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Green Analytical Chemistry Based Quality by Design Assisted UV Spectroscopic Method Development and Validation for Estimation of Chrysin

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Abstract The objective of current research work was to integrate Quality by Design (QbD) approach with the principles of green analytical chemistry with a focus on minimizing detrimental environmental effects. A cost-efficient UV spectroscopic method was developed and validated as per ICH guidelines for chrysin analysis. Water was used as a major solvent. Sonication time and scanning interval were chosen as independent variables and absorbance was selected as dependent variables. DoE was used to optimize trial runs. To identify the optimized spectroscopic method, a simple 3^2 factorial design with 2 factors and 2 levels was employed, resulting in 9 experimental runs. The optimized run was extensively validated as per ICH guidelines. A linear relationship was determined between the concentrations ranging from 2 to 10 µg/ml, with a regression coefficient of 0.998. Both precision assays showed a % RSD less than 2 indicating the precise nature of the method. The percentage recovery ranged from 99.84 to 101.26% indicating promising accuracy. Sensitivity of the proposed method is proved by its LOD and LOQ values which are 0.51 µg/mL and 1.57 µg/mL respectively. The % RSD less than 2 indicates that the developed method is rugged and robust. Additionally, the method exhibited promising repeatability. Hence, it can be concluded that integration of QbD and Green chemistry offers a promising avenue for developing a cost-effective and environment friendly analytical method for chrysin analysis.

Keywords Quality by Design, Chrysin, UV Spectroscopic, Green Analytical Chemistry, Validation

1. Introduction

Medicinal plants with therapeutic qualities remain fundamental components of different traditional medicines. Plant-derived remedies are essential for human development, playing an integral role alongside basic necessities like food, water, and shelter. Health, being a crucial requirement for individuals heavily depends on natural resources [1]. The demand for plant-based therapeutic products is on the rise due to their natural composition, which ensures fewer side effects and affordable prices. As a result, these products have proven to be beneficial for human health [2]. Chrysin is a flavonoid that occurs naturally in honey, propolis, and various plant species. The remarkable biological effects of chrysin can be attributed to its antioxidant and anti-inflammatory properties, which play a crucial role in combating inflammation and oxidative stress associated with numerous disease conditions [3]. Chrysin is officially recognized by the U.S. Food and Drug Administration as a nutraceutical that is deemed "generally recognized as safe" (GRAS) [4]. Chrysin holds great potential therapeutic

candidate that can be effectively utilized in the prevention of numerous diseases, including cancer, diabetes, and neurodegenerative disorders [5]. Substantial progress has been achieved in the analytical methods, such as chromatography, spectroscopy, DNA barcoding, and others, which have been utilized to ascertain the identification and purity of herbal products [6]. The use of modern analytical instruments is essential for maintaining the quality and standardization of phytopharmaceuticals. Spectroscopic techniques are especially vital for the accurate analysis and validation of herbal products. Spectrophotometry stands out as a preferred method due to its ease of use, precision, and cost-effectiveness [7, 8].

The utilization of the QbD approach is becoming increasingly prominent as a contemporary concept in the development and evaluation of quality pharmaceutical products [9]. The application of the QbD approach serves as a practical solution to reduce the experimental time and cost associated with drug analysis. By prioritizing the quality of the analytical process from the outset of development, the QbD approach facilitates a thorough analysis of method variables and their interactions. Ultimately, it offers a region for a highly robust and cost-effective approach [10]. In order to overcome the challenges encountered in method development, analytical scientists have progressively adopted a systematic QbD-based approach. Consequently, they have made efforts to apply the QbD approach to analytical methods [11].

Green Analytical Chemistry (GAC) is now being increasingly embraced by researchers aiming to lessen environmental hazards and enhance the safety. GAC involves the removal or reduction of harmful chemicals from analytical procedures in order to enhance their environmental and health benefits, while maintaining method efficiency [12]. The significant challenge lies in developing the suggested green analytical method by carefully selecting green, less toxic solvents to ensure better separation and quantitative analysis. This investigation utilizes green solvents for their positive impact on society, economy, and the environment, as well as their eco-friendly, non-toxic, hazard-free, and easily manageable waste disposal features in line with ICH guidelines [13].

