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Development and Validation of UV Visible Spectrophotometric Method for Estimation of Quercetin

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KEYWORDS
Quercetin; UV-visible spectrophotometer; ICH Guidelines; Method development; Analytical parameters

ABSTRACT:

Introduction: Quercetin is a naturally occurring flavonoid chemical that is found in many different plant species and has a range of pharmacological properties. Among flavonoids, quercetin is the most common, having three rings and five hydroxyl groups.

Objectives: The objective of this work is to validate a suggested methodology for quercetin in its purified form and to provide a simple UV visible spectrophotometric technique for measurement. Additionally, the investigation aims to utilize an UV visible twin beam spectrophotometer to measure pharmaceutical formulations at maximum absorption 370 nm.

Methods: The study focused on generating a specific, linear, accurate and precise UV spectrophotometric method for estimation of quercetin. Statistical validation followed International Conference of Harmonization (ICH) specifications. The method's parameters were assessed within a concentration range of 0.2-1 µg/ml, with recovery rates specifying accuracy and low % relative standard deviation (RSD) values confirming precision. Limits of detection (LOD) and quantification (LOQ) for quercetin were determined.

Results: A coefficient of correlation of 0.9995 was discovered in accordance with Beer's law. After determining the technique's sensitivity, the limit of detection and limit of quantification were determined to be 0.043µg/ml and 1.303µg/ml, respectively.

Conclusions: The developed UV spectrophotometric method proved suitable for the quantitative estimation of quercetin, offering rapid and accurate analysis. The results underscore the method's linearity, accuracy, robustness, LOD, LOQ, area under the curve and precision within the specified concentration range.

1. Introduction

In recent years, the use of unmodified plant antioxidants as natural food preservatives in place of artificial ones has drawn the attention of nutritionists. Because plant extracts include a variety of antioxidant chemicals that can take many different forms, they present a desirable alternative for chemical preservatives.

The chemicals that are extracted from edible plants also have the lowest levels of toxicity to humans. Hence, it is possible to modify naturally occurring bioactive molecules that could work in concert with medications in pharmacological applications.

Quercetin, a flavonol having three rings and five hydroxyl groups is a nutritional component that has been shown to improve health and is also a basic component of the human diet. Its biological activity is widely known. Among polyphenols, it is one of the strongest antioxidants. It has also been shown that quercetin possesses antiviral, antibacterial, anti-carcinogenic, and anti-inflammatory properties [1].

Typically, quercetin is found in plants as conjugates of glycone. One of the most abundant sources of quercetin is onion, which is both edible and medicinal.

A few more sources are buckwheat, plums, tomatoes, grapes, cherries, apples, mangoes, citrus fruits, and tea [2]. The chemical name of this compound is [3-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-chromen-4-on] depicted in Figure 1.

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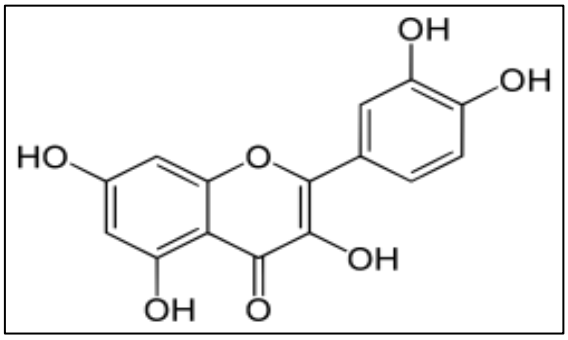

Figure 1: Chemical structure of Quercetin

Quercetin is commonly used therapeutically for a variety of allergic illnesses, including hives, eczema, cataracts, insulin resistance, peptic ulcer disease, schizophrenia, and asthmatic. In addition to conditions affecting the heart and blood vessels such "toughening of arteries" (atherosclerosis), high cholesterol, heart disorders, circulation problems, cancer, and chronic fatigue syndrome (CFS), it is also used to treat persistent infections of the prostate. It has been demonstrated that quercetin increases fibroblast proliferation, reduces immune cell infiltration, and modifies signalling in pathways associated with fibrosis [3-6]. Via a protein-mediated clotting mechanism, quercetin exerts considerable antibacterial effect against the phenol group by disrupting digestive enzymes and bursting the cell wall. As a consequence, it has a strong bactericidal property. It has been suggested that some substances belong in the vitamin category, namely "Vitamin P" [7,8].

