Original Article

# Development And Validation Of UV-Visible Spectrophotometric Method For Estimation Of Chrysin In Bulk Form

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## Abstract

The goal of this work is to establish a straight forward UV-visible Spectrophotometric approach for determining chrysin in its pure form and to validate the method that has been devised. Using a UV-visible double beam spectrophotometer and a solvent solution containing dimethyl sulfoxide, a method was developed and validated for chysin. By following the International Conference on Harmonization (ICH) recommendations, analytical properties like linearity and precision were determined. According to Beer's law, the devised method demonstrated linear response for chrysin concentrations between 1 and 5 µg/ml, and a coefficient of correlation of 0.9987 was discovered. The method's sensitivity was determined and the limits of detection (LOD) and limit of quantification (LOQ) were discovered to be 0.2155 µg/ml and 0.6531 µg/ml respectively. The study found that the method for estimating chrysin is linear, consistent, precise and affordable and can be used to analyse pharmaceutical formulations.

**Keywords:** Chrysin, phytoconstituent, method development, validation.

## INTRODUCTION

The validation procedure is a crucial step in the formulation and development of drugs because it shows if an instrument can consistently and effectively carry out its intended duties. Its primary goal is to verify that the processes have been properly established and managed. Additionally, it demonstrates that the devised approach can accurately determine the quantity of phytoconstituent contained in the sample.[1] Key components for the chemical analysis of the phytoconstituents are being revealed by the developed and validated method. Several techniques including spectrophotometry, thin layer chromatography and high pressure liquid chromatography can be used to identify and quantify phytoconstituents.[2] Among these techniques, spectrophotometry is a simple method to design and validate for flavonoid quantification. UV visible spectrophotometry assists in quantifying phytoconstituents based on the relative amounts of UV Visible radiation being absorbed by the substances in the solution using the Beer-Lambert equation. According to literature reviews, several phytoconstituents can be identified using UV spectro-photometric techniques either by themselves or in conjunction with other excipients.[3] There are numerous equipments utilized for quantitative determination of phytoconstituents including HPLC and HPTLC but their costs, complexity and lengthy procedures are their main drawbacks.

Also requirement of an highly trained personnel is required for operating the equipments. Thus UV visible spectroscopy is an better alternative when compared to other methods as it can overcome the above problems. Chrysin often referred to as 5'7 dihydroflavone is a phytoconstituent that belongs to the flavonoid class[4] and is also recognized for having anticancer activities against several types of cancer. Honey, propolis and passion flower are the main sources of the phytoconstituent chrysin.[5] Studies shows that chrysin has a potential as an anti-inflammatory, anti-diabetes, cardioprotective, hepatoprotective and anticancer agents.[6] Chrysin has the chemical formula C15H10O4 and a molecular weight of 254.24g/mol. 285.50$^0$C is its melting point.[7] Chrysin's pharmacokinetic characteristics show that it is poorly absorbed after oral administration with only traces of the substances being seen in the blood, whilst the majority of it is excreted in the faceas.[8] Most critical evaluations pay attention to the biological activitiesof chrysin and comparable plant sources in vivo and in vitro as well as their their pharmacological effects on animal and human bodies rather than the analytical techniques for identifying chrysin. Chrysin has a limited solubility in buffers and fluids, however it is well soluble in dimethyl sulfoxide. Using HPLC and HPTLC, a number of analytical techniques for chrysin estimation have been established.[9] Our study intends to develop a stable, repeatable, reliable, andtrustworthy method for determination of chrysin.

