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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Fisetin in Bulk Form

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KEYWORDS

Fisetin;

Fisetin in Bulk Form;

UV-visible spectrophotometer;

ICH Guidelines;

Method development;

Analytical parameters

ABSTRACT:

Introduction: Fisetin (FS), a flavonoid dietary element, has recently gained increasing attention. It is also known as (2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one) [2]. It is typically found in a variety of fruits and vegetables, including strawberries, onions, apples, and cucumbers.

Objectives: The aim of this work is to provide a straight forward UV-visible spectrophotometric approach for determining Fisetin in pure form and validation of the method. Using double beam UV-visible spectrophotometer and solvent using methanol, method was developed and validated for Fisetin.

Methods: The study focused on generating a specific, linear, accurate and precise UV spectrophotometric method for estimation of Fisetin in Bulk Form. Statistical validation followed International Conference of Harmonization (ICH) specifications. The method's parameters were assessed within a concentration range of 2 to 10 µg/ml, with recovery rates specifying accuracy and low % relative standard deviation (RSD) values confirming precision. Limits of detection (LOD) and quantification (LOQ) for Fisetin were determined.

Results: A coefficient of correlation of 0.998 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 0.457µg/ml and 1.386µg/ml respectively

Conclusions: The developed UV spectrophotometric method proved suitable for the quantitative estimation of Fisetin, offering rapid and accurate analysis. The results underscore the method was linear, consistent, precise, and affordable and LOD, LOQ, area under the curve were 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.

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Flavonoids are secondary metabolites of the polyphenolic class and are mostly found in a variety of fruits and vegetables. Flavonoid have been known to play a key role in biological systems because of their low toxicity, and they have demonstrated a wide range of health-endorsing views in numerous in vitro and in vivo studies.[1]

Fisetin (FS), a flavonoid dietary element, has recently gained increasing attention. It is also known as (2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one) depicted in figure 1 [2]. It is typically found in a variety of fruits and vegetables, including strawberries, onions, apples, and cucumbers, as well as in a number of Fabaceae and Anacardiaceae family trees and shrubs, as well as species of Quebracho Colorado and Pinophyta [3].

It has recently been demonstrated that it has an antiproliferative impact on a range of tumor cells, prostate cancer, human embryonal carcinoma, colon cancer, lung cancer and breast cancer [4] also exhibits antioxidant, anti-inflammatory and neuroprotective activities [5].

Schmidt (1886), who discovered the compound decades later, described its basic chemical properties. S. Kostanecki, who began a mass investigation into yellow pigments of plants in the 1890s and created new groups for their sub-categories, which are known as flavones, chromones and chalcones, clarified the compound's structure and ultimately confirmed its synthesis [6].

It has a planar structure with many carbon rings, similar to other flavonoids. Because of its ability to donate electrons and the existence of two hydroxyl groups on one ring and a hydroxyl group on another, it can scavenge free radicals [7].

The effects of Fisetin on MCF-7 and MDA-MB-231 breast cancer cells, including cytotoxicity and apoptosis were thoroughly examined in a study that was published in 2012. In MCF-7 cells lacking caspase-3, Fisetin was discovered to have anticancer properties. Fisetin also led MCF-7 cells to undergo a new kind of aberrant apoptosis, along with rupture of the plasma membrane, depolarization of the mitochondria, activation of caspases 7, 8, and 9, and cleavage of the PARP [8].

There are certain constraints such physicochemical characteristics, which are the primary causes of reduced bioavailability and include low aqueous solubility, poor chemical stability, and metabolism.

Thus, further work is going on the formulation of Liposomes to increase patient compliance and bioavailability [9].

Fisetin easily dissolves in methanol and ethanol, Dimethylformamide and certain oils. Waters and buffers don't readily dissolve Fisetin.

Specific analytical techniques are being designed to estimate the level of Fisetin utilizing an HP LC and a UV-visible spectroscopy. Also, the further work is going on the estimation of Fisetin by UPLC method.