2. Objectives

The present research work aims estimation of quercetin by developing and validating the UV spectrophotometric method.

3. Materials &Methods

Materials

Quercetin was purchased from Sigma Aldrich Chemicals Pvt. Ltd. Methanol used was analytical grade.

UV-apparatus

A Shimadzu UV visible spectrophotometer system (UV-03575 Electronics India) was used to conduct the analysis. For quercetin, sample data collection has been optimized using UV detection at 370 nm.

Method

Preparation of standard solution (10mg in 10mL)

Using a volumetric flask, 10mg of quercetin was correctly weighed. The specified amount of methanol (10mL) was added to the volumetric flask. The clear solution was produced by bath sonication process.

Determination of maximum wavelength

A UV-spectrophotometer was utilised to scan a quercetin mixture containing 10 µg/mL between 200 - 800nm in wavelength. As a blank, methanol was employed.

Standard Calibration Curve

The dilution for calibration-curve was prepared from stock solution 2 that is 100µg/ml. The dilutions were prepared in concentrations 0.2, 0.4, 0.6, 0.8, 1 µg/ml. The concentration and area are directly proportional to each other i.e., as concentration increases, the area also increases.

Analytical method validation

As per the guidelines set forth by the ICH (International Conference on Harmonization), validation is defined as the process of establishing documented proof that provides a high degree of assurance regarding the ability of a selected activity to consistently yield the intended outcome or a product that meets its predetermined specifications and quality characteristics. The following factors were evaluated in order to validate the method being used.

Preparing standard stock solution

To formulate standard stock solutions, 10mg of drug was dissolved in 10mL of methanol. The final volume was adjusted in a 10mLvolumetric flask to obtain a solution containing 100 µg/mL of drug. The UV-spectrum of 800-200nm was scanned on working standard solutions of 10 µg/mL to estimate the maximum wavelength.

Validation of the method

Linearity & Range

To ascertain linearity, the stock-solution 2 (100 µg/mL) was prepared. Five portions of the standard solution were accurately transferred (0.2, 0.4, 0.6, 0.8, 1µ/mL respectively) in five10mL of volumetric flask, methanol was added to make final volume of 10mL and absorbance was measured individually in UV-Visible

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spectrophotometer in triplicate. A concentration versus absorbance calibration curve was created, and the resulting data were analyzed using the Least-Square Method of regression, where the linearity is indicated by the square of the correlation coefficient (R^2>0.999) [9,10].

Limit of Detection and Limit of Quantification

As per the ICH recommendations, to compute the drug's LOD and LOQ, the signal-to-noise ratio (S/N) 3.3 for LOD and 10 for LOQ was used. LOD and LOQ required was calculated using the residual standard-deviation of regression-line or standard-deviation of Y-intercept of regression lines [10].

LOD = 3.3 x D / S

LOQ = 10x D / S

Where,

D- Standard deviation of y-intercept on regression lines

S- Slope of calibration curve

Precision

Precision studies were carried out to estimate the dependability of the projected analytical process. Three repetitions of the same concentration that is higher, middle, and lower concentration (0.2, 0.6, 1µg/mL) were used to firmly establish repeatability. Because of this, the absorbance was monitored throughout the day, and precision research was conducted by creating a drug resolution at a concentration of 0.2,0.6,1µg/mL and evaluating it three times throughout the day (morning, afternoon, evening). Three very distinct days were treated the same way in order to produce work that could be reported as %RSD. Despite the fact that there were two possible outcomes, the accuracy result showed genuine reliability [11,12].