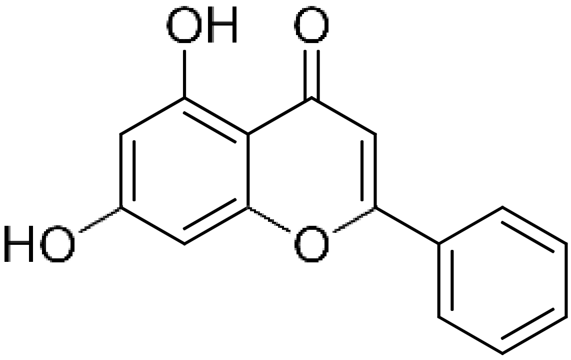

**Figure 1:** The chemical structure of chrysin

# MATERIALS AND METHODS

## Materials

Chrysin was purchased from Tokyo Chemical Industries in Japan. Spectral measurements were conducted using a double beam UV Spectrophotometric (Shimadzu 1900 Japan) and 10mm quartz curettes. The latter is less expensive andsimpler to use.[10] Only analytical-grade chemicals and reagents were used.

## Method

### Preparation of the standard solution

Chrysin was carefully weighed at 10 mg and then transferred into a 10 ml volumetric flask. A dimethylsulfoxide solution was added to the volumetric flask until it had a capacity of 10 ml. Bath sonicator was used to get a clearsolution.

### Determining maximum wavelength

In a UV spectrophotometer, a 5 µg/ml solution of chrysin was scanned at 270 nm.11 As a control solution, dimethylsulfoxide was employed.

### Creation of the reference calibration curve

By measuring the absorbance of a chrysin solution prepared from stock solutions in DMSO at 270 nm in triplicate, the standard calibration curve of chrysin was obtained. Chrysin concentration was plotted on the x-axis and absorbance on the y-axis to create the calibration curve.

### Analytical method validation

A stated definition of validation is (ICH) providing recorded proof that offers a high level of assurance that a chosen activity can consistently deliver a desired outcome or product that complies with its predetermined requirements and quality attributes. For method validation, the following variables were assessed.[12]

### Linearity and range

The analytical methodology's capacity to analyse data that are directly proportional to analyte concentration was referred to as its linearity. Chrysin standard solution ranging from 1 to 5 µg/ml was made from stock solution and examined to determine the linearity of the proposed methodology. Every measurement was made in triplicate.[13]

### Precision

To assess the validity of the proposed methodical technique, precision studies were conducted. Through the use of three triplicates of the same sample at low concentrations of 1 µg/ml, medium concentrations of 3 µg/ml, and high concentrations of 5 µg/ml, repeatability was clearly confirmed. As a result, the absorbance was measured throughout the day and a precision research was conducted by making drug resolution at concentrations of low 1 µg/ml, medium 3 µg/ml, and high 5 µg/ml and analysing it three times throughout the day. Several completely separate days were treated the same way to produce work that was reportable as %RSD. The precision result demonstrated an honest reliability, buttwo intraday and interday precision study results were measured.

### Ruggedness

By analysing low 1 µg/ml, medium 3 µg/ml, and high 5 µg/ml concentration solutions in DMSO solution three times bytwo different analyzers at 270 nm, ruggedness was assessed. The outcomes were reported as %RSD.[13]

### *Robustness*

To assess the method's robustness, chrysin solution containing mild concentrations of 1 µg/ml, medium concentrations of 3 µg/ml, and high concentrations of 5 µg/ml were analyzed 2 times at two distinct wavelengths (265 and 275).[14]

### *LOD and LOQ*

The lowest quantity of the sample's analyte which can be determined is known as the limit of detection (LOD). The lowest quantity of analyte in the sample that can be quantitatively determined with adequate precision and accuracy are termed as the limit of quantification (LOQ). The following equation was applied to determine LOD and LOQ: LOQ-10 s\m LOD-3 s\m where s is the response's standard deviation and m is the slope of the corresponding calibration curve. [15,16]

## RESULTS AND DISCUSSION

The proposed methodology offers a simple, precise, cost effective and practical method for the UV Spectrophotometric analysis of chrysin. For both intraday and interday categories, it was discovered that the approach had an RSD of under two. The RSD values were found to be less than two, which further demonstrated the strategy's toughness and robustness. The predicted methodology's limit of detection and limit of quantification which were determined to be 0.2155 µg/ml and 0.6531 µg/ml respectively, show that the method devised is sensitive.

### *Determination of maximum wavelength.*

The wavelength that has the greatest absorption in DMSO solution was found at 270nm (Figure 1). Figure 2: UV Spectrum of chrysin in DMSO.

