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A Method for Eco-sustainable Quantification of 4-Hydroxy-3-Methoxy-Cinnamic acid in the Rhizome Extract of *Alpinia galanga* (L.) Willd., using Analytical Quality by Design Approach

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KEYWORDS:

4-Hydroxy-3-Methoxy-Cinnamic acid, Alpinia galanga (L.) Willd., UV-spectrophotometry, Quality by Design, Forced Degradation.

ABSTRACT:

Introduction: A bioactive phenolic compound with considerable antioxidant and therapeutic potential, 4-Hydroxy-3-Methoxy-Cinnamic acid, also known as Ferulic Acid, is found in Alpinia galanga (L.) Willd. There are few UV-spectrophotometric methods based on Quality by Design (QbD) principles that show stability and are environmentally friendly.

Objectives: This study planned to design and validate a QbD-assisted, eco-friendly UV-spectrophotometric technique for 4-Hydroxy-3-Methoxy-Cinnamic acid measurement in the rhizome extract of Alpinia galanga (L.) Willd.,

Methods:

During method development and optimization, the environmentally friendly solvent system and a systematic QbD technique, method development and optimization were utilized. The method's stability-indicating characteristics were confirmed by investigation of forced degradation in acidic, alkaline, oxidative, thermal, and photolytic surroundings. Validation was completed following ICH Q2(R1) rules.

Results: Within a concentration range of 2–10 µg/mL, the method showed outstanding linearity ($R^2 = 0.9997$), with LOD and LOQ values of 0.920 µg/mL and 1.789 µg/mL, respectively. While precision tests showed %RSD less than 2%, the range of recovery values was 99.71% to 102.40%. In the rhizome extract of Alpinia galanga, 4-Hydroxy-3-Methoxy-Cinnamic acid's content was discovered to be 0.099 ± 0.003% w/w (n = 3). The method's environmental friendliness was confirmed by greenness assessment utilizing GAPI and AGREE.

Conclusion: The proposed UV-Spectrophotometric technique can be used for phytochemical analysis and regular quality control of 4-Hydroxy-3-Methoxy-Cinnamic acid in the extract of Alpinia galanga (L.) Willd.,

1. Introduction

Common phenolic plant chemical found naturally in seeds and leaves is 4-hydroxy-3-methoxycinnamic acid, sometimes called ferulic acid (FA). Found in plant cell walls in free and covalently bonded forms to polysaccharides, glycoproteins, polyamines, lignin, and hydroxy fatty acids, it is Apart from giving the cell wall its rigidity, FA is required in the production of other important chemical molecules including coniferyl alcohol. Among the biological actions FA exhibits are antioxidant, anti-inflammatory, antimicrobial, anti-allergenic, hepatoprotective, anticarcinogenic, antithrombotic, increase sperm viability, antiviral, vasodilatory, metal chelation, enzyme activity modulating, transcriptional factor activation, gene expression, and signal transduction [1]. Because of its

Journal of Chemical Health Risks

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numerous medical properties, the rhizomatous plant *Alpinia galanga* (L.) Willd., sometimes known as bigger galangal, is frequently utilized in Ayurvedic, Unani, and traditional Southeast Asian medicine. Phytoconstituents including flavonoids, phenolics, terpenoids, and essential oils improve the broad pharmaceutical scope of the plant. Many investigations show its antioxidant, anti-inflammatory, antibacterial, hepatoprotective, neuroprotective, anti-cancer, and cardioprotective qualities, therefore providing current scientific verification for its traditional claims [2, 3].

Quality control of herbal raw materials is absolutely necessary for Ayurvedic medicines to stay safe and efficient. As a measure of quality control, the Indian Ayurvedic Pharmacopoeia employs TLC/HPTLC. Quality control criteria for Indian medicinal plants published by the ICMR also include HPTLC, HPLC, or GC. UV-Vis spectrophotometric analysis is one of the many quality control criteria that would provide both qualitative and quantitative standards. Confirmation in both qualitative and quantitative studies, though, calls for indicators [4, 5]. With predetermined goals, QbD is a systematic development approach that stresses product and process knowledge based on solid science and quality risk management [6]. Quality, safety, and efficacy are especially important for a drug product. Scientific techniques include quality by design (QbD) and process analytical technology are used to raise product quality. Beginning with defined objectives, ICH characterizes QbD as a systematic approach to development concentrating on product and process knowledge and control based on quality risk management and good science [7].

