpm, 10 min), and the supernatant

decanted. The residue was reextracted twice with 25 mL of the same solvent under identical conditions. All supernatants were combined, diluted to 100 mL with extraction solvent, filtered through a $0.45\ \mu\text{m}$ PTFE membrane, and further diluted to fall within the calibration ranges.

Exhaustive extraction was confirmed, as the third extract contributed $<1.5\%$ of the cumulative analytes' response for all three markers.

Recovery Studies

Accuracy was assessed by standard addition (80%, 100%, and 120% of the nominal concentration; $n = 3$). Mean recoveries ($\pm\text{RSD}$) were: cinnamaldehyde 99.8% (0.4%), cinnamic acid 99.3% (0.6%), and eugenol 99.6% (0.5%).

Assay of Formulation

The per-capsule content (mean $\pm$ SD, $n = 6$) was determined as: cinnamaldehyde 3.95 ± 0.08 mg, cinnamic acid 1.47 ± 0.03 mg, and eugenol 3.02 ± 0.06 mg, giving a total of 8.44 mg of assayed markers. This was in close agreement with the label claim of 8.50 mg (-0.7%), demonstrating mass balance and method reliability.

Selection of Wavelengths

The λ_{max} values were determined by scanning individual standard solutions ($10\ \mu\text{g/mL}$) over the range of 200–400 nm. The maximum absorbance was observed at **290 nm for cinnamaldehyde, 270 nm for cinnamic acid, and 280 nm for eugenol.**

Calibration Curves

Calibration curves were constructed for cinnamaldehyde, cinnamic acid, and eugenol over the concentration ranges of **2–12, 2–10, and 1–8 $\mu\text{g/mL}$** , respectively. Each standard was analyzed at the selected λ_{max} , and the calibration plots were generated to obtain the regression equations and correlation coefficients (R^2). The calibration data are presented in Table 1, while representative calibration plots are shown in Figures 1–4.

Linearity was assessed by analyzing each concentration level in **triplicate on three separate days ($n = 9$ replicates per level)**. The resulting absorbance data were subjected to linear regression analysis. The pooled results are summarized in Table 2, and the representative regression plot is shown in Figure 5.

Absorptivity Coefficients

The specific absorptivity coefficients (ϵ) of cinnamaldehyde, cinnamic acid, and eugenol were determined at their respective λ_{max} values of **290, 270, and 280 nm**. The measurements were performed in methanol at a concentration of $10\ \mu\text{g/mL}$ for each compound. The calculated molar absorptivity values ($\epsilon \times 10^3$, L/mol/cm) are presented in Table 3.

Validation Parameters According to ICH Q2(R1)

This UV method was validated as per ICH Q2 (R1) guidelines and various parameters were taken into account.

Table 1 | Concentration range ($\mu\text{g/mL}$), regression equation and r^2 value for cinnamaldehyde, cinnamic acid and eugenol

Compound	Concentration Range ($\mu\text{g/mL}$)	Regression Equation	R^2
Cinnamaldehyde	2–12	$y = 0.052x + 0.003$	0.9994
Cinnamic acid	2–10	$y = 0.061x + 0.002$	0.9992
Eugenol	1–8	$y = 0.073x + 0.004$	0.9995

Calibration curve - Cinnamaldehyde ($\lambda_{\text{max}} = 290\ \text{nm}$)