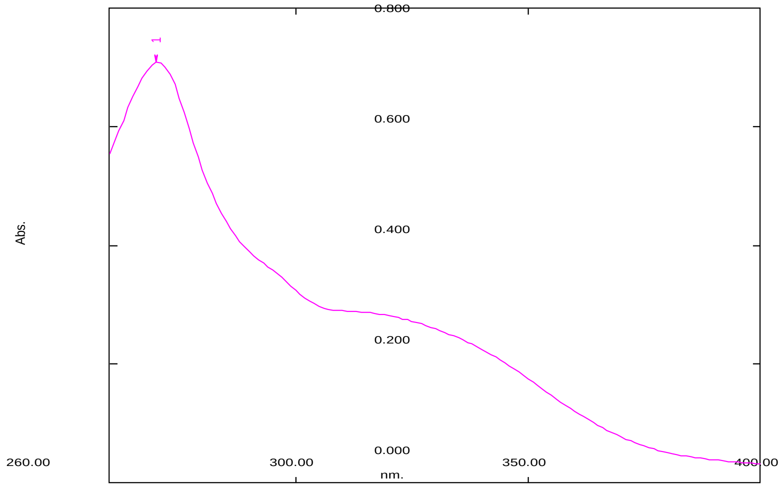

**Figure 2:** UV Spectrum of chrysin in Dimethyl sulfoxide

### *Preparation of standard calibration curve.*

Chrysin's calibration plot was determined to be linear with a 0.9987 coefficient of correlation as shown in (Figure 3).

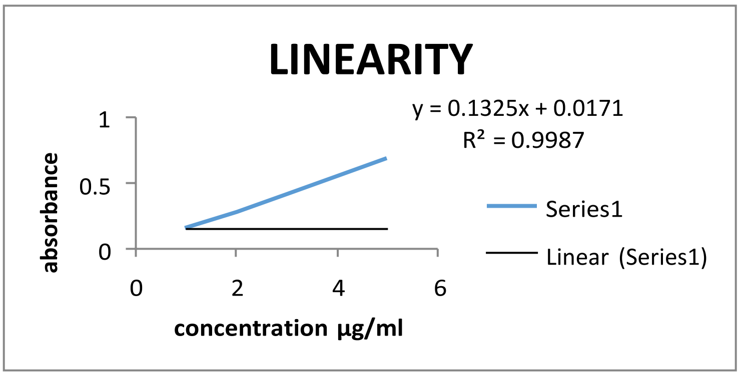

**Figure 3:** Calibration curve of chrysin in DMSO at 270nm

### *Analytical method validation*

According to ICH guidelines, the method was evaluated for a set of parameters including precision, toughness, robustness, limit of detection (LOD) and limit of quantification (LOQ).[17]

### *Linearity and Range*

The value of the correlation coefficients supported the linearity of the calibration curve (r2). According to Table 1, correlation coefficient for chrysin was determined to be 0.9987.

**Table 1:** Linearity table of chrysin

| **Concentration** µg/**ml** | **Absorbance** | |
|---|---|---|
| **1** | **0.158** | **$y=0.132x + 0.017$**<br>**$R^2 = 0.998$** |
| **2** | **0.275** | |
| **3** | **0.406** | |
| **4** | **0.552** | |
| **5** | **0.682** | |

### *Precision*

There was no significant difference between the intraday and interday because they were performed on completely different days. The outcome demonstrates the consistency of the suggested methodology. The precision outcomes are shown in Table 2 and 3. Additionally, the relative variance proportion was estimated.

