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Bioanalysis of chrysin in human plasma and analytical stability using validated UV-spectrophotometric method: a quality by design approach

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Chrysin is a flavonoid with many biological and medicinal activities, such as anti-cancer, anti-inflammatory, anti-diabetic, and antioxidant properties, and has medicinal value. Based on the Quality by Design approach, the present study used a UV-spectrophotometer to develop a bioanalytical method for chrysin in human plasma. The validation of the method was performed as per ICH M10 guidelines. The technique was performed using methanol as a solvent and the spectrum was recorded at a wavelength of 370 nm. The spiked chrysin from plasma was extracted using the protein precipitation method. The developed method was linear with an r^2 value of 0.9996 over a concentration range of 4-20 µg/ml. The results of all validation parameters are found to be within the accepted limits with a % RSD value of less than 2% and the % recovery was greater than 95%. The % RSD and % recovery results confirmed that the method was precise and accurate in the study. LOD and LOQ values in plasma samples were found to be 0.47 µg/ml and 1.43 µg/ml respectively. The stability studies like freeze-thaw, and short-term stability studies were also performed and proved the reliability of the method. Therefore, the developed bioanalytical method can effectively estimate chrysin in plasma samples within its sensitivity limits.

Keywords: Bioanalysis; Chrysin; Quality by design; Human plasma; UV-Visible Spectrophotometer.

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Introduction

Bioanalysis is a field of analytical chemistry that quantifies biomarkers, chemical entities, and metabolites in biological matrices such as blood, urine, serum, cerebrospinal fluid, and tissues (1). Identifying and concentrating new medications and their metabolites in biological fluids is crucial for pharmacokinetic, pharmacodynamic, and toxicokinetic studies that provide information about experimental medication's safety and effectiveness (2). It is a comprehensive method that has been used to support the clinical phases of drug development throughout the preclinical stages of the process (3). The extraction of analyte and metabolite in biological fluids uses various separation methods such as protein precipitation, liquid-liquid extraction, and solid phase extraction (4). According to the USFDA, the purpose of bioanalytical method development and validation is essential for sample analysis (5). Validated bioanalytical techniques are essential for obtaining trustworthy data that are easily comprehended (6).

Essential therapeutic chemicals are consistently found in plants. Since ancient times, they have been used as a source of medicines to treat various ailments. Even before modern remedies were developed, people anticipated the therapeutic effects of medicinal herbs (7). Polyphenols are intricate organic substances found in many plants. The fundamental building block in polyphenols is the phenolic ring, which is frequently split into phenolic acids and phenolic alcohols. The excellent properties of flavonoids have made them particularly valuable in formulation development and drug delivery (8,9). The broad family of polyphenols known as flavonoids includes the following classes according to their fundamental structure: flavanones, flavones, isoflavones, flavonols, and anthocyanins. Numerous biological characteristics, including anti-inflammatory, antioxidant, antiviral, antibacterial, anticancer, cardioprotective, and neuroprotective actions, are among them (10).

Chrysin (5,7-dihydroxy-2-phenyl-4H-chromen-4-one), is a flavonoid under the flavone class of polyphenolic compounds with a 15-carbon skeleton (11). This study focuses mainly on chrysin, a flavone (5,7-dihydroxyflavone) naturally present in multiple plants (12). The promising sources of flavonoids are propolis, honey, and many plant extracts, including *Passiflora caerulea*, *Passionflower incarnate*, and *Oroxylum indicum*. Chrysin has many biological and medicinal activities, such as anti-cancer, anti-inflammatory, anti-diabetic, and antioxidant properties, and has great economic and medicinal value (13,14). In 1949, Gösta Linstedt extracted chrysin, a pure flavonoid, from pine tree woods at a concentration of 0.07% at the KTH Royal Institute of Technology in Stockholm (15). Interest in plant flavonoids and their various health benefits has considerably increased in recent years, motivating several studies (16).