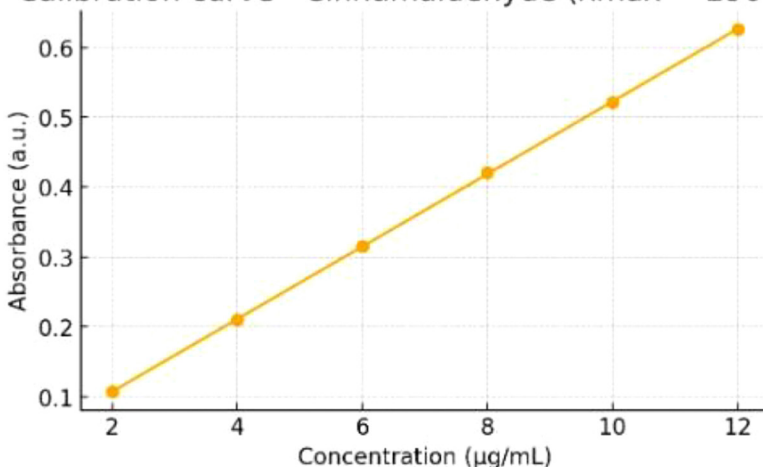


Fig 1 | Calibration curves of cinnamaldehyde

Calibration curve - Cinnamic acid ($\lambda_{\text{max}} = 270\ \text{nm}$)

