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Research

Development and Validation of UV Spectrophotometric Method for Simultaneous Quantification of Diosgenin and Fisetin in Bulk and Pharmaceutical Formulations

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Abstract

Diosgenin and fisetin are important bioactive compounds with significant pharmaceutical applications. A simple, accurate, and cost-effective analytical method is needed for their simultaneous determination in pharmaceutical formulations. The method was developed using methanol as solvent and distilled water for dilution. Standard solutions were prepared and analyzed at their respective λmax values. The method was validated according to ICH guidelines for linearity, accuracy, precision, repeatability, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The λmax for diosgenin and fisetin were found to be 210 nm and 360 nm, respectively. Linear relationships were established over concentration ranges of 1-5 µg/mL for diosgenin and 2-10 µg/mL for fisetin. The regression equations were y = 0.1865x + 0.0016 (r^2 = 0.9954) for diosgenin and y = 0.0944x + 0.0092 (r^2 = 0.9924) for fisetin. Recovery studies showed 99.67-100.13% for diosgenin and 99.93-100.37% for fisetin. The method demonstrated excellent precision with %RSD values less than 2% for all parameters. The developed UV spectrophotometric method is simple, accurate, precise, and suitable for routine quality control analysis of diosgenin and fisetin in pharmaceutical formulations.

Keywords: Diosgenin, Fisetin, UV spectrophotometry, Method validation, Pharmaceutical analysis.

INTRODUCTION

Ultraviolet (UV) spectrophotometry is a fundamental analytical technique widely employed in pharmaceutical analysis for both qualitative and quantitative determination of active pharmaceutical ingredients (APIs). Diosgenin [(3β,25R)-Spirost-5-en-3-ol] is a steroidal sapogenin with molecular formula $C_{27}H_{42}O_3$ and molecular weight 414.62 g/mol[1]. Diosgenin exhibits anti-inflammatory, antioxidant, and anticancer properties, making it valuable in pharmaceutical research and nutraceutical applications [2]. Fisetin (3,3',4',7-Tetrahydroxyflavone) is a flavonol compound with molecular formula $C_{15}H_{10}O_6$ and molecular weight 286.24 g/mol. It belongs to the flavonoid family and possesses neuroprotective, anti-inflammatory, and senolytic properties [3,4]. Fisetin is increasingly recognized for its potential therapeutic applications in neurodegenerative diseases, cancer, and metabolic disorders [5, 6]. Literature survey reveals that various chromatographic and spectrophotometric methods have been reported for individual analysis of diosgenin and fisetin. Ozkan et al. (2020) developed a simultaneous UV spectrophotometric method for diosgenin determination along with other compounds in fenugreek seed extract [7]. Ahmad et al. (2022) discussed various analytical methods for diosgenin quantification, emphasizing the importance of UV spectrophotometry due to its simplicity and cost-effectiveness [1]. However, no specific UV spectrophotometric method has been reported for the simultaneous determination of diosgenin and fisetin in pharmaceutical formulations. The objective of this study was to develop and validate a simple, precise, and accurate UV spectrophotometric method for the simultaneous determination of diosgenin and fisetin in bulk and pharmaceutical formulations, following ICH guidelines for method validation.

MATERIALS AND METHODOLOGY

Materials and Reagents

Diosgenin and Fisetin (Gift sample from Greenmeds Labs, Chennai), Methanol (Analytical grade), Double distilled water (Anichale Technologies Pvt Ltd, Haryana) Marketed Diosgenin capsules (16% - Terravita, Zooscape LLC) and Fisetin capsules (Nutricost) were purchased from online.

Instruments used

A double beam UV-visible spectrophotometer (Shimadzu 1900i) with spectra manager software was used for the analysis. Quartz cells having 1 cm path length with 3 cm length were used for spectral measurement. Sonicator (Verilux ® 0.8L Mini Ultrasonic) Weighing balance (Wensar PGB 600) with internal calibration mode was used for the accurate weighing purpose.

Method Development

Standard Curve of diosgenin& fisetin Using UV Spectra

Preparation of Standard Solutions and calibration curve of diosgenin

Accurately weighed 10 mg of diosgenin was dissolved in 100 mL methanol to prepare stock solution-1 (100µg/mL). Working standards of 1, 2, 3, 4, and 5µg/mL were prepared by appropriate dilution with distilled water.

Preparation of Standard Solutions and calibration curve of Fisetin

Accurately weighed 10 mg of fisetin was dissolved in methanol and diluted to 100 mL with distilled water to prepare stock solution-1 (100µg/mL). Working standards of 2, 4, 6, 8, and 10µg/mL were prepared by appropriate dilution.

Both drug solutions were scanned between 200-400 nm to determine their respective λmax values [8, 9]. The λmax for diosgenin was found to be 210 nm and for fisetin was 360 nm. Absorbance values were measured at respective λmax for each concentration, and calibration curves were constructed by plotting absorbance versus concentration. The linear correlation between these concentrations(x- axis) and absorbance (y-axis) were graphically presented and slope (b), intercept (a), and correlation coefficient (r^2) were calculated for the linear equation (y=bx+a).

Validation of the developed methods

The developed method was validated according to ICH Q2 (R1) guidelines for the following parameters [10]:

Linearity [11]

Linearity was evaluated by analyzing standard solutions at different concentration levels and constructing calibration curves. The correlation coefficient (r^2) was calculated.