Quality by Design (QbD) has main objectives are to understanding process performance and developing an appropriate process to attain the intended product performance. Quality is a crucial requirement for any entity to meet the requirements for regulatory clearance as a medicine, in addition to safety and efficacy (17). Incorporating QbD ultimately optimizes resources and lowers costs while improving product quality, lowering risks, and meeting regulatory requirements (18). By designing quality into processes and products, QbD reduces the likelihood of variability that can lead to batch

failures, deviations, and recalls (19). QbD encourages detailed process and product understanding by identifying critical quality attributes (CQAs) and critical process parameters (CPPs). This knowledge helps to predict how changes in the process affect product quality (20).
Ultraviolet-visible (UV-Vis) spectrophotometry, being a simple, cost-effective, and rapid technique, offers a promising alternative for the quantification of chrysin in biological samples. However, existing literature provides limited validation of UV-based bioanalytical methods for chrysin in terms of sensitivity, specificity, and accuracy within complex biological matrices such as plasma or serum. Therefore, the aim of the present study is to develop and validate a simple, sensitive, and accurate UV spectrophotometric method for the bioanalysis of chrysin in biological matrices, in accordance with bioanalytical method validation guidelines. This method is expected to facilitate pharmacokinetic and preclinical studies involving chrysin by providing a reliable tool for its quantification.

Experimental section

Instruments and apparatus

A UV spectrophotometer (Shimadzu 1800) was used to measure the absorbance. The reagents were weighed using an analytical balance. Ultrasonic bath Sonicator (Ultrasonic cleaner) was used for sonication.

Chemicals and reagents

The chemicals and reagents utilized for analytical purposes were of analytical grade and were procured from Merck, India. HPLC-grade methanol and acetonitrile were also of analytical grade. The chrysin biomarker was obtained from Yucca Enterprises, Mumbai. Plasma samples were collected from KLE's Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi, Karnataka.

Preparation of Blank Plasma

Blank plasma was prepared by adding 2 mL of methanol: ACN mixture (1:1 v/v) to 1 ml plasma. The resultant mixture was vortexed (5 min) followed by centrifugation (20 min, 10,000 rpm, 4 °C). The clear supernatant obtained after centrifugation was collected in a clean 100 ml volumetric flask and the volume was made up to 100 ml by methanol.

Preparation of standard stock solution

10 mg of Chrysin was added to 2 mL of plasma and vortexed for 10 min to obtain a spiked mixture of plasma and drug. 2 mL of methanol and ACN (1:1 v/v) was added and vortexed again for 5 min to precipitate the plasma proteins. The mixture was centrifuged for 20 min at 10,000 rpm and 4 °C, supernatant was collected with a micropipette into a 100 ml volumetric flask. The volume was made up to 100 ml with methanol to obtain a stock solution with 100 µg /mL concentration. Furtherly, serial dilutions were made to prepare 4 µg/ml, 8 µg/ml, 12 µg/ml, 16 µg/ml and 20 µg/ml[21].

Steps Involved in the Development of Bioanalytical Methods

Organizing the sequential steps for development of bioanalytical method, the experiments were conducted as follows: selection of Biological fluid; spiking of flavonoids into the biological fluid; extraction of flavonoids from biological fluid; development of the bioanalytical method; optimization of the method by QbD approach; bioanalytical method validation; method applicat.ion to quantify selected flavonoids in biological fluid.

Method development

The development of the UV spectrophotometric method started with the selection of the solvent system and the determination of the wavelength analysis. Chrysin's soluble in different solvents was evaluated, and it was found that chrysin is freely soluble in methanol. Hence, to prepare the dilutions, the whole procedure was carried out by using methanol. The solvent system was optimized using DoE software and employing QbD principles. The UV spectrophotometer Shimadzu 1800 software was used for the analysis of chrysin in human plasma. To identify the wavelength for analysis, a solution containing 10µg/ml was scanned in the UV region of 200- 800nm and the spectrum was obtained at the wavelength of 353nm.