The literature review underscores the limited availability of spectroscopic techniques for estimating chrysin. By incorporating the QbD approach and principles of GAC, a thorough comprehension of the method was attained while mitigating potential adverse environmental impacts. Accordingly, the current research efforts were directed towards the development and validation of an eco-friendly UV spectroscopic method for chrysin estimation by Quality by Design (QbD) approach.

2. Materials and Methods

2.1. Materials

Analytical grade chrysin was procured from Yucca enterprises, Mumbai, India. All other chemicals and reagents were used of analytical grade and obtained from KLE College of Pharmacy, Belagavi, India.

2.2. Instrumentation

The analysis was conducted using a Shimadzu UV-Spectrophotometer model 1900i with Labsolution software. Optimization was carried out using Design-Expert software version 13.0.

2.3. Selection of Solvents

An extensive study on various analytical solvents has been conducted, taking into account the drug characteristics. After conducting multiple experiments, water and methanol were selected as the solvent combination in the present study.

2.4. Preparation of Stock Solution

An accurately weighed quantity of chrysin (10 mg) was dissolved in methanol and water (1:1) followed by sonication for 5 minutes. Further, a series of dilutions were prepared using the above stock solution and diluted with only water up to the mark.

2.5. Selection of Wavelength

The UV Spectrophotometer was used to scan chrysin (10 µg/ml) between 200-400 nm, with the highest absorption at 270 nm.

2.6. DoE Based Method Development and Optimization

2.6.1. Defining Analytical Target Profile (ATP) and Critical Analytical Attribute (CAA)

The implementation of the QbD approach in analytical method development necessitates the initial establishment of Analytical Target Profiles (ATP). It is necessary to establish the key features that serve as the indicators of method performance. The UV spectrophotometric method was selected based on its rational justification, which lies in its simplicity and faster analysis compared to other complex analytical methods. To comply with the ATP, the absorbance of chrysin was chosen as the CAA [14,15].

2.6.2. Optimization of Method

The effect of selected process parameters and their responses were studied by optimizing the UV spectrophotometric method using DoE. To identify the optimized spectroscopic method, a simple 3^2 full factorial design with 2 factors and 2 levels was employed, resulting in 9 experimental runs. The experimental design was developed using Design-Expert software version 13.0, Stat-Ease Inc. in Minneapolis, MN, USA [16,17].

2.7. Method Validation

The validation parameters, such as linearity, specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness repeatability were assessed in accordance with the ICH Q2 (R1) guidelines [7,18-21].

2.7.1. Specificity and Selectivity

The determination of chrysin concentration in pharmaceutical products heavily relies on the specificity and selectivity of the analytical method. These factors are crucial in determining the practicality of the method. An assessment of specificity and selectivity parameters was conducted to confirm that there was no interference from any other component during the analysis of chrysin. The specificity and selectivity of the developed method were confirmed by the absence of absorbance at a wavelength of chrysin at 270 nm.

2.7.2. Linearity

The linearity of a method is determined by the relationship between the concentrations and absorbance of the sample solutions. To assess the linearity of chrysin, a series of five samples with concentrations of 2 to 10 µg/mL were prepared using a secondary stock solution. The data obtained from these samples was utilized to construct a linearity curve, from which the correlation coefficient and regression equation were derived.

2.7.3. System Precision

Analytical method accuracy is defined by the agreement between measurements obtained from multiple samplings of identical samples under similar conditions. Precision testing was conducted through intraday and interday tests. The % RSD was calculated by measuring absorbance at three different intervals on the same day and three different days.

2.7.4. Accuracy

The precision of an analytical technique is established by computing the percentage variance between the average experimental value and the actual value. To validate the precision, recovery studies were performed, and the average recovery percentage of the sample was determined at three different levels: 80%, 100%, and 120% of the sample solutions.

2.7.5. Sensitivity

The sensitivity of the proposed method was evaluated by determining the limit of quantification (LOQ) and limit of detection (LOD). Both LOD and LOQ are determined by considering the standard deviation of the response and the slope of the calibration curve by following the formula.