**Table 2:** Intraday Precision

| **Concentration** µg/ml | **Absorbance** | **Absorbance** | **Absorbance** | **Average %RSD** |
|---|---|---|---|---|
| 1 | 0.147 | 0.186 | 0.188 | 0.411286 |
| 1 | 0.148 | 0.185 | 0.187 | |
| 1 | 0.148 | 0.186 | 0.189 | |
| %RSD | 0.39098212 | 0.31096065 | 0.53191489 | |
| 3 | 0.406 | 0.445 | 0.452 | 0.26196 |
| 3 | 0.405 | 0.448 | 0.452 | |
| 3 | 0.405 | 0.445 | 0.454 | |
| %RSD | 0.14243839 | 0.3883522 | 0.25508848 | |
| 5 | 0.662 | 0.696 | 0.69 | 0.21579 |
| 5 | 0.659 | 0.696 | 0.693 | |
| 5 | 0.66 | 0.698 | 0.693 | |
| %RSD | 0.23132639 | 0.16574649 | 0.25029636 | |

**Table 3:** Intraday Precision.

| **Concentration** µg/ml | **Absorbance** | **Absorbance** | **Absorbance** | **Average %RSD** |
|---|---|---|---|---|
| 1 | 0.14 | 0.186 | 0.194 | 0.3404 |
| 1 | 0.139 | 0.185 | 0.195 | |
| 1 | 0.14 | 0.185 | 0.195 | |
| %RSD | 0.413377281 | 0.31151993 | 0.29658404 | |
| 3 | 0.388 | 0.445 | 0.457 | 0.2546 |
| 3 | 0.386 | 0.444 | 0.454 | |
| 3 | 0.386 | 0.444 | 0.455 | |
| %RSD | 0.29862945 | 0.12993629 | 0.33547406 | |
| 5 | 0.649 | 0.695 | 0.693 | 0.1330 |
| 5 | 0.649 | 0.696 | 0.695 | |
| 5 | 0.65 | 0.697 | 0.693 | |
| %RSD | 0.088914313 | 0.14367816 | 0.16646332 | |

### *Ruggedness*

By performing the assay under identical conditions on completely different days by several analysts using various tools and at various times, ruggedness was determined. The test scores fell between 99 and 101%.

**Table 4:** Result of ruggedness

| **Concentration (µg/ml) Analyst 1** | **Absorbance** | **Statistical Analysis** |
|---|---|---|
| 1 | 0.145 | Mean-0.146333333SD-<br>0.001527525<br>%RSD- 1.043866901 |
| 1 | 0.148 | |
| 1 | 0.146 | |
| 3 | 0.452 | Mean-0.454333333SD-<br>0.002081666<br>%RSD-0.458180337 |
| 3 | 0.456 | |
| 3 | 0.455 | |
| 5 | 0.672 | Mean-0.672666667SD-<br>0.001154701<br>%RSD-0.17166014 |
| 5 | 0.672 | |
| 5 | 0.674 | |

| | | |
|---|---|---|
| **Analyst 2** | | |
| 1 | 0.146 | ean-0.14466667SD-<br>0.0011547<br>%RSD-0.7981801 |
| 1 | 0.144 | |
| 1 | 0.144 | |
| 3 | 0.458 | ean-0.45766667SD-<br>0.00152753<br>%RSD-0.33376371 |
| 3 | 0.456 | |
| 3 | 0.459 | |
| 5 | 0.679 | ean-0.67733333SD-<br>0.00152753<br>%RSD-0.22552046 |
| 5 | 0.676 | |
| 5 | 0.677 | |

***Robustness:***

By securing the assay throughout the modification wavelength, robustness was firmly established. The sharp RSD was found to be less than 2%, which was within the permissible range as demonstrated in Table 5.