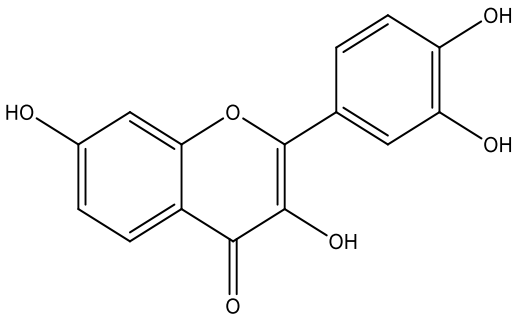

Figure 1: The chemical structure of Fisetin

2. Objectives

This study's objective is to create, develop and validate a precise and detailed method for determination of Fisetin by UV-visible spectroscopy method.

3. Materials & Methods

Materials

The supplier of Fisetin was Carbanio chemicals Hyderabad, Telangana, India. Methanol used was analytical grade.

UV-apparatus

A Shimadzu Double beam UV- spectrophotometer system (UV-03575 Electronics India), with 10 mm quartz cuvettes was used to conduct the analysis.

Method

Preparation of standard solution (10mg in 10mL)

Fisetin in the amount of 10 mg was precisely measured and put into 10ml volumetric flask. The volumetric flask was completely filled by Methanol. The solution was then sonicated on bath sonicator for 5 minutes. The

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concentration of the solution was 1000µg/ml [10,11,12,13].

Preparation of 2nd Stock Solution

From the above standard solution 1ml of solution was obtained and placed into 10ml volumetric flask and filled up to the mark with Methanol. The solution was sonicated on bath sonicator for 5 minutes. The concentration of this solution was 100µg/ml.

Determination of maximum wavelength

From the above 2nd stock solution 1ml of solution was taken and transferred into 10ml volumetric flask and filled up to mark by Methanol. Hence the concentration obtained is 10µg/ml. In a UV spectrophotometer, the solution was scanned between 200 and 400 nm. Methanol solution was used as a blank.

Standard Calibration Curve

By measuring the absorbance of a solution, the standard calibration curve for Fisetin was established in range of concentration 2µg/ml to 10µg/ml which was prepared from the 2nd stock solution of Fisetin. The Fisetin calibration curve was then drawn, with the concentration on the X-axis and absorbance on the Y-axis.

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. A particular procedure will reliably result in a product that meets the required standards and quality criteria. Other variables were assessed for method validation.

Validation of the method

Linearity & Range

In analytical approach linearity is used to check whether the results obtained are directly proportional to the analyte concentration. It was observed within 2µg/ml to 10µg/ml concentration which was prepared from the 2nd stock solution. It should be linear within the specified range and correlation coefficient should not be less than 0.99. 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).

Limit of Detection and Limit of Quantification

The smallest amount of analyte that can be detected in the sample is known as the limit of detection (LOD). The smallest quantity of analyte that can be quantitatively detected in the sample with the necessary precision and accuracy are known as the limit of quantification (LOQ). 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.

$$LOD = 3.3\ X\ \frac{D}{S}$$

$$LOQ = 10\ X\ \frac{D}{S}$$

Where,

D- Standard deviation of y-intercept on regression lines

S- Slope of calibration curve

Precision

The study of precision is carried out by repeatability to know the reliability of the method. Also the Intraday and Interday variation is known by this method. In Intraday the sample concentration is considered as Low (2µg/ml) Medium (6µg/ml) and High (10µg/ml) and each sample is run in triplicate at 3 different times during day i.e., from morning, afternoon and evening. The same procedure is followed for Interday here the sample concentration is considered as Low (2µg/ml) Medium (6µg/ml) and High (10µg/ml) and each sample is run in triplicate at different consecutive days. From the result of absorbance Mean, Standard Deviation and %RSD was calculated. The acceptable range for intraday and interday variation should be within 2%.

Robustness

Robustness study was carried out under different wavelengths the sample concentration is considered as Low (2µg/ml) Medium (6µg/ml) and High (10µg/ml) and run at five different wavelengths. The obtained

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results showed %RSD was within the acceptance criteria i.e. 2%. The method was hence robust in given conditions.