Method Optimization by Quality by Design Approach

QbD is defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on quality risk management." In the present investigation, we have worked on various elements of AQbD and implemented them in the practical methodology. It was implemented by following the steps: (22)

Defining Analytical Target Profile (ATP)

The Quality by Design (QbD) approach defined the Analytical Target Profile (ATP), which aligned with the Quality Target Product Profile (QTPP). The ATP outlined the goals for analytical method development, linking method outcomes to the objectives of the QTPP. It was established using scientific reasoning and process knowledge. Key components of the ATP included the target drug in plasma, sample types and applications, bioanalytical method and instruments, sample preparation, and method quality attributes. A comprehensive review of the literature and drug properties supported the creation of the ATP, summarizing the quality characteristics of the analytical method.

Identification of Critical Quality Attributes (CQAs

Critical Quality Attributes (CQAs) are measurable parameters of the analyte that must fall within specified limits, ranges, or criteria to ensure the bioanalytical procedure meets the desired quality and performance. For example, in a UV method, the CQA could be the absorbance.

Identification of critical method parameters (CMPs)

Critical Method Parameters (CMPs) are factors influencing the sensitivity of bioanalytical methods. These vary by technique, such as UV, GC, HPLC, or HPTLC. Common CMPs include the % organic modifier, mobile phase pH, column temperature, flow rate, UV scan speed, and injection volume. For UV methods, key CMPs include solvent variation, detection wavelength, scan speed, sampling interval, sample integrity, slit width, and centrifuge time.

Risk Assessment (RA) by employing a fish-bone approach

Risk assessment identifies potential interactions with CMPs and evaluates the likelihood of failures. A fishbone diagram was created to highlight method variables potentially affecting the attributes of the UV spectrophotometric method for Chrysin. A literature review revealed that critical method variables (CMVs) such as solvent variation, detection wavelength, scan speed, sampling interval, sample integrity, and slit width had high final scores, indicating they are high-risk factors.

Method optimization by Design of Experiment (DOE) Software

After completing the risk analysis to identify Critical Method Variables, the focus shifted to optimizing two key variables: scanning speed and centrifugation time. Other method variables were maintained at their lowest or optimal values. Using Central Composite Design (CCD) within the Design of Experiment (DoE) framework, the study aimed to determine optimal levels for scanning speed (A) and centrifugation time (B) to improve method robustness. The CCD approach involved 9 experimental runs, including a center point, for these selected variables.

Method validation

The developed method was validated according to ICH M10 guidelines. The validation parameters, such as linearity and range, accuracy, precision, LOD and LOQ, robustness, ruggedness, and matrix effect, were evaluated[23].

Selectivity and Specificity

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peak of Chrysin. The specificity and selectivity were evaluated by running the solvent and comparing the spectrum of chrysin.

Linearity and Range

The calibration curve was plotted against the ratio of analyte concentration versus absorbance. Using MS Excel software, the slope, intercept, and regression coefficient were calculated and reported.

Matrix Effect

The matrix effect is defined as the change in the analyte response due to interfering and undetectable components in the sample matrix. The matrix effect is mainly due to alteration in the ionization efficiency. The matrix effect is basically evaluated by analyzing LOQ and HQC samples (n=3) prepared using a plasma matrix. The accuracy and precision should be between $\pm$ 15%.

Accuracy

The accuracy study was performed based on the percentage absolute recovery of chrysin from three quality control samples viz lower quantifiable concentration (80%, LQC), middle quantifiable concentration (100%, MQC), and high quantifiable concentration (120%, HQC) Percentage absolute recovery of chrysin from LQC, MQC, and HQC was calculated by the formula mentioned below and is used to calculate the accuracy of the method. Each concentration was analyzed three times (n=3) and mean, standard deviation (SD), and percentage relative standard deviation (%RSD) were calculated to confirm the accuracy.