**Table 5:** Results showing robustness

| Concentration (µg/ml) at wavelength 270nm | Absorbance | Statistical Analysis |
|---|---|---|
| 1 | 0.152 | ean-0.1526667SD-<br>0.0011547<br>%RSD-0.7563541 |
| 1 | 0.152 | |
| 1 | 0.154 | |
| 3 | 0.502 | ean-0.5006667SD-<br>0.0015275<br>%RSD-0.3050982 |
| 3 | 0.499 | |
| 3 | 0.501 | |
| 5 | 0.675 | ean-0.6766667SD-<br>0.0015275<br>%RSD-0.2257426 |
| 5 | 0.678 | |
| 5 | 0.677 | |
| **Concentration (µg/ml) at wavelength 265nm** | | |
| 1 | 0.234 | ean-0.23333333SD-<br>0.00305505<br>%RSD-1.30930734 |
| 1 | 0.23 | |
| 1 | 0.236 | |
| 3 | 0.518 | ean-0.51766667SD-<br>0.00057735<br>%RSD-0.11152935 |
| 3 | 0.517 | |
| 3 | 0.518 | |
| 5 | 0.696 | ean-0.69533333SD-<br>0.00057735<br>%RSD-0.08303216 |
| 5 | 0.695 | |
| 5 | 0.695 | |
| **Concentration (µg/ml) at wavelength 275nm** | | |
| 1 | 0.135 | ean-0.1353333SD-<br>0.0005774<br>%RSD-0.4266135 |
| 1 | 0.136 | |
| 1 | 0.135 | |
| 3 | 0.486 | ean-0.4856667SD-<br>0.0005774<br>%RSD-0.1188779 |
| 3 | 0.485 | |
| 3 | 0.486 | |
| 5 | 0.662 | Mean-0.663 SD-<br>0.0017321<br>%RSD-0.2612445 |
| 5 | 0.665 | |
| 5 | 0.662 | |

***LOD AND LOQ:***

LOD and LOQ values were found to be 0.2155 and 0.6531 µg/ml, respectively

**Table 6:** Optical characteristics.

| Parameters | Result |
|---|---|
| Absorption maxima | 270nm |
| Beers law range | 1-5 µg/ml |
| Correlation coefficient | 0.9987 |
| Regression equation | 0.132x + 0.017 |
| Slope | 0.1325 |
| Intercept | 0.0171 |
| Precision | 0.26196 |
| LOD µg/ml | 0.2155 µg/ml |
| LOQ µg/ml | 0.6531 µg/ml |

Chrysin at 270 nm was chosen as the phytoconstituent and the method was designed and validated in accordance with ICH requirements for linearity, accuracy, specificity, robustness, ruggedness, LOD and LOQ. Chrysin concentration was measured at three distinct concentrations: low (1 µg/ml), middle (3 µg/ml), and high (5 µg/ml). Beer's law is followed by all triplicates concentration of the phytoconstituent chrysin in the concentration range of 1 to 5 micrograms/ml. Chrysin was determined using the suggested approach which revealed linear regression with the equation y=0.1325x + 0.0171 and a coefficient correlation (r2) of 0.9987 (Figure 1). The relative standard deviation of the three test samples of chrysin was used to measure the precision. Each assay was calculated, and the resulting relative standard deviation of %assay was less than 1.5%. Limits of detection (LOD) and limit of quantification were discovered to be 0.2155 µg/ml and 0.6531 µg/ml respectively. Chrysin phytoconstituent can be analysed using the suggested method when it is taken in bulk dosage form.

## CONCLUSION

The suggested UV Spectrophotometric approach which has been used in numerous research can be regarded as straightforward, quick and affordable. With outstanding accuracy, precision and linearity, the method is valid conformity with ICH criteria and appropriate for chrysin estimation.

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## CONFLICT OF INTEREST: Nil