Robustness

Robustness was measured using two completely distinct wavelengths, 368nm and 372nm, to analyze concentration solutions in methanol at 0.2, 0.6, and 1 µg/mL. %RSD was used to indicate the results.

Determining maximum-wavelength

The wavelength of quercetin with maximum absorption in methanol was found on UV spectrophotometer.

4. Results

A scanning between 200-800nm wavelengths of a quercetin mixture of 10 µg/mL was utilized to get UV spectrum of quercetin which is depicted in Figure 2.

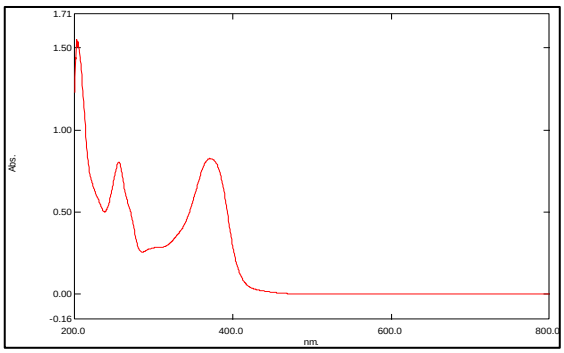

Figure 2. UV Spectrum of Quercetin

Analytical method validation:

The validation parameters of the method observed in this study are as follows:

Linearity and Range

Linearity is conveyed in terms of variance around the direction of the regression line, which is calculated based on mathematical equations of data obtained from the test results of analytes in samples with various series of concentration. The parameter used in linearity testing is the correlation coefficient (r) in linear regression analysis. The value of the correlation-coefficient (R^2) confirmed the calibration curve's linearity [13]. As seen in Table 1, the linear regression line equation obtained is y = 0.913x - 0.0084 with a correlation coefficient (r) 0.9995 presented in Figure 3. The wavelength with the greatest absorption in methanol was discovered to be 370nm depicted in Figure 4.

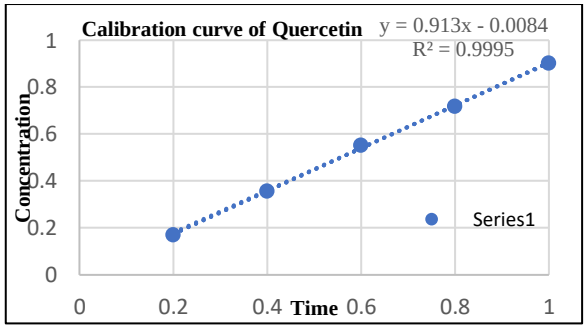

Figure 3. Linearity and range of Quercetin

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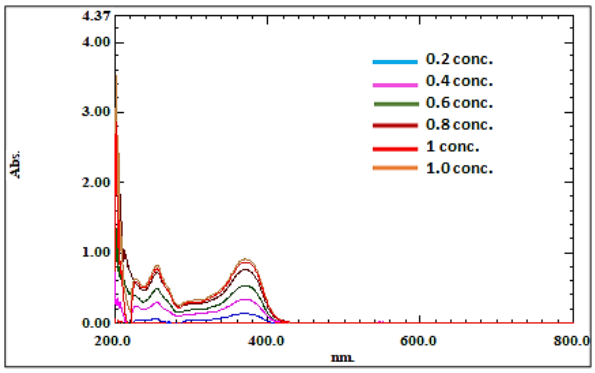

Figure 4. Overlay of maximum absorption of Quercetin on UV spectrophotometer at various concentrations

Precision

A precision method was conducted repeatedly by the same analyst in a shorter time interval; thus, the precision can also be described as repeatability or reproducibility. Precision was measured as a standard deviation or coefficient of variation. The intraday & interday were conducted on completely different days with few differences between them. The outcome demonstrates that the suggested methodology is reliable. The results of precision are shown in Tables 2, 3. Calculations were also done for the relative variance proportion [14-18].