Forced degradation research offers a tool for assessing the stability of medicine samples in the pharmaceutical industry. Oxidation, photolytic damage, acid, and base were applied to samples to determine the stability of the traditional UV- spectroscopic method. In every experiment, the percentage decrease was determined. Reasonable and permissible threshold characterizes the research on forced degradation [8]. The chemical stability of the molecule affects the efficacy and safety of pharmaceutical products. Guidelines (Q1A) of the International Conference on Harmonization (ICH) stipulate that stability studies should be undertaken to provide fresh medical components and/or the shelf life of goods [9].

Designed goods and processes that minimize environmental effects by decreasing trash, conserving energy, and substituting safer materials form the basis of sustainable chemistry also known as green chemistry (GC) [10]. This is mostly attributable to a constant awareness of the state of the ecosystem and the damaging impact analytic methods could have on it. The most important approach developed to address this issue is green analytical chemistry (GAC). GAC is an environmentally friendly analytical chemistry technique aiming to minimize the negative consequences of analytical methods on public health and the environment [11].

A commonly researched phenolic substance, 4-hydroxy-3-methoxy-cinnamic acid is known for its antioxidant and medicinal characteristics; hence, measuring its presence in plant extracts is crucial for herbal formulations' quality control. Older analytical approaches mostly used conventional UV and chromatographic techniques without methodical optimization or environmental concerns. Ensuring robustness and dependability [12–13], the Quality by Design (QbD) method provides a scientific and risk-based framework for method development. Hence, the present research seeks to create and confirm a QbD-assisted UV spectrophotometric technique for the measurement of 4-Hydroxy-3-Methoxy-Cinnamic acid in *Alpinia galanga* (L.) Willd., rhizome extract, as well as to assess the greenness of the created process utilizing eco-analytical methodologies [14].

2. Materials and Methods

Instruments and apparatus

An ultrasonic bath Sonicator was used to sonicate the sample; absorbance was measured with a UV-Spectrophotometer (Shimadzu 1800); the analyte was weighed using a weighing scale (SARTORIUS).

Chemicals and reagents

Deionized water produced by a Milli-Q® Direct system at KLE College of Pharmacy, Belagavi served as the diluent, and analytical-grade methanol was employed as the solvent. Yucca Enterprises, Mumbai India, graciously gave 4-Hydroxy-3-Methoxy-cinnamic acid.

Method Development

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Choosing a solvent solution and identifying the analytical wavelength is important step in the UV-spectrophotometric technique. The solubility of 4-Hydroxy-3-Methoxy-Cinnamic acid was studied in several solvents like Methanol. Consequently, Methanol was the first stock used in the dilution process. For the secondary stock solution and working standards for 4-Hydroxy-3-Methoxy-Cinnamic acid, we employed Methanol: Deionised Water (50:50% v/v) as solvent system. DoE software and QbD concepts helped to optimise the solvent system [15, 16].

Selection of solvent and wavelength of analysis

A 10 µg/ml solution was scanned in the ultraviolet (UV) region between 200 and 400 nm to define the analysis wavelength. The spectrum obtained showed that 314 nm which was the wavelength with the most absorption [17].

Preparation of standard solutions

Ten milligrams of the compound were dissolved in a 10 ml volumetric flask to create a conventional stock solution of 4-Hydroxy-3-Methoxy-Cinnamic acid at a concentration of 1000 µg/ml. Methanol was used to thin the solution to volume. As solvents, mixtures of Methanol and distilled water were employed for the further dilutions. The stock solution was further serial dilution to create concentrations ranging from 2 µg/ml to 10 µg/ml [18].

Method Optimization by QbD Approach

Defining analytical target profile

The QbD method begins by establishing an analytical target profile matching the Quality Target Product Profile (QTPP). Linking the outcomes of the analytical method development process to attaining QTPP, the Analytical Target Profile (ATP) clarifies its goals. To develop an ATP, an extensive analysis of current literature including reports and drug profiles describing the physical and chemical features was carried out [15].

Discover fundamental quality analytical characteristics

Measurable analyte values called Critical Quality Attributes (CQAs) or Critical Analytical Attributes (CAAs) must lie within an appropriate and tolerable limit, range, or threshold for the analytical approach to function as planned. Absorbance distinguishes the UV approach.

Finding of important methodological variables**.** The sensitivity levels related with a certain analytical process are known as Critical Method Parameters (CMPs). Important technical aspects to keep in mind while creating UV methods are differences in solvent used, detection wavelength, scan speed, sampling interval, sample integrity, and slit width [15].