Ruggedness

Ruggedness was analyzed by change of UV instrument (Shimadzu 1900) and the sample concentration is considered as Low (2µg/ml) Medium (6µg/ml) and High (10µg/ml) and ruggedness was determined.

Determining maximum-wavelength

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

4. Results

This method presents an easy, accurate, inexpensive and convenient method for investigation of Fisetin using UV spectrophotometry. 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 Limit of Detection and Limit of Quantification of the method was found to be 0.0457 µg/ml and 0.1386 µg/ml respectively demonstrating the sensitivity of the method.

Determination of maximum wavelength

The maximum absorbance of the concentration 10 µg/ml was found at 363nm (Figure 2).

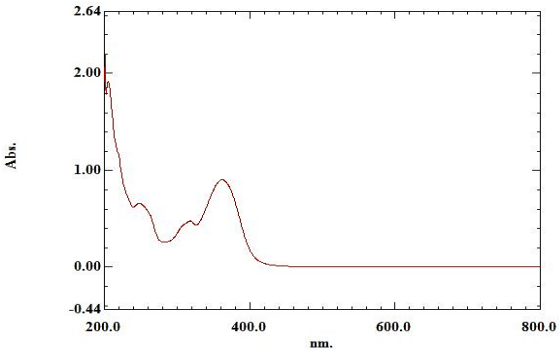

Figure 2: The UV spectrum of Fisetin in methanol

Preparation of standard calibration curve

Fisetin calibration charting is linear, having a 0.998 coefficient of correlation.as shown in (Figure 3) also the overlay plotting of Fisetin is linear as shown in (Figure 4).

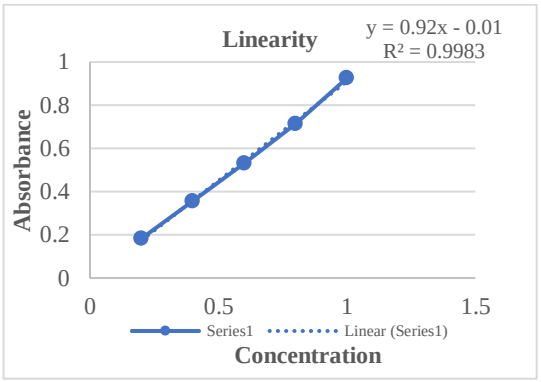

Figure 3: Calibration curve of Fisetin in methanol at 363nm

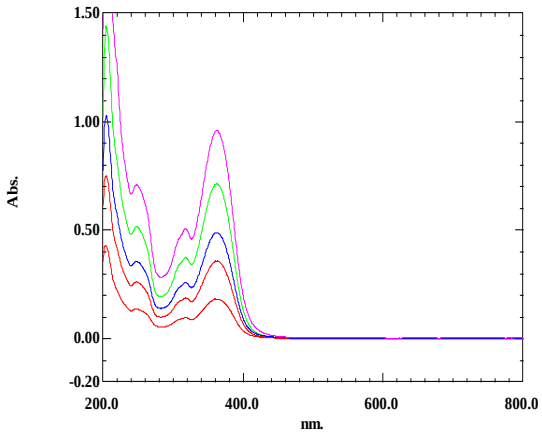

Figure 4: Overlay plotting of Fisetin in methanol

Analytical method validation

According to ICH recommendations, this method has been verified for factors like linearity, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ) 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 [14]. The magnitude of the correlation coefficients supported the linearity of the calibration curve (R^2). Fisetin was shown to have a correlation coefficient of 0.998 as shown in (Table 1).

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Table 1: Linearity table of Fisetin.

Concentration (µg/ml)	Absorbance	
2	0.183	
4	0.357	Y= 0.92x-0.01
6	0.532	R^2 = 0.998
8	0.713	
10	0.925	

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 and Interday were conducted on different days and different timings where there was no much distinction found. This proves that the method carried out is consistent. The results are displayed in following tables. Additionally, the relative variance proportion was estimated [15-19] (Table 2, 3).

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 Fisetin, LOD and LOQ values were discovered to be 0.45765 µg/ml and 1.38682 µg/ml respectively [20,21].