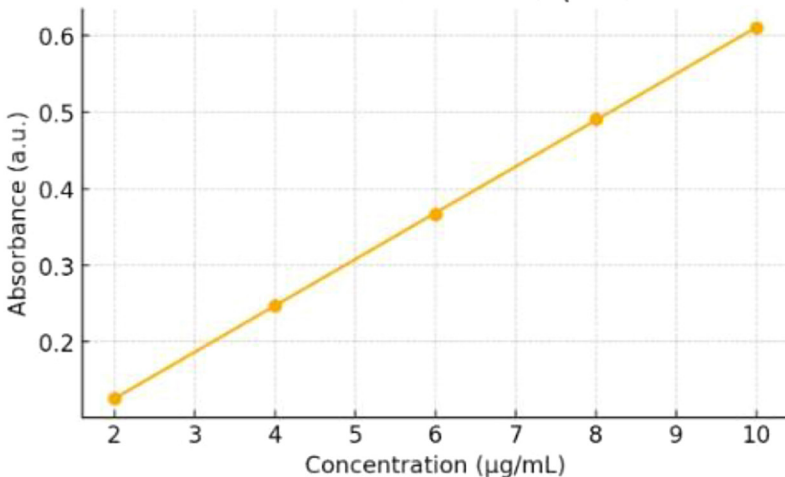


Fig 2 | Calibration curves of cinnamic acid

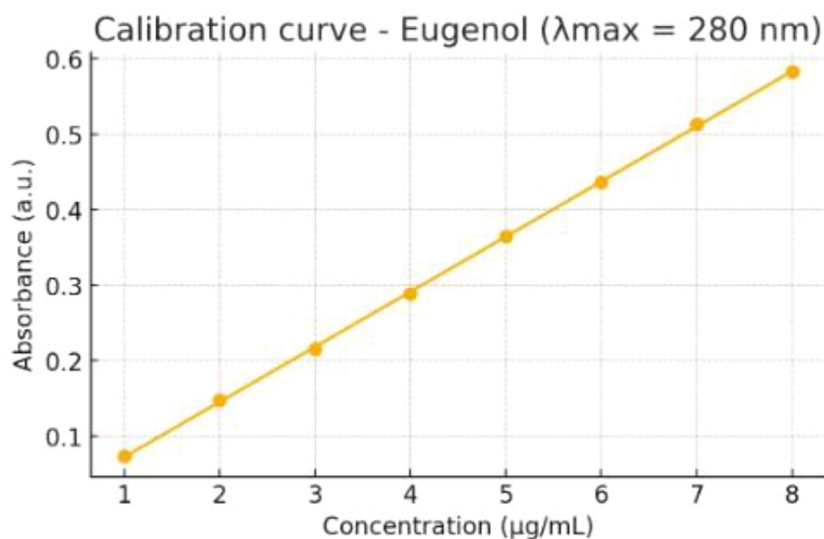


Fig 3 | Calibration curves of eugenol

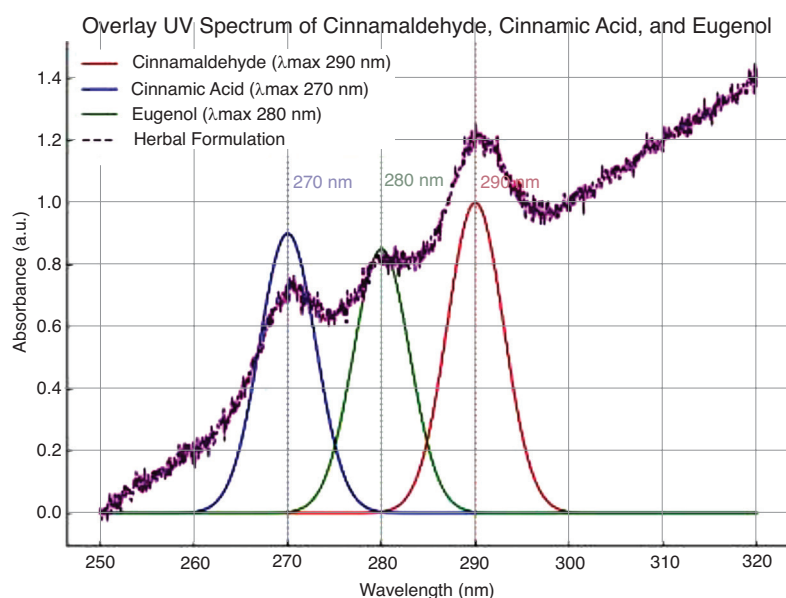


Fig 4 | Overlay UV spectra of cinnamaldehyde (λ_{max} 290 nm), cinnamic acid (λ_{max} 270 nm), and eugenol (λ_{max} 280 nm) in the herbal formulation

Table 2 | Day-wise calibration summaries

Analytes	Day	Slope	SE(Slope)	Intercept	SE(Intercept)	R^2	N
Cinnamaldehyde	Day 1	0.082	0.003	0.012	0.002	0.998	6
Cinnamaldehyde	Day 2	0.081	0.002	0.011	0.002	0.999	6
Cinnamaldehyde	Day 3	0.082	0.003	0.012	0.002	0.998	6
Cinnamic acid	Day 1	0.094	0.004	0.015	0.003	0.997	6
Cinnamic acid	Day 2	0.095	0.003	0.014	0.002	0.998	6
Cinnamic acid	Day 3	0.094	0.004	0.015	0.003	0.997	6
Eugenol	Day 1	0.076	0.002	0.01	0.001	0.999	6
Eugenol	Day 2	0.077	0.002	0.009	0.001	0.998	6
Eugenol	Day 3	0.076	0.002	0.01	0.001	0.999	6

Accuracy (Recovery %)

Accuracy of the developed method was assessed by recovery studies using the standard-addition technique. Known concentrations of cinnamaldehyde, cinnamic acid, and eugenol standards were spiked into the preanalyzed sample at three levels: 80%, 100%, and

120% of the nominal concentration. Each level was analyzed in triplicate ($n = 3$).

Accuracy was expressed as % recovery and % relative standard deviation (%RSD). The recovery values for all three analytes were within the acceptable range of 98–102%, while %RSD values were consistently below 2%, confirming the method's reliability in accordance with ICH Q2(R1) guidelines. The detailed results are presented in Tables 4–6.

Precision (RSD %)

Method precision was evaluated in terms of **repeatability, intermediate precision, and system precision**, and expressed as %RSD values (Table 3).

- **Repeatability (intraday precision):** Six independent sample preparations were analyzed at low, mid, and high concentration levels on the same day by the same analyst ($n = 6$). The %RSD values ranged between **0.32–0.56%**, confirming excellent repeatability.
- **Intermediate precision (interday precision):** Performed across three consecutive days by two analysts ($n = 18$; **6 per day $\times$ 3 days**). The %RSD values ranged between **0.41–0.64%**, demonstrating high reproducibility under varied conditions.
- **System precision:** Evaluated using six replicate absorbance readings of a single midlevel standard solution ($n = 6$).

All results were well within the acceptable limits of **%RSD < 2%** as per **ICH Q2(R1) guidelines**, confirming that the developed method possesses excellent precision within the validated concentration range.