Fish-bone analysis of risk assessment

Risk analysis finds probable contacts with CMPs and judges the probability of future failure. Highlighting different methodological elements that might affect the method properties of the UV spectrophotometric technique of 4-Hydroxy-3-Methoxy-Cinnamic acid, a fishbone diagram was built in this regard [15].

Optimization of method by Department of Energy software

After a risk analysis found the Critical Method Variables, the focus turned on maximizing two key methodological elements: scanning speed and solvent ratio. The remaining method variables were maintained either at their lowest or best levels. Using the CCD within the DoE framework with an alpha value of one, the goal was to identify the best levels for scanning speed (A) and solvent ratio (B) to improve the robustness of the method. With one centered point, the CCD approach required 9 runs for the two chosen variables. One response variable absorbance at 314nm for 4-Hydroxy-3-Methoxy-Cinnamic acid was the focus of the investigation of the chosen independent variables, namely solvent ratio (A) and scanning speed (B). All tests carried out during the optimization research were done three times to guarantee reliability and precision [15].

Model confirmation and data analysis

Using the Design Expert program, the ANOVA was conducted to gather important metrics including p-value, f-value, R2 value, sufficient accuracy, among others. Several polynomial equations were created, all with a notable p-value lower than 0.5. For both critical method variables, graphs for normal graphs, predicted vs actual graphs, Residual vs Run Graphs, Residual vs Predicted graphs, and Cook's Distance graphs were

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

created. 2-D contour graphs and 3-D response surface charts were produced to show graphically the correlation among the selected independent technique response variables. Furthermore, overlay graphs were created to show the design space across all experimental areas [15].

Studies of Forced Degradation

Stress tests were carried out under conditions advised by ICH 9–11 to assess the stability-indicating capacity of the suggested UV method. Thermal, UV irradiation, alkaline, acidic, and oxidative conditions were applied to the bulk sample to induce forced degradation of 4-Hydroxy-3-Methoxy-Cinnamic acid [19].

Irradiation with ultraviolet light

UV light for 48 hours was applied to a sample powder of 10 mg of 4-Hydroxy-3-Methoxy-Cinnamic acid. Ten milliliters of methanol and distilled water evaporated the material. Claiming a concentration of 1 µg/ml, the solution was filtered using a syringe filtration disk.

Thermal decay

10 mg sample powder of 4-Hydroxy-3-Methoxy-Cinnamic acid was kept at 70°C for 48 hours in a hot air oven. 10 mL of methanol and distilled water were used to dissolve the analyte to prepare 1 mg/ml, the analyte solution and absorbance was measured.

Oxidative Degradation

Round-bottomed flask was loaded with precisely 10 µg/ml of 4-Hydroxy-3-Methoxy-Cinnamic acid solution. 9 mL of 30% hydrogen peroxide solution were then combined with the contents, which were then permitted to react at room temperature (25 degrees C) with intermittent shaking for two hours; absorbance was measured.

Hydrolysis using alkaline and acidic solutions

Ten µg/ml aliquots of 4-Hydroxy-3-Methoxy-Cinnamic acid solution were poured to a tiny round-bottom flask. Nine ml of either 0.1 N HCl or 0.1 N NaOH were combined with the answer. The prepared solutions were refluxed for two hours in a boiling water bath. Absorbance was measured and the samples were kept for 30 minutes to cool-down to room temperature (25 degrees Celsius).

Methods of Analytical Validation

Selectivity and specificity

Eliminating the chance of solvent interference especially in the area of highest absorption peak of 4-Hydroxy-3-Methoxy-Cinnamic acid, specificity testing was conducted. The specificity and selectivity of 4-Hydroxy-3-Methoxy-Cinnamic acid were determined by comparing spectra acquired from running both the drug solution and the solvent [20-25].

Linearity and range

To get concentrations between 2 µg/ml and 10 µg/ml, the standard stock solution of 4-Hydroxy-3-Methoxy-Cinnamic acid was diluted. UV analysis was done on the created solutions [20].

Precision

The absorbance of solutions of 4-Hydroxy-3-Methoxy-Cinnamic acid at three different concentrations was used to calculate the precision. Respectively, by analysis on the same day at two different times and three separate days, the intraday and interday accuracy were evaluated. For the absorbance values found, the %RSD was computed following every study [21].