Precision

Precision studies were performed with LQC, MQC, and HQC samples to evaluate the consistency of results between many samples of the same concentration on the same day and on other days of analysis. These studies can be performed by injecting (n=3) quality control samples on the same day for three consecutive days under the same experimental conditions. Inter-analyst precision was performed by carrying out the samples on the same day by two different analysts. Their mean, SD, and %RSD were calculated.

Determination of LOD and LOQ

LOD and LOQ is calculated based on SD of intercept and slope of linear regression equation using Equations;

$$LOD = \frac{(3.3)\ SD}{S}$$

$$LOQ = \frac{(10)\ SD}{S}$$

Where SD is the standard deviation of the intercept, S is the slope of the linearity curve.

Stability study

Freeze thaw method

To perform freeze thaw stability study of chrysin in plasma, plasma (3ml) was taken in a vial and 3mg of chrysin was added to get the concentration of 1000ug/ml. The resultant mixture was vortexed for 5 min and kept in a deep freezer at -20 ℃ for 12 hours. After the sample had frozen the vial was removed and allowed to thaw at normal temperature. 1ml plasma was withdrawn from the thawed samples (cycle 1) using a micropipette. After 12 hr the remaining 2 ml sample was again kept in the deep freezer for 2nd cycle. The collected 1ml plasma containing chrysin was precipitated using acetonitrile and centrifuged. The clear supernatant was collected and diluted with 100 ml of methanol to get the concentration of 100µg/ml. After the extraction of drugs, the sample is processed for the preparation of LQC, MQC, and HQC samples after each interval. Similarly, cycle 3 was carried out. The mean, SD, and %RSD were calculated for each concentration.

Short-term stability

The short-term stability studies of plasma spiked with chrysin were determined at room temperature after 1hr, 2hr, and 3hr before extraction. For short-term stability, 1000ug/ml solution was prepared by adding 3mg of chrysin to a vial containing 3 ml of plasma and vortexed for 5 min. The vial containing chrysin-spiked plasma was kept at room temperature. After 1hr, 2hr, and 3hr samples were withdrawn and extracted the drug from the plasma. The clear supernatant was diluted with 100 ml of methanol to get the concentration of 100 µg/ml. After the extraction of drugs, the sample is processed for the preparation of LQC, MQC, and HQC samples after each interval. Samples were prepared in triplicate and analyzed. The mean, SD, and % RSD were calculated for each concentration.

Results and Discussion

Method Development

The solvent development step involves the use of methanol as solvent system, in which chrysin showed a spectrum with maximum absorbance at 353nm (Figure 1).

The method was optimized using quality-by-design principles. The Optimized method parameters are presented in Table 1.

Figure 1. UV-Spectrum of chrysin in a range of 200-800 nm.

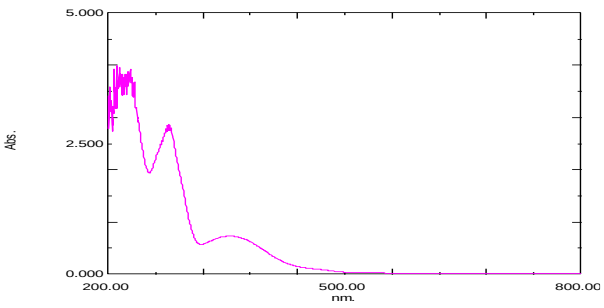


Table 1. Analytical parameters for the UV-spectrophotometric method proposed for chrysin.

Sr. No.	Parameters	Specifications
1	Method	Spectrophotometric
2	Instrument	UV-Spectrophotometer
3	Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Analyte	Chrysin
7	Solvent	Methanol
8	Lambda Max.	353nm

Method Optimization by Quality by Design Approach

The systematic approach of QbD-based method optimization involved defining the Analytical Target Profile, identifying Critical Quality Attributes and critical method parameters, conducting a risk assessment using the fishbone approach, and optimizing the method through Design of Experiments software. The outcomes are summarized below.

