Estimation of Fisetin in Strawberry (*Fragaria ananassa*) by UV-Vis Spectrophotometry

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Abstract

Flavonoids are naturally occurring plant molecules. Fisetin belongs to a class of flavonoids; it is a bioactive polyphenolic flavanol molecule found in fruits and vegetables with potential pharmacological activities. This study was designed to validate a simple method for estimating and evaluating fisetin in the strawberry extract by spectrophotometry. Methodology: Isolation of fisetin from the methanolic extract of *Fragaria ananassa* with chloroform by liquid-liquid extraction technique. The wet granulation method was formulated for making the tablet by incorporating pure fisetin as a biomarker. Spectrophotometric detection was carried out at an absorption maximum of 362 nm using methanol as solvent. The method was validated by linearity, accuracy, and precision studies. In the validation study, fisetin obeyed Beer-Lambert's law in the concentration range of 1 to 8 μg/mL, and it was found to be linear with a correlation coefficient of 0.99, and the regression equation was found to be Y = 0.10x -0.04. Found the percentage relative standard deviation among all the responses was less than 2%, indicating the method's precision. The percentage recovery of fisetin was found to be in the range of 96.61 to 101.63%. The present study was statistically confirmed. Therefore, the proposed approach can accurately measure the active marker compound in the crude drug (fisetin). The analysis allows the optimization of a precise method for determining the fisetin in the fruit of *Fragaria ananassa*, which can be used to support the quality assessment of this herbal material.

Introduction

Flavonoids are physiologically active agents in plants, notable due to their crucial health benefits. Fisetin (Fig. 1) is a bioactive polyphenolic flavonoid found in many plants (Maher, 2021), serving as pigment and color-color for fruit and vegetables (Kimira *et al.*, 1998). It is present in strawberries, apples, persimmons, onions, and cucumbers. Among them, the highest concentrations of fisetin are present in strawberries (160 μg/g) and the least concentration in apples (26.9 μg/g) and persimmons (10.5 μg/g). The strawberry's scientific name is *Fragaria ananassa*; such species are available in the universe, such as diploids like *Fragaria viridis*, *Fragaria daltoniana*, *Fragaria nubicola*, *Fragaria nipponica*, *Fragaria vesca*, Fragaria nilgerrensis, *Fragaria iinumae*; tetraploids like *Fragaria* orientalis, Fragaria moupinensis; hexaploids like *Fragaria moschata*; octaploids like *Fragaria X ananassa*, *Fragaria virginiana*, *Fragaria ovalis*. *Fragaria ananassa* is an accessory fruit stimulated by cold weather as a berry. It is reported to constitute phenolic acid, ellagic acid and its derivatives EA-4-arabiniside, EA-4-acetylarabinoside, EA-4-acetylxylo -side, β-carotenoids (Lutein, β-carotene), omega-3 fatty acids and derivatives. It shows essential dietary antioxidant properties, and the polymers comprise one of the largest phytonutrients that afford beneficial health effects (Khan *et al.*, 2013). It elevates Glutathione metabolism, which can protect from glutamate (Ishige *et al.*, 2001) and prevents inflammatory cytokine production for bone marrow-derived circulating leukocytes, signifying that fisetin causes a reduction in the age-related decline in brain function. Fisetin is protective against cancer, by inhibiting cancer cell proliferation and inducing JAK/STAT3 signaling pathways with oxidative damage to the cancer cell (Liang *et al.*, 2020). It's also able to show effects on Parkinson's disease, neurological disorders, asthma and accumulation of specific proteins starts neurodegeneration (Maher, 2017; Mitra *et al.*, 2022). As a dietary supplement flavonoid fisetin can be able to suppress rRNA biogenesis(Kammerud *et al.*, 2021). Dietary phytochemicals