LOD and LOQ

Limit of Detection is the smallest number of analytes in the sample, which still gives a significant response compared to others. Limit of Detection is a limit test parameter. Meanwhile, the limit of Quantification can be interpreted as the smallest quantity of analytes in a sample that had fulfilled the criteria of precision and accuracy. The LOD and LOQ can be calculated statistically based on the standard deviation response and the standard slope (S) curve. For quercetin, the results showed the LOD and LOQ were, 0.043 µg/mL and 1.303 µg/mL respectively [19,20].

Robustness

Table 4 presents the outcomes from the robustness investigation. There are two distinct wavelengths in the parameters- 368 nm and 372 nm. Moreover, table 4 displays the results of the statistical analysis.

Area under curve

In order to determine the area under the curve, 10µg/mL of the third stock solution was utilized. Furthermore, Table 5 and figure 5 displays the results of area under curve [21].

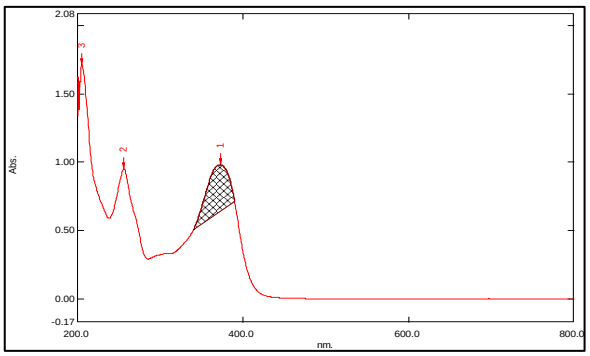

Figure 5. Area under curve of Quercetin

The optical characteristic [22,23,24] of the developed method for quercetin is mentioned in table 6.

Table 1. Linearity of quercetin

Concentration (µg/mL)	Absorbance	Equation
0.2	0.17	y = 0.913x - 0.0084 R^2 = 0.9995
0.4	0.356	
0.6	0.551	
0.8	0.718	
1	0.902	

Table 2. Intraday Precision for Quercetin

Concentration (µg/mL)			Absorbance			%RSD		
Morning 9:00 am	Afternoon 2:00 pm	Evening 5:00 pm	Morning 9:00 am	Afternoon 2:00 pm	Evening 5:00 pm	Morning 9:00 am	Afternoon 2:00 pm	Evening 5:00 pm
0.2a	0.2a	0.2a	0.203	0.198	0.148	0.565	1.744	1.824
0.2b	0.2b	0.2b	0.205	0.201	0.143			
0.2c	0.2c	0.2c	0.205	0.205	0.144			

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0.6a	0.6a	0.6a	0.43	0.384	0.388	1.521	0.539	0.392
0.6b	0.6b	0.6b	0.418	0.385	0.391			
0.6c	0.6c	0.6c	0.42	0.388	0.389			
1a	1a	1a	0.747	0.715	0.712	0.743	0.080	0.214
1b	1b	1b	0.737	0.714	0.711			
1c	1c	1c	0.738	0.714	0.718			

Table 3. Interday Precision for Quercetin

Day 1			Day 2			Day 3		
Concentration µg/mL	**Absorbance**	**%RSD**	**Concentration µg/mL**	**Absorbance**	**%RSD**	**Concentration µg/mL**	**Absorbance**	**%RSD**
0.2a	0.138	0.38	0.2a	0.167	1.02	0.2a	0.135	1.11
0.2b	0.139		0.2b	0.17		0.2b	0.138	
0.2c	0.139		0.2c	0.17		0.2c	0.137	
0.6a	0.376	0.45	0.6a	0.167	0.63	0.6a	0.452	0.58
0.6b	0.376		0.6b	0.17		0.6b	0.456	
0.6c	0.379		0.6c	0.17		0.6c	0.457	
1a	0.807	0.37	1a	0.817	0.64	1a	0.739	0.77
1b	0.811		1b	0.825		1b	0.739	
1c	0.813		1c	0.827		1c	0.749	