Defining Analytical Target Profile (ATP)

ATP describes the method requirements which are expected to be the measurements. The rationale justification for the selection of the UV spectrophotometric method was owing to the simple and rapid analysis of chrysin in human plasma as compared to other sophisticated analytical methods. Table 2 represents ATP for the proposed UV-spectrophotometric method for chrysin.

Table 2. Analytical target profile (ATP) for UV spectrophotometric method applied to chrysin.

Sl. No.	ATP Parameters	Target
1	Target Analyte	Chrysin
2	Target Sample	Plasma
3	Method category	UV-spectrophotometric method
4	Instrument Requirement	UV-spectrophotometer
5	Nature of analyte	Solid (Solution)
6	Standard stock solution preparation	Dilution of main drug in linear manner
7	Application of Method	Estimation of Chrysin
8	Process parameters	Specificity, selectivity, linearity, range, precision, robustness, ruggedness, accuracy, LOD, LOQ.

Identification of Critical Quality Attributes (CQAs)

The CQAs of an analytical method include method parameters and attributes, defined as measurable analyte parameters within acceptable limits to ensure quality. For the UV method, drug absorbance was identified as the critical quality attribute.

Identification of critical method parameters (CMPs)

In this study, several potential Critical Method Parameters (CMPs) for the UV analytical method were initially identified based on prior knowledge and risk assessment, including solvent composition, detection wavelength, scan speed, centrifugation time, sampling interval, sample integrity, and slit width. However, through experimental evaluation (e.g., using design of experiments or robustness studies), the parameters that were found to significantly impact the Critical Quality Attribute (CQA), specifically absorbance, were [e.g., detection wavelength, solvent composition, and slit width].

Risk Assessment by employing fish-bone approach

Risk assessment evaluates potential interactions with CMPs and the likelihood of failures. An Ishikawa fishbone diagram was used to identify risks and root causes affecting the performance of the UV method for chrysin, highlighting various method variables influencing its attributes (Figure 2).

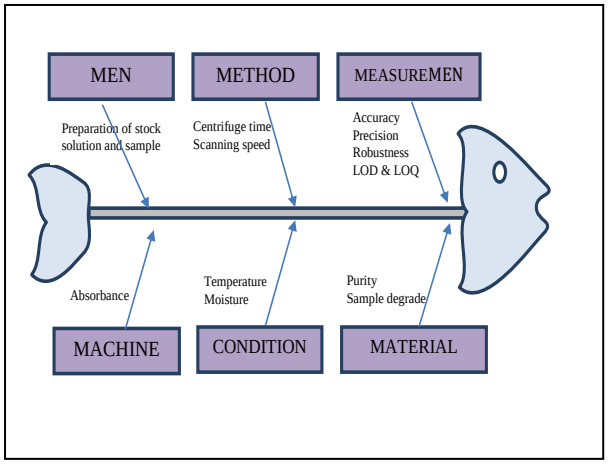


Figure 2. Representation of Ishikawa fishbone diagram.

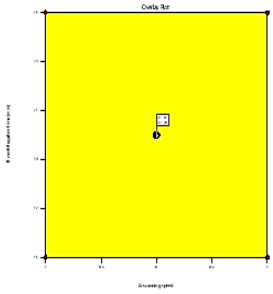
Method optimization by Design of Experiment (DOE) Software

The method was further optimized by using CCD within the Design of Experiment (DoE) framework, the study aimed to determine optimal levels for scanning speed (A) and centrifugation time (B) to improve method robustness. The selection of levels for CCD design is given in Table 3.

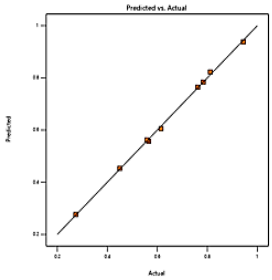
Table 3. Selection of levels for CCD Design.