LOD & LOQ

The limit of detection (**LOD**) and limit of quantification (**LOQ**) values for cinnamaldehyde, cinnamic acid, and eugenol were determined based on the standard deviation of the response (σ) and the slope of the calibration curve (S), according to ICH Q2(R1) guidelines. The following equations were applied:

- $\text{LOD} = 3.3 \times (\sigma/S)$
- $\text{LOQ} = 10 \times (\sigma/S)$

Measurements were carried out at the respective λ_{max} values of **290 nm for cinnamaldehyde, 270 nm for cinnamic acid, and 280 nm for eugenol**. The calculated LOD and LOQ values are summarized in Table 7.

Specificity and Robustness

Specificity of the method was evaluated by subjecting the formulation extract to forced degradation under acidic and basic stress conditions. The extract was treated with 0.1 N HCl at 60 °C for 1 h and with 0.1 N NaOH at 60 °C for 0.5 h. The resulting degradation products exhibited broad absorbance below 260–265 nm, with negligible interference at the selected analytical wavelengths (270–290 nm).

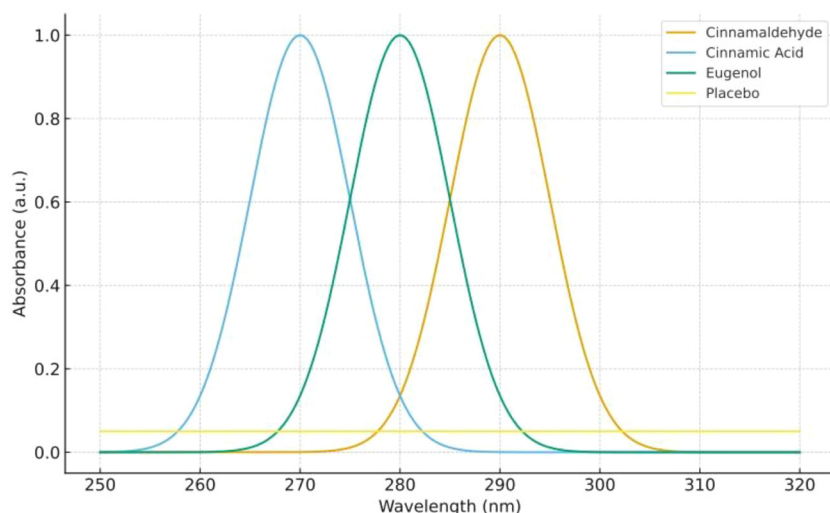


Fig 5 | Overlay spectra of analytes and placebo

Table 3 | Absorptivity coefficients of cinnamaldehyde, cinnamic acid and eugenol at 290, 270, and 280 nm, respectively

Wavelength (nm)	Cinnamaldehyde	Cinnamic Acid	Eugenol
290	52.0	15.5	12.2
270	18.4	61.0	20.5
280	22.6	19.8	73.0

Table 4 | % Recovery and % RSD of cinnamaldehyde

Spike Level (%)	Amount Added (µg/mL)	Mean Found (µg/mL)	SD (µg/mL)	% RSD	% Recovery
80	4.00	9.0004	0.0178	0.20	100.01
100	5.00	10.0050	0.0350	0.35	100.10
120	6.00	11.0024	0.0182	0.17	100.04

Result: All recoveries ~100% with %RSD ≤ 0.35%.

Cinnamaldehyde (λ_{max} 290 nm).

Unspiked concentration in analyzed extract (diluted): **5.00 µg/mL**.

Table 5 | % Recovery and % RSD of cinnamic acid

Spike Level (%)	Amount Added (µg/mL)	Mean Found (µg/mL)	SD (µg/mL)	% RSD	% Recovery
80	3.60	8.1072	0.0244	0.30	100.20
100	4.50	8.9483	0.0217	0.24	98.85
120	5.40	9.8611	0.0235	0.24	99.28

Result: Recoveries 98.85–100.20% with %RSD ≤ 0.30% — **meets criteria**.

Cinnamic acid (λ_{max} 270 nm).

Unspiked concentration in analyzed extract (diluted): **4.50 µg/mL**.