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fisetin can extend longevity by regulation of metabolism and showing effectiveness during multiple treatments via evolutionarily conserved mechanisms (Shen et al., 2022). The scientific proven emphasizes pharmacological activities are cardiotonic, anti-inflammatory, anti-bacterial, anti-cancer, anti-viral, anti-depressant, and anti-hepatotoxic (Lozada-Ramírez et al., 2021). Based on literature surveys, a few methods are reported for determining and isolating the fisetin, such as HPLC and UV (Kumar et al., 2020) approaches in self-nano emulsifying drug delivery systems, Identification and determination of fisetin in black and green teas by ZIC-HILIC column coupled with UV detection (Abdulrahman et al., 2021), quantification in the formulation and in-vitro release study (Liu et al., 2021), preliminary screening and quantification of flavonoids in selected seeds of Apiaceae by UV-Visible spectrophotometry (Aparna & Hema, 2022), determination of total flavonoids in watermelon by spectrophotometry (Wei et al., 2019). The present work aims to develop a validated novel, accurate, and robust analytical procedure for estimating the fisetin in the strawberry extract by UV-Visbile spectrophotometry.
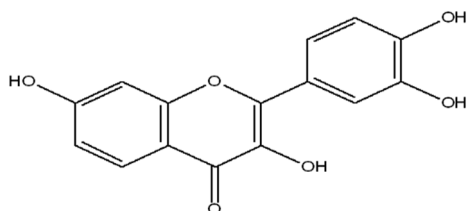

Fig. 1. Structure of fisetin

Materials and methods

All analytical grade chemicals and reagents are employed. Methanol was procured from GlaxoSmithKline Pharmaceuticals Limited, Mumbai, India. We received chloroform from Ideal Chemicals Pvt. Ltd, Gujarat, India. Standard fisetin was purchased from Sigma- Aldrich, Mumbai, India.

Experimental work
Apparatus

The research was carried out using a UV-Visible spectrophotometer, SHIMADZU model 1800 (Mumbai, India), with a spectral width of 2 nm, and a pair of 10 mm matched quartz cells used to test the absorption of all the solution. Shimadzu, model AUX 220 (Mumbai, India) electronic balance was used to weigh the sample.

Preparation of crude extract

350 g of strawberries were thinly sliced and dried for 40 h at 100 °C in an oven. The strawberries were ground in an electrical grinder (Retsch GmbH - Haan, Germany) and 30 g of fine powder was taken in a Petri plate followed by adding 100 mL hydro-alcoholic mixture, in the dark at room temperature for 72 h, renewal the solvent every 24 h. Further, the solution was filtered, evaporated at 50 °C and refrigerated for 48 h (Bouzid et al., 2014; Surnis et al., 2016).

Estimation of fisetin in strawberry extract

Extraction of the active component utilizing is a powerful enrichment strategy of liquid-liquid extraction by separating the concentration of analytes. By liquid-liquid extract, using chloroform as a solvent system, a total of 0.1 g methanolic extract was dissolved in 100 mL of distilled water and poured into a separating funnel. 30 mL of chloroform was added to the separation funnel apparatus and left for 2 min. The colorless part of the mixture was taken, which contains flavonoids. The filtrate was evaporated to remove excess chloroform and preheated. Transfer it to 10 mL of volumetric flask and make up the volume with methanol to get desired concentration (5 μg/mL). The concentration was observed from the calibration curve with respective absorbance values. Statistical analysis was carried out to verify the uniformity (Chen et al., 2014).

Alkaline reagent test

5 mg of strawberry powder was added in 10 mL of distilled water. The solution was heated using a burner until the solution became warm and then it was filtered through Whatman No 42-filter paper. To perform this filtration, 2 mL of 10% sodium hydroxide solution were added, showing itself through yellow color. A change in color from yellow to colorless on the addition of dilute HCl, indicates the presence of flavonoid (Shah & Hossain, 2014).

Preparation of a standard stock solution

Weighed about 10 mg of fisetin and transferred it to a 100 mL volumetric flask, dissolved with enough methanol by shaking manually for 10 min. The volume was adjusted with the methanol up to the mark to give the final strength, i.e., 100 μg/mL. Furthermore, the solutions with the requisite concentrations for procedures by diluting with the phosphate buffer (pH, 7.0) made from the working standard.