Independent variable	Levels	
	-1	+1
Scanning speed(A)	-1(Low)	+1(High)
Centrifuge time(B)	15	25

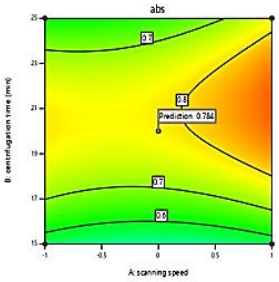
The CCD approach entailed 9 runs for the two selected variables, including one centered point (Table 4). After performing the experiments suggested by CCD, the responses obtained were filled into the software and further analyzed. The optimized graphs for the values are shown in Figure 3.



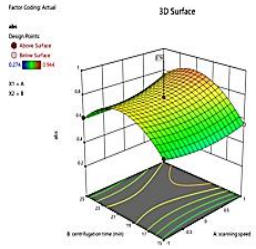
(a) Overlay plot



(b) Actual vs. predicted plot



(c) 2D Counter plot



(d) 3D surface plot

Figure 3. Optimized graphics for CCD design applied to method development.

Table 4.CCD approach for 9 runs with two variables applied in the method development.

Std	Run	Factor 1 A: Scanning speed	Factor 2 B: Centrifugation time	Response 1 Absorbance
8	1	0	27	0.45
2	2	1	15	0.559
5	3	-1	20	0.812
6	4	1	20	0.944
9	5	0	20	0.784
7	6	0	12	0.274
4	7	1	25	0.762
3	8	-1	25	0.615
1	9	-1	15	0.565

In the Table 5 a statistical analysis by ANOVA is illustrated, being possible to visualize the responsed from scanning speed and centrifugation time

Table 5. ANOVA testing applied to quadratic model employed for scanning speed and centrifugation time.

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	0.34	5	0.06	641.79	< 0.0001 (significant)
A-scanning speed	0.0134	1	0.0134	126.61	0.0015
B-centrifugation time	0.0315	1	0.0315	297.05	0.0004
AB	0.0059	1	0.0059	55.21	0.0050
A^2	0.0068	1	0.0068	64.05	0.0041
B^2	0.1279	1	0.1279	1206.64	< 0.0001
Residual	0.0003	3	0.0001		
Cor Total	0.3405	8			

Method validation

Specificity and Selectivity

The solvent spectrum obtained showed no interference of absorbance 353nm which shows the specificity and selectivity of the method. UV spectrum of blank plasma and plasma spiked chrysin were shown in Figure 4 and Figure 5.

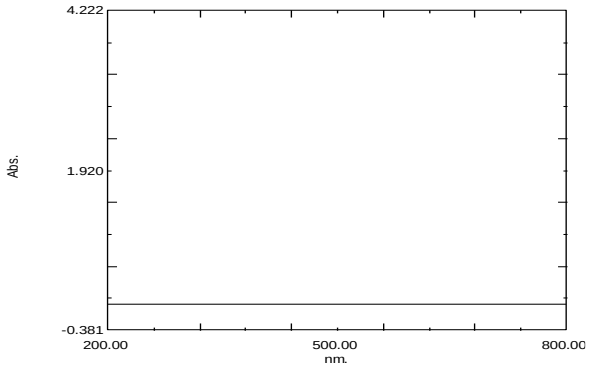


Figure 4. UV-Spectrum of solvent used in the method development for chrysin analysis.

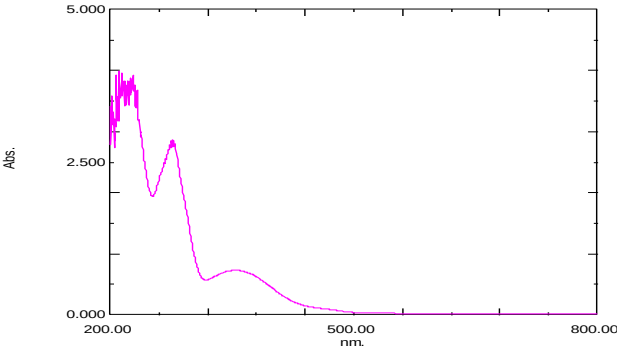


Figure 5. UV-Spectrum of chrysin obtained in the method development.