## REFERENCES:

1. Ramanunny AK, Wadhwa S, Gulati M, Gupta S, Porwal O, Jha NK, et al. Development and validation of RP-HPLC method for 1′- Acetoxychavicol acetate (ACA) and its application in optimizing the yield of ACA during its isolation from *Alpinia galanga* extract as well as its quantification in nanoemulsion. South African Journal of Botany. 2022 Sep;149:887-98.
2. Kustrin SA, Doyle E, Gegechkori V, Morton DW, High-performance thin-layer chromatography linked with (bio)assays and FTIR-ATR spectroscopy as a method for discovery and quantification of bioactive components in native Australian plants. Journal of Pharmaceutical and Biomedical Analysis. 2020 May;184:113208.
3. Bansal, A., Srivastava, N. & Nagpal, K. Development and Validation of UV Spectrophotometric Method for Determination of Chrysin and Its Solubility Studies. *J Appl Spectrosc. 2022;89:150-58.*
4. Kudatarkar N, Jalalpure S, Kurangi B. Formulation and Characterization of Chrysin Loaded Phytosomes and its Cytotoxic Effect against Colorectal Cancer Cells. Indian Journal of Pharmaceutical Education and Research. 2022 Jun;56(3): DOI: 10.5530/ijper.56.3s.148.
5. Naz S, Imran M, Rauf A, Orhan I E, Shariati M A, Haq I U, Chrysin: Pharmacological and therapeutic properties. Life Sciences. 2019 Oct;235:116797.
6. Kasala E R, Bodduluru L N, Madana R M, Athira K V, Gogoi R, Barua C C. Chemopreventive and therapeutic potential of chrysin in cancer: mechanistic perspectives. Toxicology Letters. 2015 Mar;233(2):214-25.
7. Bansal, A., Srivastava, N. & Nagpal, K. Development and Validation of UV Spectrophotometric Method for Determination of Chrysin and Its Solubility Studies. *J Appl Spectrosc. 2022;89:150-58.*
8. Majumder K K, Sharma J B, Kumar M, Bhatt S. Development and validation of UV-Visible spectrophotometric method for the estimation of curcumin in bulk and pharmaceutical formulation. Pharmacophore. 2020;11(1):115-21.
9. Sharma T, Kaur R, Borges K B, Kaur R, Katare O P, Singh B. An HPTLC densitometric method for simultaneous quantification of sorafenib tosylate and chrysin: Analytical method development, validation and applications. 2021 Mar;162:105821.
10. Tsvetkova D, Ivanova S. Estimation of validation parameters of UV-Spectrophotometric method for analysis of Valsartan. Journal of Advanced Pharmacy Education & Research| Jul-Sep. 2018;8(3).
11. Bansal, A., Srivastava, N. & Nagpal, K. Development and Validation of UV Spectrophotometric Method for Determination of Chrysin and Its Solubility Studies. *J Appl Spectrosc. 2022;89:150-58*
12. Warule Pooja S, Patel Vipul P, Gosavi Seema. A. Development and Validation of UV Spectrophotometric Methodfor the Estimation of Curcumin in an Ayurvedic Formulation Haridrakhand. Int J. Pharma and Drug. Anal. 2017;5(5): 193-197.
13. Castro GT, Ferretti FH, Blanco SE. Determination of the overlapping pKa values of chrysin using UV–vis spectroscopy and ab initio methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2005 Nov 1;62(1-3):657-65.
14. Breaux J, Jones K, Boulas P. Analytical methods development and validation. Pharm. Technol. 2003;1:6-13.
15. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2018 Nov 7;10(6):8-15.
16. Taleuzzaman M. Limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ). Org. Med. Chem. Int. J. 2018;7(5).
17. Kumari B, Khansili A. Analytical method development and validation of UV-visible spectrophotometric method for the estimation of vildagliptin in gastric medium. Drug Research. 2020 Sep;70(09):417-23.
18. Kaur I, Wakode S, Singh HP. Development and validation of UV spectroscopic method for determination of canagliflozin in bulk and pharmaceutical dosage form. Pharmaceutical Methods. 2015 Jul 29;6(2):82-6.
19. Sharma K, Agrawal SS, Gupta M. Development and validation of UV spectrophotometric method for the estimation of curcumin in bulk drug and pharmaceutical dosage forms. International Journal of Drug Development and Research. 2012;4(2)
20. Pradhan KK, Panigrahi G, Sahu KC. Method development, validation and stability study of irbesartan in bulk and pharmaceutical dosage form by UV-spectrophotometric method. hypertension. 2010;1(2):3-4.
21. Dosani F, Hakam N, Vyawahare A, Patharkar S, Gajbhiye K. Development and validation of stability indicating method of UV-Spectrophotometry for the estimation of flavoxate in bulk and tablet dosage form. Asian Journal of Pharmaceutical Analysis. 2022 Mar 1;12(1).