Robustness

The roughness points to differences inside the laboratory conditions (varying analyst/instrument). By various analysts, three replicates of 2µg/ml, 6µg/ml, and 10µg/ml 4-Hydroxy-3-Methoxy-Cinnamic acid solutions were made and UV tested on the same instrument. % RSD numbers were computed [20-24].

Accuracy

Three different degrees of recovery studies were carried out to assess the correctness of the predetermined strategy. To evaluate recoveries, samples were spiked with 80%, 100%, and 120% standard solution and the mix was UV analyzer tested. The three-fold analysis of the recovery [22-25].

Quantification of 4-Hydroxy-3-Methoxy-Cinnamic acid in the Rhizome Extract of *Alpinia galanga* (L.) Willd.

Then, 10 mg of extract form was weighed and put into a 10 mL volumetric flask, sonication with an appropriate methanol as solvent for ten minutes was performed, then filtered. Serial dilutions were made to get a 4-

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Hydroxy-3-Methoxy-Cinnamic acid concentration of 10 µg/ml, which was then spectrophotometrically determined.

Measuring Greenness Using AGREE Score

Environmental sustainability of the proposed analytical approach was assessed using the AGREE measure, which is founded on the 12 principles of Green Analytical Chemistry. The method shown excellent greenness with a total AGREE score of 0.74. High ratings (1.0) were given to low energy consumption, reagent renewable use, process integration, and minimum sample requirements. Though in-situ analysis and operator safety did fairly, direct analysis, derivatization prevention, automation, and multi-parameter capability showed excellent compliance. Lower scores were given to analytical waste management and the usage of hazardous chemicals, therefore pointing regions that still need work [26,27].

3. Results and Discussion

4-Hydroxy-3-Methoxy-Cinnamic acid showed a spectrum with maximum absorbance at 314 nm (Figure 1) in a Methanol: Distilled water (50:50% v/v) solvent mixture. As detailed in the methodology section, QbD principles helped to maximize the approach. Table 1 lists the optimized process parameters.

Table 1: Developed UV-Spectrophotometric method for 4-Hydroxy-3-Methoxy-Cinnamic acid

Sr. No.	Parameters	Specifications
1	Method	Spectrophotometric
2	Instrument	UV-Spectrophotometer
3	Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Analyte	4-Hydroxy-3-Methoxy-Cinnamic acid
7	Solvent	Methanol: Distilled water (50:50% v/v)
8	Lambda Max.	314nm

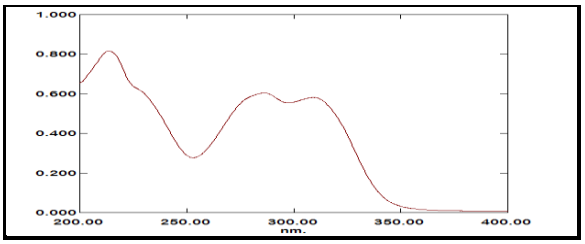

Figure 1: UV-Spectrum of 4-Hydroxy-3-Methoxy-Cinnamic acid

Method Optimization Using a QbD Method

The several phases in the QbD-based approach optimization were performed methodically. ATP specifies the expected measurement process needs. Knowledge and scientific reasoning about the analytical approach help to create the analytical target profile. Depending on the main objective of this study, a UV spectrophotometric approach was therefore used for the rapid measurement of 4-Hydroxy-3-Methoxy-Cinnamic acid. For the suggested UV-spectrophotometric technique for 4-Hydroxy-3-Methoxy-Cinnamic acid, table 2 shows ATP. The absorbance generated by the medication was found in the suggested UV technique to be a key quality trait of the analyte. Highlighting several method factors that could affect the UV spectrophotometer's performance for 4-Hydroxy-3-Methoxy-Cinnamic acid, an Ishikawa fishbone diagram was developed to pinpoint possible hazards and underlying causes.

Table 2: ATP for UV analysis of 4-Hydroxy-3-Methoxy-Cinnamic acid

Sl. No.	ATP Parameters	Target
1	Target Analyte	4-Hydroxy-3-Methoxy-Cinnamic acid
2	Target Sample	*Alpinia galanga* extract
3	Method category	UV-spectrophotometric method
4	Instrument	UV-spectrophotometer

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

5	Nature of analyte	Solid (Solution)
6	Standard stock solution preparation	Dilution of the main drug in a linear manner
7	Application of Method	Estimation of 4-Hydroxy-3-Methoxy-Cinnamic acid
8	Validation parameters	Accuracy, Selectivity, ruggedness, precision, linearity, specificity, LOD and LOQ

DoE Software and method improvement

Using the CCD inside the DoE framework with an alpha value of one, the method was refined. To increase the method's resilience, the best parameters for solvent ratio (B) and scanning speed (A) had to be found. Table 3 presents the choosing of CCD design levels. The CCD technique included 9 runs for the two designated variables, including one centered point (Table 4). The data were entered into the computer and subjected to additional analysis following the CCD-indicated tests.