Development of Calibration Curve (Linearity and Range)

Standard calibration curve was plotted using concentration vs absorbance obtained by linear dilution of Chrysin. Each concentration showed linear absorbance range between the concentration range of 4, 8, 12, 16, 20µg/ml (Table 6). The calibration curve for chrysin prepared in plasma indicated excellent linearity with R^2 Value of 0.9996 (Figure 6 and 7).

Table 6. Linearity data obtained from UV method development applied to chrysin in biological sample.

Sr. No.	Concentration (µg/ml)	Absorbance
	4	0.257
2	8	0.438
3	12	0.598
4	16	0.771
5	20	0.932

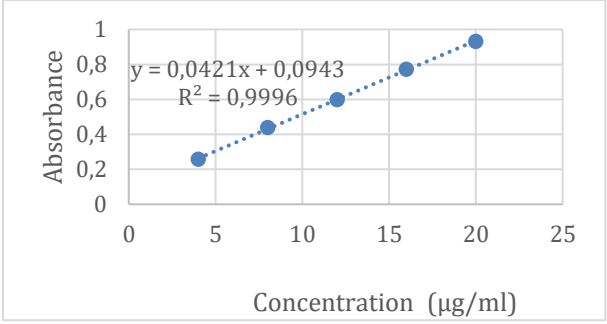


Figure 6. Calibration curve obtained from UV spectrophotometric determination of chrysin in biological sample.

Table 7. Results of precision obtained from UV method development applied to chrysin in biological sample.

Precision	Concentration (µg/ml)	Mean Absorbance	Standard Deviation	%RSD
Intraday (Morning)	4	0.250	0.001	0.4
	12	0.561	0.005	0.89
	20	0.879	0.002	0.23
Intraday-2 (Afternoon)	4	0.275	0.004	1.4
	12	0.586	0.002	0.35
	20	0.906	0.0005	0.06
Intraday-3 (Evening)	4	0.293	0.001	0.39
	12	0.593	0.001	0.19
	20	0.902	0.001	0.19
Interday-1	4	0.250	0.001	0.4
	12	0.561	0.005	0.89
	20	0.879	0.002	0.23
Interday-2	4	0.364	0.001	0.47
	12	0.701	0.002	0.35
	20	0.942	0.003	0.32
Interday-3	4	0.234	0.003	1.37
	12	0.593	0.004	0.77
	20	0.905	0.005	0.60

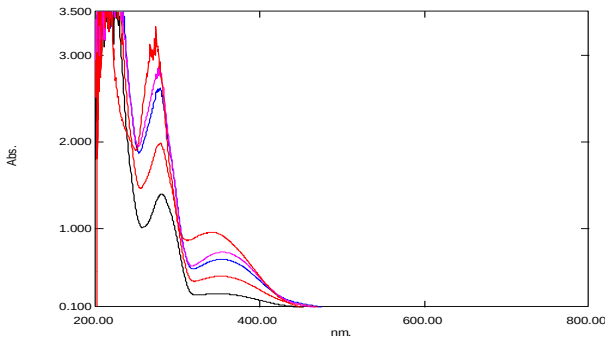


Figure 6. Overlay plot obtained from analysis of chrysin in biological sample by UV method, ranging different concentration.

Limit of Detection and Limit of Quantification

The Limit of Detection and Limit of Quantification were found to be 0.47 µg/ml and 1.43 µg/ml respectively.

Precision

The method was found to be precise, as the % RSD calculated for three replicate solutions of Chrysin at each precision level (4, 12, and 20 µg/ml) was found to be less than 2 %. Table 7 represents the data of interday and intraday precision.