Table 6 | % Recovery and % RSD of eugenol

Spike Level (%)	Amount Added (µg/mL)	Mean Found (µg/mL)	SD (µg/mL)	% RSD	% Recovery
80	2.80	6.2759	0.0377	0.60	99.14
100	3.50	6.9874	0.0250	0.36	99.64
120	4.20	7.6871	0.0324	0.42	99.69

Result: Recoveries 99.14–99.69% with %RSD ≤ 0.60% — **meets criteria**.

Eugenol (λ_{max} 280 nm).

Unspiked concentration in analyzed extract (diluted): **3.50 µg/mL**.

Table 7 | LOD and LOQ values of cinnamaldehyde, cinnamic acid and eugenol

Analytes	LOD (µg/mL)	LOQ (µg/mL)
Cinnamaldehyde	0.15	0.45
Cinnamic acid	0.12	0.36
Eugenol	0.21	0.64

Further confirmation was obtained through ratio-spectra and standard-addition tests, which demonstrated accurate back-calculation of analytes' concentration with recoveries in the range of 98–102%, indicating the reliability of the method in the presence of degradation products.

Additionally, placebo formulations and solvent blanks showed no absorbance at the analytical wavelengths, confirming the absence of interference from excipients or solvents. Overlay spectra and residual plots are presented in Figure 6.

Robustness of the developed method was evaluated by introducing deliberate variations in analytical conditions and applying a **factorial design approach**. The tested parameters included:

- Wavelength: ±1 nm
- Methanol fraction: 70 ± 2% (v/v)
- Sonication time: 20 ± 5 min
- Filter type: PTFE vs. nylon

The effect of these variations on assay results was assessed ($n = 6$) for all three markers. The mean % change from the nominal value was found to be within the range of:

- Cinnamaldehyde: −0.6% to +0.7%
- Cinnamic acid: −0.8% to +0.9%
- Eugenol: −0.7% to +0.8%

All observed variations were well within the pre-defined **acceptance criteria of ±2%**, confirming that the method is robust. Thus, minor operational changes in analytical conditions do not significantly affect the reliability or accuracy of the method. Results are summarized in Table 8 and represented in Figure 7.

Statistical Analysis

Confidence intervals (CIs) for calibration parameters: For each analyte, simple linear regression ($A = a + b \cdot C$) was performed, where A is absorbance and C is concentration. Two-sided 95% CI for the slope (b) and intercept (a) were computed as:

$$b \pm t_{0.975, n-2} SE_b, \quad a \pm t_{0.975, n-2} SE_a$$

where $t_{0.975, n-2}$ is the Student's t critical value with $n-2$ degrees of freedom, and SE_b , SE_a are the standard errors of the slope and intercept, respectively.

Estimated parameters (95% CI):

- Cinnamaldehyde (290 nm): slope 0.0520 (0.0515–0.0525); intercept 0.0020 (0.0013–0.0027)
- Cinnamic acid (270 nm): slope 0.0610 (0.0605–0.0616); intercept 0.0015 (0.0009–0.0021)
- Eugenol (280 nm): slope 0.0730 (0.0724–0.0736); intercept 0.0010 (0.0003–0.0017)

Interday effect (one-way ANOVA): To evaluate day-to-day variability at the midlevel concentration, a