Analysing data

Using a statistical approach, notably the CCD, the crucial analytical factors were best tuned. Using the Design Expert tool, 9 experimental runs for the identified independent variables—scanning speed and solvent ratio using the CCD technique generated a design plan. Table 4 presents the specifics of this experimental plan. For investigation, the absorbance at 314nm was chosen as the response or dependent variable. Several linear regression analysis was applied to the experimental data. The data was analyzed using a second-order quadratic model created through a coded equation following the development of the mathematical model employing software.

Table 3: Selection of levels for CCD

Independent Variable	Levels		
	+1	**0**	**-1**
Scanning Speed (A)	**+1 (High)**	**0 (Medium)**	**-1 (Low)**
Solvent Ratio (B)	100%	50%	0%

Table 4: Experimental design matrix by CCD

Run	Factor 1 (A: Solvent Ratio)	Factor 2 (B: Scanning Speed)	Response 1 (Absorbance)
1	0	1	0.945
2	100	1	1.118
3	100	0	1.120
4	100	-1	1.129
5	50	-1	0.952
6	50	0	0.946
7	0	0	0.948
8	0	-1	0.947
9	50	1	0.965

Responses equation in coded form

Various levels, categorised as low (-1) and high (+1), coded factor equations were used to forecast the correlation between independent and dependent variables. ANOVA was employed for statistical research of the chosen components. With a p-value less than 0.05 showing the model's appropriateness, the model was judged using the mismatch, R2, and adjusted R2 values. A major lack of fit implies that the model falls short to explain the variations between predicted and observed data points. With values approaching 1 being ideal, ANOVA R2 values close to 1 reveal how well the expected model corresponds with the experimental one. For the selected response, Absorbance at 314 nm, Table 5 shows several ANOVA studies taking into account p-value, lack of fit, and R2 value among others. The results in Table 4 show p-values under 0.05 for both the model and the variables, therefore highlighting the importance of the created model. The response's F-values of 179.23 emphasize the need of the model. In the created model, the probability of mistakes depending on the F-value is found to be 0.01 percent.

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Table 5: ANOVA results for UV method at 314 nm

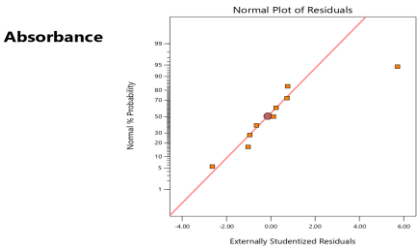
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0592	5	0.0118	179.23	0.0007	**Significant**
A-solvent ratio	0.0463	1	0.0463	700.65	0.0001	
B-scanning speed	0.0000	1	0.0000	0.0000	1.0000	
AB	0.0000	1	0.0000	0.3065	0.6184	
A^2	0.0000	1	0.0000	0.6593	0.4763	
B^2	0.0129	1	0.0129	194.56	0.0008	
Residual	0.0002	3	0.0001			
Cor Total	0.0594	8				

Shown in Figure 2a-c are several graphs created in the design—the predicted versus actual graph, box-cox graph, and normal graph—all illustrating acceptable reaction criteria relative to parameters. Two-dimensional contour maps and 3-D response surface diagrams made with Design Expert software were included in Figure 3a-b to show how variables and reaction interact. Analysis of these graphs yielded the conclusion that absorbance declines with low and moderate values of both chosen factors: scanning speed and solvent ratio.