Development of calibration curve

To develop calibration curve, first working standard solutions were prepared. From this solution, suitable aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL were taken into the ten different 10 mL volumetric flasks and volume was made up to 10 mL using phosphate buffer (pH, 7.0). The final resultant concentration of the samples was 1, 2, 3, 4, 5, 6, 7, and 8 μg/mL. These samples were used to develop a calibration curve for UV-Visible spectrophotometry. The calibration curve was plotted between concentration and absorbance by Microsoft Excel 2016 software.

Selection of wavelength for analysis of fisetin

Appropriate volume of 0.5 mL of standard stock solution of fisetin was transferred into a 10 mL volumetric flask, diluted to a mark with phosphate buffer (pH, 7.0) to give a concentration of 5 μg/mL. The resulting solutions was scanned in the UV-Vis range (200-500 nm). In the spectrum fisetin showed the maximum absorbance at 362 ± 1.2 nm.

Method validation

The developed method were evaluated according to the ICH guidelines (ICH, 2005) for validation study in terms to determine the linearity, precision, and accuracy. As per the ICH

guidelines validation study, if the percentage of RSD less than 2% and the correlation coefficient value of the linearity study is 0.99 or more, its indicating developed method is accepted and accurate for evaluation.

Development of formulation

Tablets were prepared by wet granulation technique, using ingredients fisetin, PVPK30, and lactose mixed uniformly. An enough granulating agent as a water was added slowly to prepare the wet mass. Granules were prepared by sieving method using sieve No. 10. Further, granules were dried at 35-40 °C for 2 h and passed through sieve number 16. Then the fine granules were collected and, talc and magnesium stearate are added to the granules. The required number of granules were weighed and compressed using a tablet punching machine, model RDHSTP (Riddhi Pharma Machinery Limited, Mumbai) having a 6 mm punch diameter.
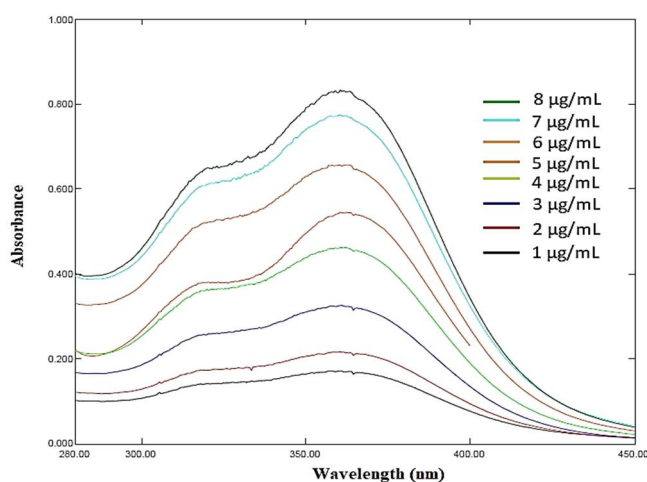

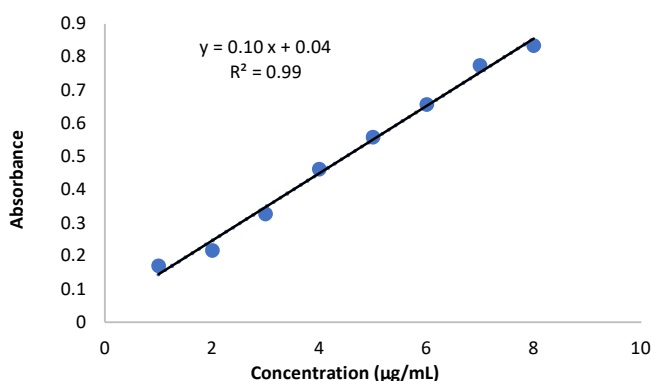**Fig. 2.** Overlain spectra for linearity of fisetin (1-8 μg/mL).

Fig. 3. Linearity of fisetin in different concentration (μg/mL).