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

Where σ is the standard deviation and S is the slope of the calibration curve.

2.7.6. Ruggedness

In order to assess ruggedness, the method was replicated on two instruments, and independent analysts were assigned to examine the repeatability. Subsequently, the repeatability data was utilized to calculate the percentage relative standard deviation (RSD).

2.7.7. Robustness

The developed method's robustness is exemplified by its capacity to withstand minor deliberate variations in the optimal parameters without being affected. To evaluate its robustness, analysis was conducted at different wavelengths and the percentage relative standard deviation (RSD) was calculated.

2.7.8. Repeatability

The term intra-assay precision, also known as repeatability, is used to define this specific characteristic. To evaluate the repeatability of the UV method, the absorbance of the chrysin (6 µg/mL) was measured six times. Subsequently, the percent relative standard deviation (RSD) was determined.

3. Results and Discussion

An ecofriendly QbD assisted UV Spectroscopic method for chrysin estimation was successfully developed and validated as per ICH guidelines.

3.1. Selection of Solvents

A thorough literature survey was done on available resources. Many trials and experimentation were carried out. Initially, chrysin was dissolved in methanol followed by water. The subsequent dilutions were dissolved only in water up to the mark and used for the analysis. Water was used as a major solvent throughout the experiments.

3.2. Selection of Wavelength

The maximum absorbance wavelength of chrysin was detected at 270 nm by scanning 10 µg/ml at 400-200 nm with water as a blank.

3.3. Method Development and Optimization

According to the ICH Q8 (R2) guidelines, QbD was utilized to optimize the UV-Spectroscopic method. The decision to employ the QbD approach for optimizing the quality characteristics of the UV-visible spectrophotometer was driven by the need to obtain relevant experimental data. The QbD principle strategy facilitated the development of the analytical target profile, which could assist in determining the concentration in samples. In order to achieve the analytical profile, chrysin absorbance was identified as the critical analytical attribute (CAA). Additionally, face centered design was chosen as the suitable QbD for assessing and optimizing the UV-visible spectrophotometer method by adjusting parameters such as sonication time and scanning interval.

3.3.1. Optimization of Method

Extensive optimizations were conducted to pinpoint the optimal choice within the given design space. Furthermore, the effect of variables like sonication time and scanning interval on the response (absorbance) was assessed. The optimization process involved overlaying the contour of the critical response with contour plots using design expert software. Table 1 specifies ANOVA analysis of the response to the absorbance of chrysin at 270nm with a significant p-value < 0.0001. Figure 1 represents a 3D response plot of chrysin. Figure 2 shows a 2D contour plot showing the correlation between the selected variables i.e., sonication time and scanning interval and response. Figure 3 illustrates the overlay plot of the optimized method. Optimum conditions were determined based on the overlay plot and desirability criteria. i.e., sonication time of 10 mins with medium scanning interval.

Table 1. ANOVA analysis of response i.e., Absorbance at 270nm

Responses	**Absorbance at 270 nm**		**Remarks**
Source	F-value	p-value	
Model	447.30	0.0002	Significant
A-Sonication time	2121.95	< 0.0001	
B-Scanning Interval	7.73	0.0689	
AB	1.49	0.3087	
A^2	0.2441	0.6552	
B^2	105.09	0.0020	