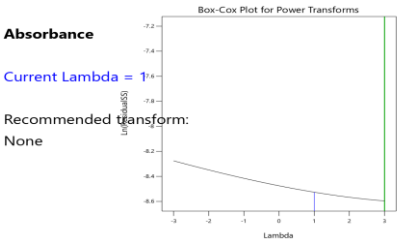
Design Space

Figure 4 highlights the design space essential for maximizing the chosen response, absorbance at 314 nm, regarding the selected parameters (solvent ratio and scanning rate). The overlay graph shows the design space in dark yellow within the experimental area in grey; the region in light yellow marks the location where several variables can be changed.

a. Normal Graph



b. Box-Cox



c. Predicted vs actual graph

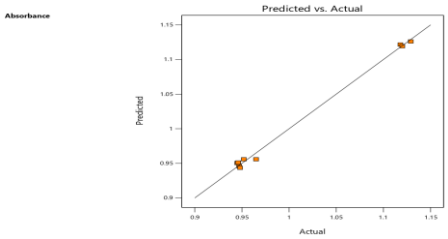
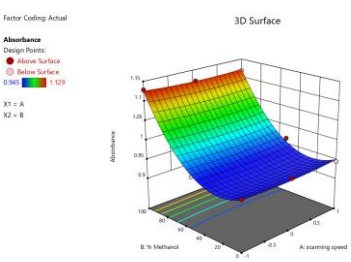


Figure 2: (a) Normal graph (b) Box-Cox graph (c) Predicted vs actual grap

(a) 3-D Response surface graph



Journal of Chemical Health Risks

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

(b) 2-D contour graph

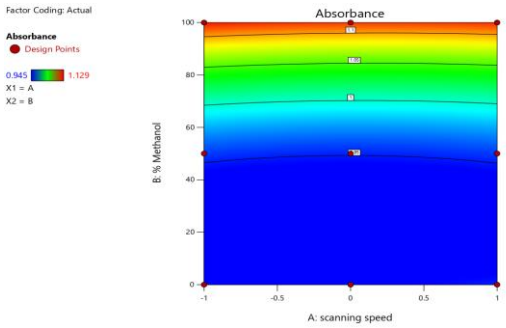

Figure 3: (a) 3-D Response surface graph (b) 2-D contour graph

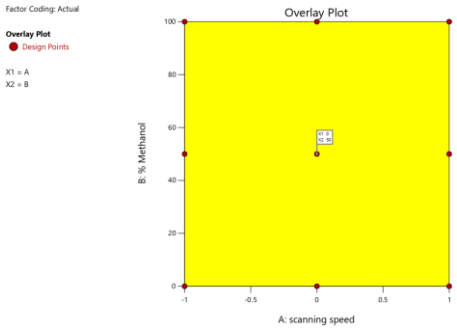

Figure 4: Overlay graph showing the design space from experimental area

Analytical Method Validation [16-25]

Selectivity and specificity

The method's specificity and selectivity are shown by the solvent spectrum's absence of absorbance interference at 314nm. The UV spectrum of the solvent and 4-Hydroxy-3-Methoxy-Cinnamic acid is shown in Figures 5 and 6.

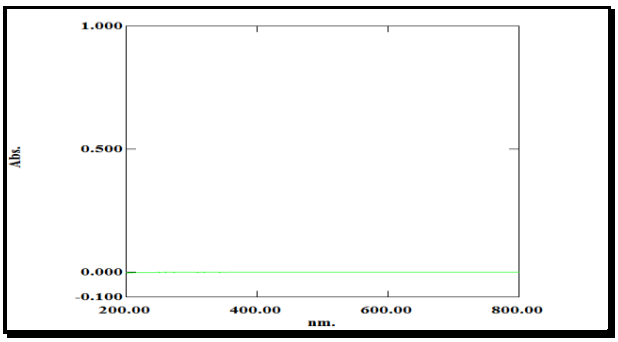

Figure 5: UV Spectrum of blank solvent

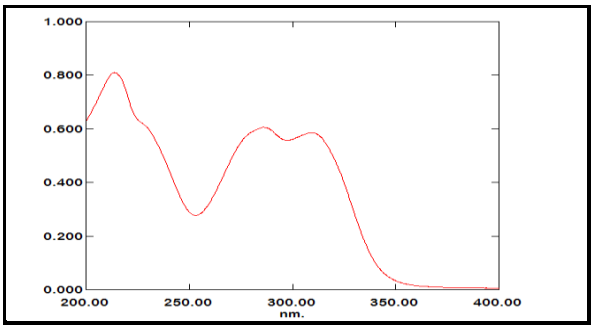

Figure 6: UV-Spectrum of 4-Hydroxy-3-Methoxy-Cinnamic acid (10µg/ml)

Linearity and Range

Plotting 4-Hydroxy-3-Methoxy-Cinnamic acid 's linear dilution concentration against absorbance yields a typical calibration curve. With an r2 of 0.9997, 4-Hydroxy-3-Methoxy-Cinnamic acid had a linear absorbance range of 2, 4, 6, 8, and 10 µg/ml. Linearity data is shown in Table 6. Standard calibration curves are illustrated in Figure 7. Figure 8 shows the overlay spectrum of linearity for 4-Hydroxy-3-Methoxy-Cinnamic acid.