Results and discussion

Fisetin was soluble in both organic and aqueous solvents; based on the study of stability, solubility, and the appearance of the spectrum, methanol was initially chosen to develop this novel method for the determination of fisetin. Because of the high absorbance of methanol in UV, phosphate buffer (pH, 7.0) was chosen for future dilution after being dissolved in methanol. The maximum absorbance (λmax) of fisetin was found to be $362 + 1.2$ nm. From the linearity study, it was found that fisetin obeys Beer–Lambert law within the concentration range of 1-

10 μg/mL, and the coefficient correlation was found to be 0.99. The regression equation was y = 0.10x-0.03, as shown in Fig. (2), and the overlay spectrum of linearity was represented in Fig. (3). The detection and quantification limits were calculated, which were found to be 0.13 and 0.39 μg/mL, respectively. The precision results of intraday and interday measurements showed significant reproducibility with the percent relative standard deviation (% RSD) below 2.0 (Table 1). It indicates that the method is highly precise. The accuracy of the developed method was demonstrated by the percent recovery value, which was greater than 96%, and is shown in Table (2). In the alkaline reagent test, the filtrate solution was treated with a few drops of sodium hydroxide solution and observed an intense yellow colour in the test tube. The formed colour disappeared to colourless by adding the hydrochloride solution that indicated the presence of flavonoids. In the evaluation of fisetin 5 mg tablet formulation, not observed any significant differences from the standard drug. The percentage of fisetin found and the obtained %RSD values are 87% and 1.49%, respectively. The estimation of fisetin in the strawberry extract was found to be 5.76%.

Table 1. Precision studies

Component	Concentration (μg/mL)	Intraday precision[a]	Interday precision[a]
Fisetin	5	0.55	0.54
	5	0.55	0.55
	5	0.55	0.54
	5	0.54	0.55
	5	0.55	0.53
	5	0.55	0.52
	% RSD	1.10	1.78

[a] Average of six estimations

Table 2. Accuracy of the proposed method

Component level	Amount of drug added (μg/mL)	Amount recovered (μg/mL)	% Recovery	% RSD
50 %	2.50	2.46	98.21	
	2.50	2.44	97.55	0.82
	2.50	2.42	96.61	
100 %	5	5.08	101.63	
	5	4.96	99.11	1.88
	5	4.90	97.98	
150 %	7.50	7.34	97.82	
	7.50	7.41	98.86	1.70
	7.50	7.58	101.13	

Su-Hwan Kim and Chang-Ki Huh study conducted for isolation and identification fisetin from *Rhus verniciflua* Seeds (Kim & Huh, 2022). Naeimi and Alizadeh (2017), provide a concise review of fisetin and its beneficial properties. In the Jang *et al.* (2005) study, flavonoids purified from *Rhus verniciflua* and reported the stokes actively inhibit cell growth and induce apoptosis in human osteosarcoma cells. In this study, the quantification and extraction of the fisetin from the *Fragaria ananassa*, as well as Strawberry, was successfully developed and validated by this novel method. The analytical method was verified according to the procedure such as accuracy, precision, specificity, and linearity presented in the ICH guidelines. As a result, the validated UV-spectrophotometric analysis method is suitable for the determination of fisetin in the different origins of the developed natural sources.

Conclusion

Recently, scientists have increasingly investigated the health security of fisetin in food and vegetables. Developing the ideal methodology with better analytical characteristics, which may be applied to the analysis of complex foods matrices and may be accommodated to suit the necessities of a certain fisetin. A simple, precise UV-Visible spectrophotometric method for determining fisetin from strawberry extract was successfully developed and validated. Fisetin is present abundantly in strawberries. The extraction of fisetin was carried out using methanol as a solvent system and its isolation was performed in chloroform by liquid-liquid extraction technique and a high amount of fisetin was found. The proposed method can be applied for the routine quality analysis of fisetin from herbal formulations. The low solvent consumption and replacing hazardous solvents with greener ones, made these methods eligible for use in different laboratories as existing alternative methods for routine and fast determination of fisetin from various sources.

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Author contributions

Nalanda Baby Revu: Data collection, Data analysis, and interpretation, Writing the draft of the manuscript; **Subhadip Chakraborty:** Presenting the research idea and study design, Revising, and editing the manuscript, Supervising the study, Approval of the final version; **Geeta Surisetty:** Data collection, Data analysis.

Conflicts of interest

The authors declare that there is no conflict of interest.

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