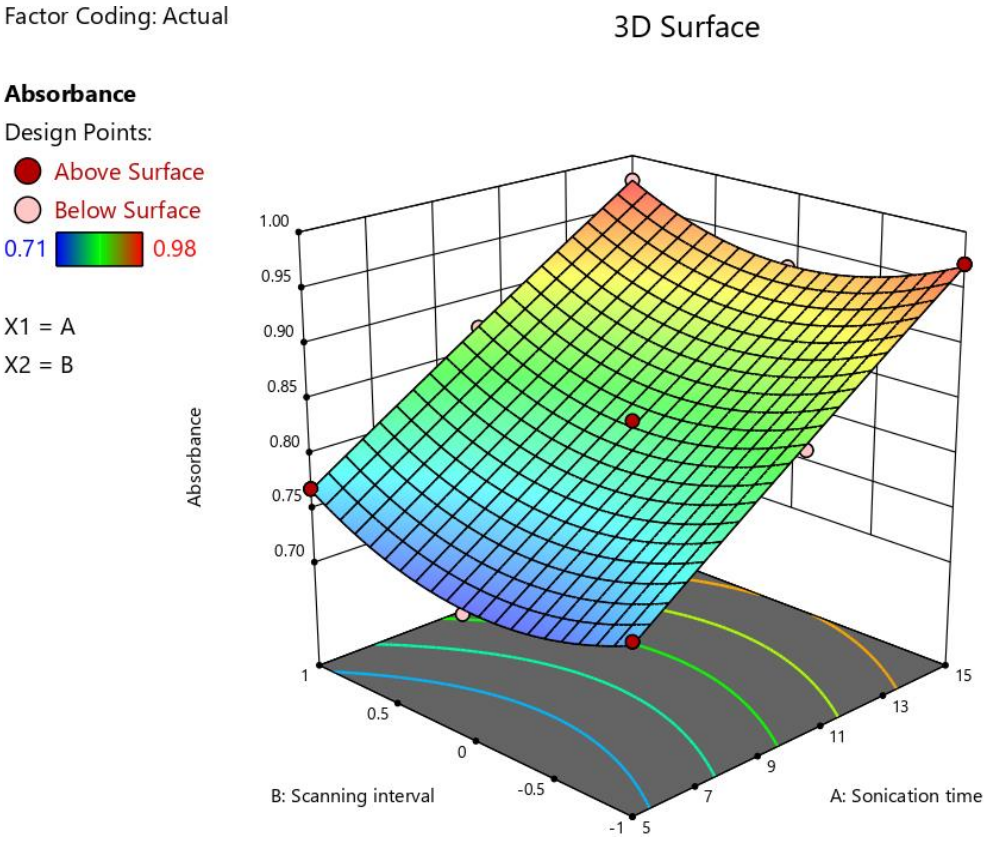

Figure 1. 3D plot of response of chrysin

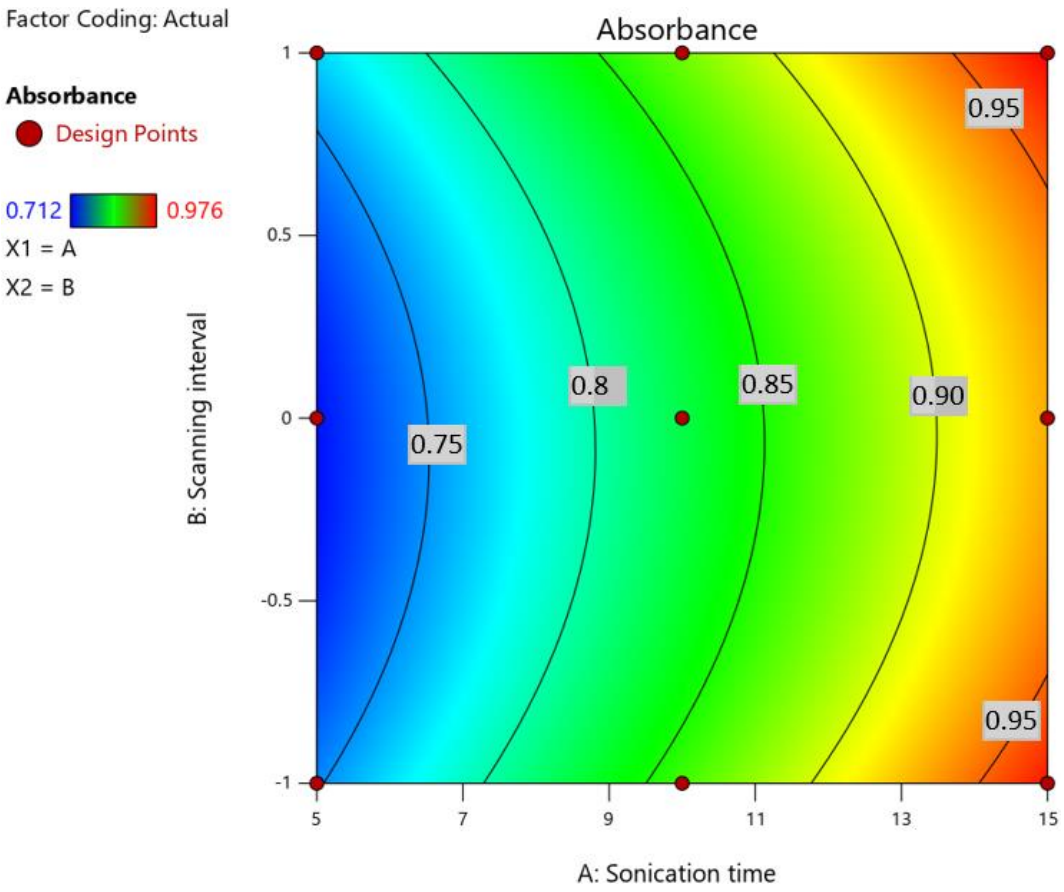

Figure 2. 2D contour plot showing the correlation between the selected variables i.e., sonication time and scanning