Table 4. Robustness

Concentration µg/mL	**Absorbance at 368nm**	**Statistical Analysis**	**Concertation µg/mL**	**Absorbance at 372nm**	**Statistical Analysis**
0.2a	0.158	Mean- 0.159 SD- 0.0015	0.2a	0.617	Mean- 0.17 SD- 0.003
0.2b	0.16		0.2b	0.17	
0.2c	0.159	%RSD- 0.95	0.2c	0.173	%RSD- 1.76
0.6a	0.393	Mean- 0.39 SD- 0.0015	0.6a	0.411	Mean- 0.41 SD- 0.006
0.6b	0.392		0.6b	0.419	
0.6c	0.395	%RSD- 0.38	0.6c	0.423	%RSD- 1.46
1a	0.709	Mean- 0.714 SD- 0.004	1a	0.741	Mean- 0.75 SD- 0.008
1b	0.716		1b	0.754	
1c	0.718	%RSD- 0.66	1c	0.756	%RSD- 1.085

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Table 5. Results of area under curve

Region	Color	Start	End	Devisor	Area
1	340	389.5	1	10.6	10.6

Table 6. Optical characteristics

Statistical	Results
Absorption maxima	370
Beer's law range	2-12 µg/mL
Correlation coefficient	0.994
Regression equation	0.835x-0.010
Slope	0.835
Intercept	0.01
LOD µg/mL	0.043 µg/mL
LOQ µg/mL	1.303 µg/mL

Discussion

Quercetin is a plant flavonoid present in many vegetables, fruits, leaves, and grains. It has antioxidant properties. It inhibits non-specific protein kinase enzymes. Additionally, it has been noted to have estrogenic properties through the activation of estrogen receptors. It is used to treat cardiac conditions, respiratory issues brought on by exercise, high cholesterol, diabetes, asthma, gout, and malignancies like pancreatic, ovarian, and lung cancer. Quercetin is known by its IUPAC nomenclature, 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy4H-chromen-4-one (Fig. 1). It is a yellow crystalline powder with the molecular weight of 302.236 g/mol and the formula C15H10O7. It is easily dissolved in ether and methanol, as well as in ethanol, acetone, pyridine, and acetic acid. According to the literature review, quercetin has been estimated using a variety of analytical techniques both alone and in combination with other medications. We have created a straightforward, verified, and optimized UV analysis technique for quercetin estimate in this study. The International Conference on Harmonization (ICH) requirements were followed in the validation of the approach. This method presents an easy, accurate, inexpensive and convenient method for investigation of quercetin using UV spectrophotometry. A scanning between 200-800nm wavelengths of a quercetin mixture of 10 µg/mL was utilized to get UV spectrum of quercetin which is depicted in Figure 2. The wavelength with the greatest absorption in methanol was discovered to be 370nm depicted in Figure 3. For both intraday and interday techniques, the data were determined to have an RSD of less than 2%. The findings achieved were less than 2% RSD, demonstrating the method's robustness and durability. The method's sensitivity was demonstrated by the finding that its limit of detection and limit of quantification were 0.043 µg/mL and 0.303µg/mL respectively. Validation of the assay method is conducted to ensure that the analytical methods are accurate, specific, reproducible, and resistant to the analyte range to be analyzed. The method was validated for many parameters like one-dimensionality, linearity and range, precision, robustness, LOD, LOQ and area under curve with ICH guidelines.

To estimate the amount of quercetin in *Tagetes erecta* extract, Rajashri Sumbe et al. devised and validated a UV visible spectrophotometric method. The technique specified in this study fulfils the Beer-Lambert law and offers a practical means of testing quercetin simultaneously in the 2–12µg/ml concentration range at λmax 369 nm. Quercetin was estimated from an extract of *Tagetes erecta*. The content of quercetin in the extract was determined to be 0.82 ± 0.020 w/w. Strong linearity between concentration and absorption was suggested by the validated parameters of quercetin, which had a correlation coefficient (R^2) value of 0.999. The percentage RSD value for quercetin was determined to be 0.38±0.020 for intraday accuracy and 0.33±0.015 for interday accuracy. The low standard deviation value demonstrated that the approach is precise [25].