Accuracy

The method was found to be accurate as all the level recovery values were well within the acceptance. Table 8 represents the data of accuracy.

Table 8. Recovery data obtained from accuracy testing applied to UV method for quantification of chrysin.

Concentration added (µg/ml)	Level	Mean	Concentration found	% Recovery
9.6	80%	0.54	9.73	101.44
12	100%	0.64	12.23	101.97
14.4	120%	0.73	14.63	101.65

Ruggedness

The method was found to be rugged through a good perfromance testing different analysts and instruments within the acceptable range (% RSD<2) (Table 9).

Table 9. Ruggedness data of Chrysin obtained during by UV Method development.

Precision Test	Conc. (µg/ml)	Mean Absorbance	SD	%RSD
Change in Analyst	4	0.272	0.002	0.76
	12	0.529	0.002	0.39
	20	0.987	0.001	0.10
Change in Instrument	4	0.27	0.001	0.56
	12	0.522	0.002	0.48
	20	0.981	0.001	0.15

Robustness

The results obtained are found to be reliable and within the acceptable range even when performed by slight variation in the wavelength. (Table 10).

Table 10. Robustness data obtained from UV method development applied to chrysin in biological sample.

Concentration (µg/ml)	Wavelength (nm)	Mean	%RSD
4	351	0.253	0.99
4	353	0.267	0.99
4	355	0.274	0.21

Matrix effect

To evaluate the matrix effect, the LQC and HQC samples were run three times, and the percentage recovery and % RSD were calculated. The percentage recovery was found to be above 95 % in all cases indicating there is no interference from any other unidentified compound in the sample. It is also confirmed by % RSD less than 2 (Table 11). For this purpose, non-hemolyzed and non-lipemic plasma samples were used to eliminate the possibility of interference from hemoglobin or lipids, which could affect absorbance. The results confirmed that there were no significant absorbance peaks at the detection wavelength in the blank plasma, indicating high specificity of the method for chrysin.

Table 11. Matrix effect observed during UV method development applied to chrysin in biological sample.

Conc. added (µg/ml)	Level	Mean	Conc. found	% Recovery
9.6	80%(LQC)	0.542	9.65	100.52
14.4	120%(HQC)	0.742	14.21	98.68

Stability study of plasma samples

Freeze-thaw and short-term stability studies were conducted for plasma samples. The results indicated that the % RSD was less than 2. The outcome of these studies indicated the stress as well as stability of drugs in plasma samples (Table 12 and 13).

Table 12. Results of freeze thaw stability from plasma sample of chrysin.

Concentration (µg/ml)	Absorbance	Mean	%RSD
4(cycle 1_12hr)	0.231	0.228	0.91
	0.227		
	0.228		
4(cycle 2_12hr)	0.243	0.241	0.63
	0.24		
	0.242		
4(cycle 3_12hr)	0.235	0.236	0.42
	0.236		
	0.237		

Table 13. Results of short- term stability from sample of chrysin.

Concentration (µg/ml)	Absorbance	Mean	%RSD
4_(1Hour)	0.215	0.217	0.95
	0.219		
	0.218		
4_(2Hour)	0.23	0.229	0.9
	0.231		
	0.227		
4_(3Hour)	0.229	0.23	0.501
	0.231		
	0.231		

Conclusions

The UV spectrophotometric method developed for estimating chrysin in biological matrices is simple,

accurate, precise, and cost-effective. This method can be used at the laboratory level to estimate the plasma concentration of Chrysin. The method showed good linearity, sensitivity, and reproducibility within the selected concentration range. Validation parameters such as accuracy, precision, specificity, limit of detection (LOD), and limit of quantitation (LOQ) were within acceptable limits, confirming the method's reliability for routine analysis. This validated method can be effectively employed for the quantitative analysis of chrysin in various bioanalytical applications, including pharmacokinetic and therapeutic monitoring studies.

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Conflict of interest

The authors declare no conflict of interest related to this study.

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