interval with absorbance of chrysin

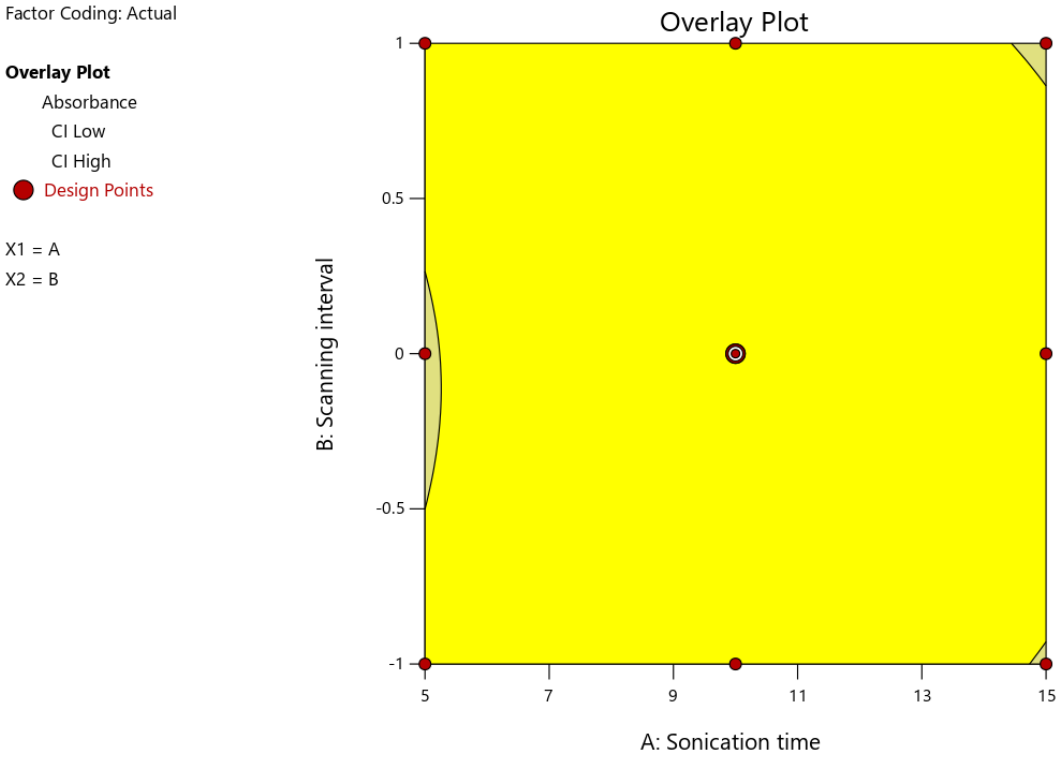

Figure 3. Overlay plot showing the design space from experimental area

3.4. Method Validation

As per ICH Q2 (R1) guidelines, the optimized method was validated.