Sensitivity

Respectively, the LOD and LOQ values were determined to be 0.520 µg/ml and 1.789 µg/ml.

Precision

A % RSD of under 2% for three duplicate 4-Hydroxy-3-Methoxy-Cinnamic acid solutions at each precision level showed the method's accuracy. Data for precision are presented in Table **7**.

Ruggedness

The method was shown to be reliable as the %RSD of each concentration carried out by several analysts and examined on different instruments remained within acceptable limits Table 8.

Robustness

Robustness is the capacity of an analytical technique to stay unchanged by little, deliberate changes in variables, hence showing its dependability under typical conditions. Table 9 presents UV spectroscopic technique Robustness results for 4-Hydroxy-3-Methoxy-Cinnamic acid.

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Accuracy

The recovery values are within acceptable limits and hence, the approach was found to be correct. Table 10 shows the accuracy statistics.

Studies of Degradation

The varied degrees of degradation seen under all applied stressors showed that 4-hydroxy-3-methoxy-cinnamic acid was prone to stress-induced degradation. With the least degree of degradation (6.94%), photolytic (19.79%) and alkaline (18.01%) conditions displayed the most, followed by oxidative (13.07%) and thermal (16.83%) stress. The behavior of deterioration shows that oxidative and hydrolytic processes produce degradation. Based on these data, the UV spectrophotometric method shows stability. The forced deterioration findings are summarized in Table 11.

Table 6: Linearity data of 4-Hydroxy-3-Methoxy-Cinnamic acid

Sl. No.	Concentration (µg/ml)	Absorbance at 314nm
1	2	0.184
2	4	0.366
3	6	0.557
4	8	0.764
5	10	0.989
r^2		0.9997

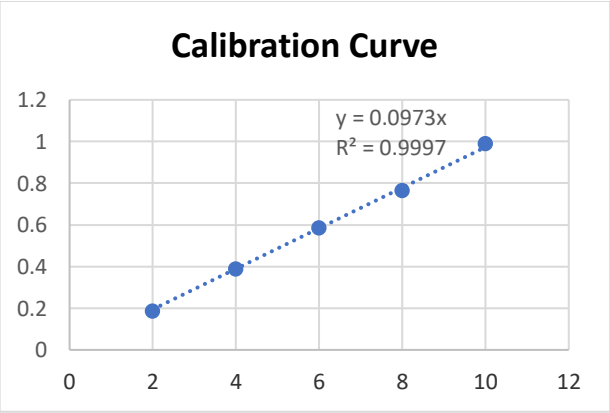

Figure 7: Calibration Curve of 4-Hydroxy-3-Methoxy-Cinnamic acid

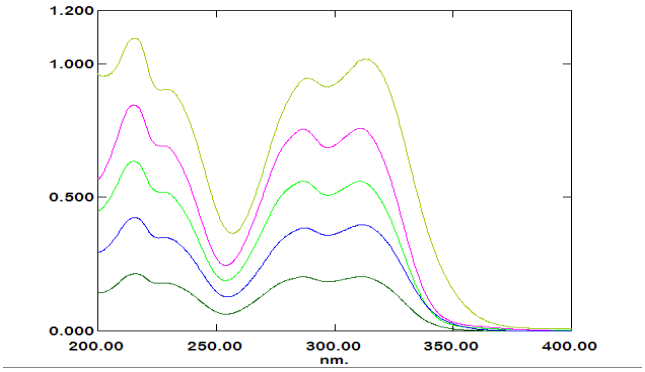

Figure 8: Linearity Overlay Spectrum of 4-Hydroxy-3-Methoxy-Cinnamic acid

Table 7: Precision data of 4-Hydroxy-3-Methoxy-Cinnamic acid

Precision	Concentration (µg/ml)	Mean Absorbance	Standard Deviation	%RSD
Intraday	2	0.190	0.001	0.53
	6	0.580	0.001	0.52
	10	1.00	0.003	0.49
Interday	2	0.203	0.001	1.02
	6	0.590	0.001	0.42
	10	1.050	0.016	0.72

Table 8: Ruggedness data of 4-Hydroxy-3-Methoxy-Cinnamic Acid

Precision Type	Concentration (µg/ml)	Mean Absorbance	SD	%RSD
Change in Analyst	2	0.210	0.001	0.74
	6	0.612	0.003	0.43
	10	1.004	0.011	0.51
Change in Instrument	2	0.193	0.002	1.03
	6	0.614	0.004	0.50
	10	1.00	0.021	0.31