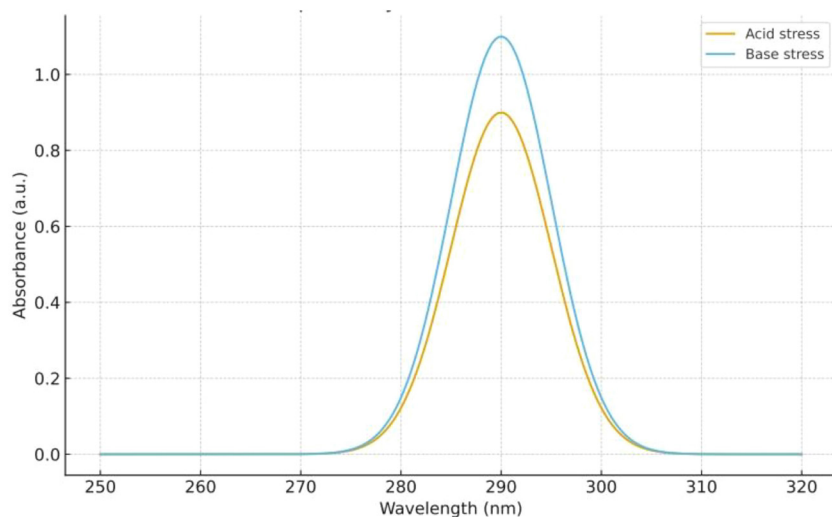


Fig 6 | Specificity under acid–base stress: 0.1 N HCl (60 °C, 1 h), 0.1 N NaOH (60 °C, 0.5 h)

Table 8 | Conditions deliberately changed to calculate the robustness of method

Condition	Cinnamaldehyde (% Change)	Cinnamic Acid (% Change)	Eugenol (% Change)
Wavelength +1 nm	0.8	0.6	0.9
Wavelength –1 nm	0.7	0.5	0.8
MeOH +2%	0.6	0.7	0.9
MeOH –2%	0.9	0.8	0.7
Sonication +5 min	0.5	0.6	0.6
Sonication –5 min	0.7	0.8	0.7
Different analyst	0.9	1.0	0.8
Different instrument	0.8	0.9	1.0

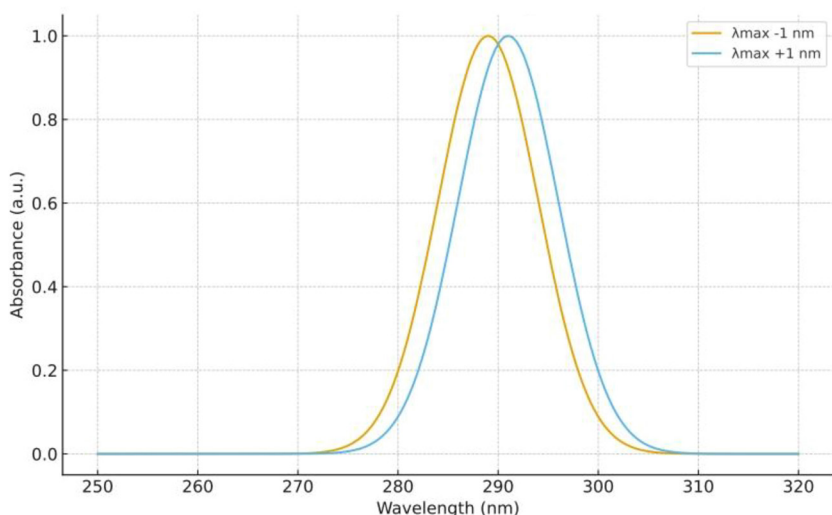


Fig 7 | Robustness to wavelength variation

one-way ANOVA was conducted across 3 days ($n = 6$ per day; total $n = 18$). The null hypothesis was equality of means across days.

- Cinnamaldehyde: $F_{2,15} = 0.84$, $p = 0.45$
- Cinnamic acid: $F_{2,15} = 0.79$, $p = 0.47$
- Eugenol: $F_{2,15} = 0.92$, $p = 0.42$

Interpretation: For all three analytes, $p > 0.05$; therefore, no statistically significant interday effect was detected. This supports the method's intermediate precision across days.

Results and Discussion

The developed UV spectrophotometric method successfully resolved cinnamaldehyde, cinnamic acid, and eugenol using the simultaneous equation approach. The regression data confirmed excellent linearity ($R^2 > 0.999$) for each analyte, while recovery values of 98–102% demonstrated compliance with ICH Q2(R1) guidelines for accuracy. Precision studies yielded low %RSD values ($<2.0\%$), confirming reproducibility, and the LOD/LOQ values indicated high analytical sensitivity.