3.4.1. Specificity and Selectivity

Selectivity is a typical problem in UV-vis spectroscopy when dealing with complex samples, as a few components can cause interference with the absorption spectra of the desired analytes. The specificity of the technique is evident as chrysin exhibits the highest absorbance at 270nm and the selectivity of this method was confirmed by the lack of absorbance at the wavelength of 270 nm in the solvent's spectra. Figure 4 represents UV spectrum of solvent (water) and Figure 5 represents UV spectrum of chrysin showing absorbance at 270nm.

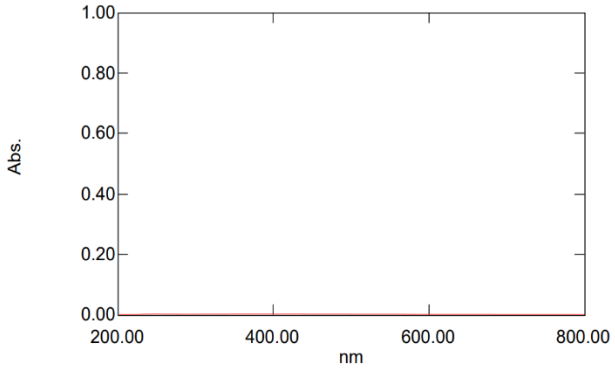

Figure 4. UV spectrum of blank (Water)

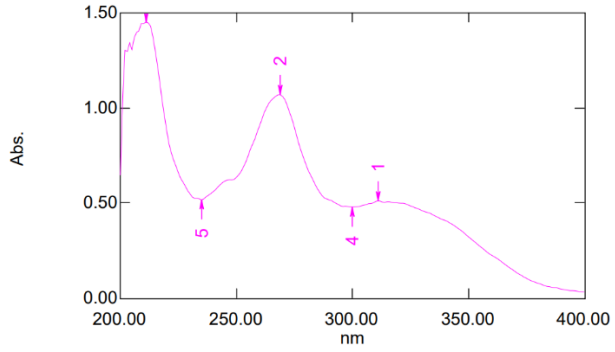

Figure 5. UV spectrum of chrysin at 270nm

3.4.2. Linearity

Linearity is the effectiveness of the analytical method in producing test results that are directly linked to the concentration of the analyte within a given range, as determined by the variance of the slope of the regression line. A linear relationship was determined between the concentration ranging from 2 to 10 µg/ml, with a regression coefficient of 0.998 (Figure 6 and Table 2), demonstrating a strong correlation between concentration and absorbance.

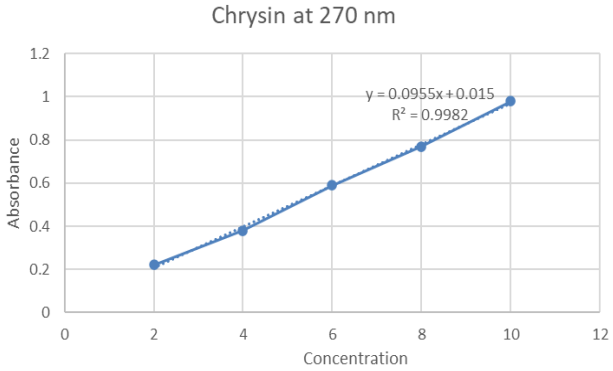

Figure 6. Linearity curve for chrysin

Table 2. Linearity of chrysin

Sr. No	Conc (µg/ml)	Abs (nm)
1.	2	0.22
2.	4	0.38
3.	6	0.59
4.	8	0.77
5.	10	0.98
R^2		0.9982
LOD		0.51 µg/ml
LOQ		1.57 µg/ml

3.4.3. System Precision

Validation of the method included evaluating its precision in terms of both intra-day and inter-day measurements. Intra-day precision was determined by measuring absorbance in the morning, afternoon, and evening on the same day, while inter-day precision was assessed over three consecutive days. The % RSD values were found to be below 2%, demonstrating the excellent precision of the method. Results are depicted in Table 3 and Table 4.