Erma Yunita et al., evaluated the quercetin content of the ethanolic tamarind leaf extract using the UV-Vis Spectrophotometric technique. The outcomes of the investigation indicate that the UV-Vis spectrophotometry approach met a number of requirements including precision, accuracy, LOD, LOQ, and linearity for figuring out the amount of quercetin in the tamarind leaf extract. The tamarind extract has a quercetin content of 21.52 mg / g. Regression function coefficient (Vxo) ≤ 5% is 0.59545%, and correlation coefficient is 0.9999. The LOD and LOQ are 0.1515 and 0.4592 ppm, respectively. For precision parameters, the

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variation coefficient values have been less than 2%. In the meanwhile, the accuracy values fall between 97% and 103% [26]. V.C. Yeligar et al. validated the UV spectrophotometric approach for simultaneous measurement of Melatonin and Quercetin in liposome formulation utilizing a calibration curve method with methanol as a solvent. The wavelength range of the UV spectrum is 200–400 nm. The standard solutions of 10 µg/ml melatonin and 10 µg/ml quercetin were found to have λmax values of 276 nm and 372 nm, respectively. The simultaneous equation method was used to find the absorption and absorptivity coefficients [27]. Singh Upendra et al. verified UV spectrometric methods for the simultaneous measurement of quercetin and silymarin using a double beam UV-Vis spectrophotometer by producing a reference solution of 0.01 g of each drug in methanol and scanning in the 200-400 nm range. For quercetin and silymarin, the greatest absorption is detected at 256 and 288 nm, respectively [27]. The simultaneous estimation of piperine, quercetin, and curcumin by UV spectrometry has been validated by Ginpreet Aneja et al. They made a standard stock solution of 1000 ppm of each drug in methanol and scanned in the 200–400 nm range. The λmax of piperine, curcumin, and quercetin were found to be 371.31 nm, 424.68 nm, and 343.76 nm, respectively [27].

Viswanath et al. developed a UV spectrometry method for estimating Quercetin in *Ipomoea sepiaria* Koenig. The two-extraction scanned for absorption maxima. The standard quercetin absorbs at 350 nm, acetone extract at 328.2 nm, and methanolic extracts at 323.4 nm [27].

Marzanna Kurzawa et al. used a UV–Vis double beam spectrometer to identify the quercetin and rutin present in a variety of botanicals and medicinal formulations. Methanol and ethanol were used to make the standard solution of quercetin and rutin. The absorbance of rutin is 362 nm while quercetin is 425 nm at its maximum absorbance [27].

5. Conclusion

In summary, the establishment and validation of an UV spectrophotometric method for the estimation of quercetin have been successfully accomplished. The method demonstrated excellent linearity, accuracy, precision, and robustness, meeting the requirements set forth by ICH guidelines. This analytical approach offers a valuable means of assessing quercetin content in bulk powders and formulations. Overall, this UV spectrophotometric method serves as a reliable, straightforward, accurate, sensitive, cost-effective and practical tool for the analysis of quercetin, contributing to the advancement of pharmaceutical research and development. Based on the developed method, we recommend further analysis of the estimated quercetin for its sensitivity and in vitro and in vivo several cancer studies.

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Author Contributions

All authors have reviewed the final version to be published and agreed to be answerable for all aspects of the work.

Concept and design: FH, PS

Analysis, or interpretation of data: FH, PS, RG

Drafting of the manuscript: FH, MK, RG

Critical review of the manuscript: PS, MK

Supervision: PS, FH

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflict of interest: All the authors declare that there is no conflict of interest.

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