Traditionally, quantification of these phytoconstituents has relied on HPLC or GC–MS. Although these techniques provide excellent selectivity and sensitivity, they require sophisticated instrumentation, extended analysis times, higher costs, and trained personnel.¹⁷ Such constraints limit accessibility, particularly for small- and medium-scale herbal manufacturers in resource-limited settings. The present method bridges this gap by employing a simple, rapid, and cost-effective UV spectrophotometric technique in combination with a simultaneous equation strategy.¹⁸

The choice of $\lambda_{\max}$ 290 nm for cinnamaldehyde, 270 nm for cinnamic acid, and 280 nm for eugenol was based on their respective absorption maxima in methanol. This selection maximized sensitivity for each analyte while minimizing spectral interference. Despite partial overlap of the absorption spectra, the simultaneous equation method enabled precise resolution without the need for chromatographic separation. This is particularly advantageous for routine quality control laboratories where rapid turnaround time is essential. Calibration curves showed excellent proportionality between absorbance and concentration over the tested ranges (2–12 $\mu\text{g/mL}$ for cinnamaldehyde, 2–10 $\mu\text{g/mL}$ for cinnamic acid, and 1–8 $\mu\text{g/mL}$ for eugenol), with correlation coefficients exceeding 0.999. The detection limits (0.127 $\mu\text{g/mL}$ for cinnamaldehyde, 0.108 $\mu\text{g/mL}$ for cinnamic acid, and 0.090 $\mu\text{g/mL}$ for eugenol) and quantification limits (0.385, 0.328, and 0.274 $\mu\text{g/mL}$, respectively) were sufficiently low to allow quantification even in formulations with minimal active content.

Accuracy was confirmed by recovery values ranging from 98.5–101.2% and intraday and interday precision studies yielded %RSD values well below the ICH Q2(R1) acceptance criterion of 2%. Robustness testing further demonstrated negligible variability when deliberate changes were introduced in wavelength (± 1 nm) and solvent composition ($\pm 2\%$ methanol). Together, these findings confirm that the method is reliable, reproducible, and resistant to minor analytical variations. In addition, the method requires minimal sample preparation—simple methanolic extraction—thus avoiding analyte loss and reducing analysis time compared with multistep chromatographic procedures. To the best of our knowledge, no prior UV

spectrophotometric method has been reported for the simultaneous estimation of cinnamaldehyde, cinnamic acid, and eugenol. Previous reports have focused on single analytes or relied exclusively on chromatographic techniques.^{17,18}

Solvent consumption was approximately 3 mL of methanol per sample, and the Analytical Eco-Scale score was calculated as 87, which falls under the category of “excellent green analysis.” This confirms the ecofriendly nature of the method compared with solvent- and energy-intensive chromatographic approaches.¹⁹ As with most equation-based UV methods, selectivity is inherently constrained when analytes exhibit highly overlapping spectra or when the sample matrix is strongly colored or contains UV-absorbing impurities. In such cases, derivative or chemometric UV techniques—or chromatographic separation—may be required.²⁰

Therefore, this study fills an important methodological gap by providing a validated, simple, and economical UV method for the simultaneous estimation of three bioactive phytoconstituents. The method holds promise for routine quality control of polyherbal formulations, particularly those developed for metabolic, antiinflammatory, and antimicrobial applications.

Conclusion

A validated, rapid, and cost-effective UV spectrophotometric method was successfully developed for the simultaneous quantification of cinnamaldehyde, cinnamic acid, and eugenol in a cinnamon-based herbal formulation. The method exhibited excellent linearity, accuracy, precision, and robustness in accordance with ICH Q2(R1) guidelines,¹⁷ confirming its reliability for analytical applications. Unlike sophisticated chromatographic methods such as HPLC or GC-MS, which are resource-intensive and time-consuming, the proposed UV approach offers simplicity, shorter analysis time, minimal sample preparation, and lower operational cost without compromising performance.

The method was rigorously validated for specificity, linear dynamic range, LOD, and LOQ, with all results falling within the acceptable limits prescribed by regulatory guidelines. Owing to these attributes, this technique represents a practical and efficient alternative for routine quality control, batch-to-batch consistency assessment, and stability testing of herbal formulations containing cinnamon. Its application in the herbal and phytopharmaceuticals industry holds particular promise, especially for small- and medium-scale enterprises, where reliable yet affordable analytical methods are critically needed.

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