Robustness

Robustness was carried out for different wavelengths and the results obtained were firm. The %RSD was not found to be more than 2% (Table 4).

Ruggedness

Ruggedness tests were conducted under identical conditions on different days, with different model of instruments, and at different times. The %RSD was not more than 2% (Table 5).

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

Table 2: Intraday Precision

	Concentration µg/ml	**Absorbance 1 (9:00 am)**	**Absorbance 2 (1:00 pm)**	**Absorbance 3 (5:00 pm)**	**Average % RSD**
Low	2	0.183	0.184	0.184	0.39091446
	2	0.184	0.183	0.183	
	2	0.183	0.184	0.185	
	%RSD	0.31491833	0.31434679	0.54347826	
Medium	6	0.475	0.474	0.472	0.15161803
	6	0.475	0.473	0.473	
	6	0.476	0.473	0.474	
	%RSD	0.12146219	0.12197541	0.21141649	
High	10	0.971	0.974	0.978	0.098686
	10	0.972	0.976	0.976	
	10	0.971	0.976	0.976	
	%RSD	0.05943894	0.11839035	0.11822872	

Table 3: Interday Precision

	Concentration µg/ml	**Absorbance 1 (Day 1)**	**Absorbance 2 (Day 2)**	**Absorbance 3 (Day 3)**	**Average % RSD**
Low	2	0.183	0.184	0.183	0.419319567
	2	0.183	0.183	0.183	
	2	0.185	0.184	0.184	
	%RSD	0.62869358	0.31434679	0.31491833	
Medium	6	0.484	0.474	0.472	0.12113807
	6	0.484	0.473	0.472	
	6	0.485	0.473	0.473	
	%RSD	0.11920515	0.12197541	0.12223365	
	10	0.962	0.974	0.909	

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High	10	0.965	0.976	0.908	0.113480103
	10	0.964	0.976	0.909	
	%RSD	0.158511778	0.118390348	0.063538183	

Table 4: Results of Robustness

	Concentration µg/ml	**Wavelength 357nm**	**Wavelength 360nm**	**Wavelength 363nm**	**Wavelength 366nm**	**Wavelength 369nm**
Low	2	0.176	0.18	0.182	0.179	0.175
	2	0.176	0.18	0.181	0.178	0.175
	2	0.175	0.181	0.181	0.178	0.174
	Mean	0.175666667	0.180333333	0.181333333	0.178333333	0.174666667
	SD	0.00057735	0.00057735	0.00057735	0.00057735	0.00057735
	%RSD	0.328662392	0.320157266	0.318391693	0.323747814	0.330544047
Medium	6	0.459	0.469	0.473	0.467	0.456
	6	0.462	0.473	0.476	0.47	0.459
	6	0.46	0.469	0.473	0.468	0.455
	Mean	0.460333333	0.470333333	0.474	0.468333333	0.456666667
	SD	0.001527525	0.002309401	0.001732051	0.001527525	0.002081666
	%RSD	0.331830246	0.491013695	0.365411563	0.326161971	0.45583927
High	10	0.883	0.9	0.908	0.898	0.876
	10	0.882	0.901	0.908	0.894	0.873
	10	0.878	0.9	0.906	0.896	0.873
	Mean	0.881	0.900333333	0.907333333	0.896	0.874
	SD	0.002645751	0.00057735	0.001154701	0.002	0.001732051
	%RSD	0.300312294	0.064126279	0.127263101	0.223214286	0.19817515

Table 5: Results of Ruggedness

	Concentration µg/ml	**Shimadzu 1900**	**Shimadzu 1800**
Low	2	0.182	0.186
	2	0.18	0.185
	2	0.182	0.185
	Mean	0.181333333	0.185333333
	SD	0.001154701	0.00057735
	%RSD	0.636783385	0.311519929
Medium	6	0.475	0.461
	6	0.473	0.461
	6	0.476	0.46
	Mean	0.474666667	0.460666667
	SD	0.001527525	0.00057735
	%RSD	0.321810091	0.125329291
High	10	0.901	0.907
	10	0.901	0.906
	10	0.898	0.907
	Mean	0.9	0.906666667
	SD	0.001732051	0.00057735
	%RSD	0.19245009	0.063678339