Table 3. Intraday precision assay

Intraday Precision				
Conc (µg/ml)	%RSD	M	A	E
2		1.26	1.49	1.36
6		1.80	1.22	0.97
10		1.01	1.21	1.06

Table 4. Interday precision assay

Interday Precision				
Conc (µg/ml)	**%RSD**	**Day 1**	**Day 2**	**Day 3**
2		1.35	1.32	1.84
6		0.96	1.20	1.23
10		1.16	1.02	1.11

3.4.4. Accuracy

Precision indicates the proximity of the correlation between the actual value and the measured value. The chrysin's percentage recovery ranged from 99.84 to 101.26%. The consistent recovery percentages at varying concentrations highlighted the efficacy of the developed method with a satisfactory level of accuracy. The results are depicted in Table 5.

Table 5. Accuracy/ percentage recovery of chrysin

Level	Absorbance	% Recovery
80%	1.117	101.25
	1.208	
	1.201	
100%	1.432	101.26
	1.487	
	1.478	
120%	1.725	99.84
	1.732	
	1.737	

3.4.5. Sensitivity

In order to evaluate the sensitivity of the method, the limit of detection (LOD) and the limit of quantification (LOQ) were computed. The LOD was calculated by multiplying 3.3 with the ratio of the standard deviation of the intercept to the slope of the calibration curve. Likewise, the LOQ was determined by multiplying 10 with the same ratio. The analysis revealed that the LOD and LOQ for chrysin were 0.51 µg/mL and 1.57 µg/mL respectively, demonstrating the excellent sensitivity of the proposed method.

3.4.6. Ruggedness and Robustness

The ruggedness of the method was confirmed through analysis carried out by two distinct analysts, with the absorbance for a concentration of 10 µg/ml being recorded. The robustness was assessed by measuring the absorbance at 270 ±2. The % RSD values were calculated, which were below 2%, demonstrating the method's repeatability, reproducibility, and robustness. Table 6 and Table 7 represent results of ruggedness and robustness respectively.

Table 6. Ruggedness of chrysin with change in analyst

Ruggedness			
Conc (µg/ml)	**%RSD**	**Change in analyst**	
		Analyst I	**Analyst II**
2		1.63	1.70
6		1.03	1.47
10		0.97	1.41

Table 7. Robustness of chrysin with change in wavelength

Robustness				
Conc (µg/ml)	**%RSD**	**Change in Wavelength (±2nm)**		
		268 nm	270 nm	272nm
2		1.83	1.79	1.82
6		0.98	0.80	0.97
10		1.16	0.76	0.76

3.4.7. Repeatability

Intra-assay precision is another term used to describe repeatability. Precision is characterized as the ability to consistently produce the same results in a brief timeframe under identical conditions. Results are depicted in Table 8.

Table 8. Repeatability of chrysin analysis

Sr. No	Conc (µg/ml)	Abs (nm)	% RSD
1.	6	0.628	0.74
2.	6	0.627	
3.	6	0.623	
4.	6	0.619	
5.	6	0.621	
6.	6	0.616	

4. Conclusions

Chrysin is a well-known herbal bioactive compound valued for its therapeutic potential. Extensive research has been conducted on its clinical applications. Due to the lack of quality assessment in phytomedicines, there is a need for a readily accessible analytical method. Although some literature exists, none has combined the principles of QbD and green analytical chemistry. Many studies have utilized hazardous chemicals that could have adverse effects on environmental sustainability. Through our research efforts, we have pinpointed the requirement for an analytical method that adheres to the principles of QbD and Green analytical chemistry. As a result, we have successfully devised a UV spectroscopic method utilizing water as the solvent through a QbD approach. The method developed was validated in accordance with ICH guidelines. This study stands unique due to the fact that the developed

method is itself a simple, robust, eco-friendly cost-effective analytical method for chrysin estimation. Hence, it can be concluded that integration of QbD and GAC offers a promising avenue for developing a cost-effective and environment friendly analytical method for chrysin analysis. This approach has the potential to yield an efficient and sustainable solution that meets both economic and environmental requirements.

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