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Table 9: Robustness data of 4-Hydroxy-3-Methoxy-Cinnamic Acid

Parameter	Concentration	Wavelength			Mean	SD	%RSD
		312	314	316			
Robustness	2	0.194	0.197	0.201	0.20	0.00	1.52
	6	0.580	0.586	0.586	0.58	0.00	0.59
	10	1.023	1.012	0.986	1.01	0.02	1.89

Table 10: Accuracy data of 4-Hydroxy-3-Methoxy-Cinnamic Acid

Level	Original (µg/ml)	Standard Added (µg/ml)	Total Found (µg/ml)	%Recovery
80%	10	8	17.9	98.75%
100%	10	10	19.8	98.00%
120%	10	12	21.9	99.17%

Table 11: Degradation data of 4-Hydroxy-3-Methoxy-Cinnamic Acid

Concentration (µg/ml)	Stress	Absorbance	Amount found	Amount degraded	% Degraded
10	Acid	0.941	9.30649	0.69351	6.9351
	Base	0.829	8.19881	1.80119	18.0119
	Thermal	0.841	8.31749	1.68251	16.8251
	Oxidative	0.879	8.69331	1.30669	13.0669
	Photolytic	0.811	8.02079	1.97921	19.7921

Quantification

The estimated 4-Hydroxy-3-Methoxy-Cinnamic acid in the *Alpinia galanga's* rhizome extract benefited from the developed approach. The concentration of 4-Hydroxy-3-Methoxy-Cinnamic acid in the rhizome extract of Alpinia galanga was discovered to be 0.099 ± 0.003% w/w (n = 3), showing precise, accurate, and repeatable quantification inside permissible analytical limits.

Greenness Evaluation of the Developed Procedure

AGREE

Using a contour to score an element or parameter, an AGREE pictogram defines the total safety score following the lowering of penalties for straying from safety, therefore it is more important. Usually seen on the scale (Figure 9), a mark greater than 0.7 is thought of as green. Out of the answers of twelve parameters relevant to the developed instrumental approach, the black line on the scale showed that the developed method was safer for the environment; the AGREE score was discovered to be near to the green zone. (26)

GAPI

Figure 10 shows the results of the Green Analytical Procedure Index (GAPI), a pictograph used to evaluate the environmental friendliness of the already established UV spectrophotometric methods for 4-Hydroxy-3-Methoxy-Cinnamic acid. Employing methanol:water (50:50, v/v) as the solvent system, the Q-absorbance approach showed six green zones, seven yellow zones, and one red zone. This suggests an organic solvent had a negligible environmental impact. In contrast, the mixed hydrotropy method displayed superior green performance with eleven green zones, three yellow zones, and just one red zone, therefore pointing to a lesser environmental load. Taking everything into consideration, the GAPI results validate the mixed hydrotropy technique as being more environmentally benign than the Q-absorbance method. (27)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2796-2808 | ISSN:2251-6727

Figure 9: Greenness score for the developed method

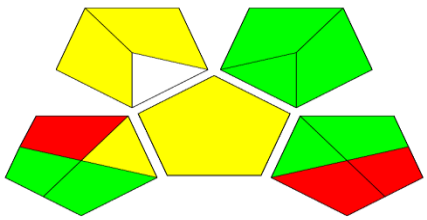

Figure 10: GAPI scores for the determination of 4-Hydroxy-3-Methoxy-Cinnamic Acid

4. Conclusion

This study shows how to quantify 4-Hydroxy-3-Methoxy-Cinnamic Acid using an inexpensive and repeatable UV-spectrophotometric technique. Response surface methodology and central composite design optimization explained the interaction and impacts among important response factors. According to ICH standards, the method was thoroughly validated. The validation parameters showed the uniqueness, linearity, accuracy, precision, and improved sensitivity as seen by reduced LOD and LOQ values. Combining QbD with ICH standards prepares this method for dependable use in pharmaceutical quality control labs to measure 4-Hydroxy-3-Methoxy-Cinnamic Acid. For routine quantification of 4-Hydroxy-3-Methoxy-Cinnamic acid in herbal extracts or products, the technique is found to be suitable.

Conflicts of Interest: The authors declare no conflicts of interest, financial or otherwise.

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Consent for Publication: Not applicable.

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