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Table 6: Characteristics

Parameter	**Result**	
Absorption maxima	363 nm	
Beers Law Range	2-12 µg/ml	
Correlation coefficient	0.998	
Regression equation	0.92 x - 0.01	
Slope	1.08511	
Intercept	0.01187	
Precision	Intraday	Interday
	Low - 0.390	Low - 0.419
	Medium - 0.151	Medium - 0.121
	High - 0.098	High - 0.113
LOD	0.457 µg/ml	
LOQ	1.386 µg/ml	

5. Discussion

Flavonoids are secondary metabolites of the polyphenolic class that are commonly found in fruits and vegetables. Fisetin (FS), a flavonoid dietary component, has lately received more attention. It's also called (2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one). It is a polyphenolic substance that is commonly found in fruits, vegetables, seeds, nuts, and tea. It exhibits excellent pharmacological properties, including antioxidant, neurological, antiviral, anticancer (breast, ovarian, lung, colon, prostate, and oral cancer), cardiovascular, antimicrobial, anti-inflammatory, anti-Alzheimer's, and anti-diabetic (type 2 diabetes mellitus) properties [25]. In this work, we developed a simple, tested, and optimised UV analysis approach for assessing fisetin levels. The technique was validated in accordance with the International Conference on Harmonisation (ICH) criteria. This approach provides an easy, accurate, affordable, and convenient way to investigate quercetin using UV spectrophotometry. Figure 2 shows the UV spectra of a fisetin combination with a concentration of 10 µg/mL, scanned between 200-800nm. Both intraday and interday approaches were shown to have an RSD of less than 2%. The results obtained were not more than 2%, indicating the method's resilience and longevity. The method's sensitivity was proven by its limits of detection and quantification, which were 0.457 µg/ml and 1.386 µg/ml respectively. The assay technique is validated to verify that it is accurate, specific, repeatable, and resistant to the analyte range to be analysed. The approach was tested against ICH criteria for a variety of metrics, including one-dimensionality, linearity and range, accuracy, robustness, LOD, LOQ, and area under curve.

Kumar Rajan, Singh Sachin Kumar, et al. devised, developed, and validated a simple and cost-effective approach for estimating fisetin in a liquid self-nanoemulsifying drug delivery system (LSNEDD). Fisetin is a polyphenolic flavonoid that has been shown to have a variety of pharmacological actions in both crude extract and formulation form. To generate the calibration curve, solutions with varying concentrations of fisetin were produced in methanol. The solutions were scanned at 362nm using a UV spectrophotometer. Similarly, to validate the procedure, drug recovery was assessed at three levels: 80%, 100%, and 120% of the mid concentration, with data recorded. Furthermore, this approach was applied to determine fisetin drug loading in L-SNEDDS. The data was linear from 4-12µg/mL, with a regression coefficient of 0.999. The drug recovery ranged from 95% to 105%, indicating the method's accuracy. The percentage relative standard deviation among all replies was less than 2%, showing that the approach is precise. The LOD and LOQ were determined to be 0.28 and 0.86µg/mL, respectively. Drug loading in the L-SNEDDS was shown to be 93.66%. A simple, accurate, and cost-effective approach for estimating fisetin in pharmaceutical dosage forms was devised and validated using ultra violet spectroscopy [26].

6. Conclusion

In summary, a UV spectrophotometric method for the estimation of fisetin has been successfully developed and validated. The method demonstrated excellent linearity, accuracy, precision, and robustness, adhering to ICH guidelines. This analytical technique offers a reliable approach for evaluating fisetin content in bulk powders and formulations. Widely employed in various studies, the proposed UV spectrophotometric method is simple, fast, cost-effective, and practical, making it a valuable tool for pharmaceutical research and development. Based on this method, further investigation into the sensitivity of fisetin, including in vitro and in vivo cancer studies, is recommended.

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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: RG, AA

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

Drafting of the manuscript: RG, SP, MK

Critical review of the manuscript: PS, MK, RG, AA

Supervision: PS, AA, RG

Disclosures

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

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