Accuracy (Recovery Studies)

Recovery studies were performed by spiking known amounts of standard solutions at 100% and 120% levels to pre-analyzed samples. The percentage recovery was calculated.

Precision

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 2, 4 and 6 µg/ml of diosgenin solutions for three times in the same day. Interday precision was determined by analyzing the 2, 4 and 6 µg/ml of diosgenin solution daily for 3 days over the period of week. The same procedure was adapted to fisetin with 2, 4, and 6µg/ml concentrations.

Repeatability

Solutions of 6 µg/mL concentration were analyzed six times to assess repeatability.

Ruggedness

The method was tested by two different analysts under identical conditions using 6 µg/mL concentrations.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated using the formulas:

- LOD = 3.3 × (σ/S)
- LOQ = 10 × (σ/S)

Where σ is the standard deviation of intercept and S is the slope of calibration curve.

Application to Pharmaceutical Formulations [12]

The validated method was applied to determine diosgenin and fisetin content in commercial pharmaceutical formulations. Sample preparation involved extraction with methanol followed by appropriate dilution to achieve concentrations within the linear range.

RESULTS AND DISCUSSIONS

Determination of Wavelength of Maximum Absorbance

Identifying the wavelength of maximum absorbance is essential for quantitative UV analysis. A solution with an absorbance value less than 1 is typically considered suitable for this determination. Considering these criteria, the maximum wavelength for a diosgenin solution (10 µg/ml) and fisetin (15µg/ml) were identified using the full scan mode of a UV-Visible spectrophotometer (Figure 1&2).

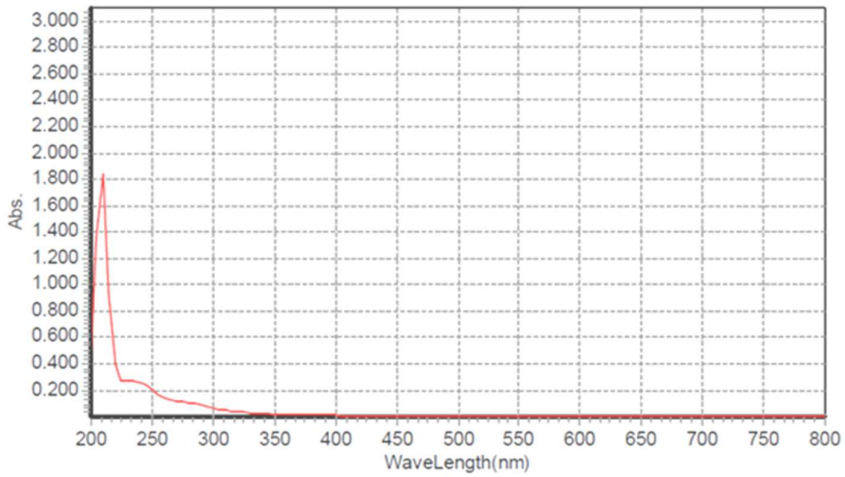

Fig 1: λ max of diosgenin

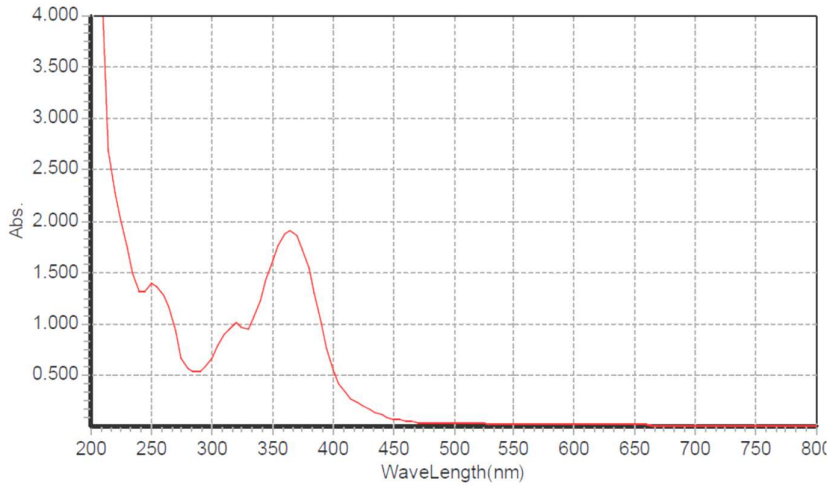

Fig 2: UV-visible spectra of fisetin

The UV spectral analysis revealed that diosgenin showed maximum absorbance at 210 nm, while fisetin exhibited maximum absorbance at 360 nm. These wavelengths provided optimal sensitivity for quantitative analysis. The calibration curve was generated five times, and the mean values ± standard deviation was calculated.

Table1: Calibration data of Diosgenin and Fisetin

Sl no	Diosgenin		Fisetin	
	Concentration (µg/mL)	Absorbance @210nm	Concentration (µg/mL)	Absorbance @360nm
1	1	0.182±0.03	2	0.201±0.02
2	2	0.4±0.02	4	0.376±0.03
3	3	0.59±0.01	6	0.559±0.01
4	4	0.75±0.04	8	0.754±0.02
5	5	0.901±0.01	10	0.958±0.04

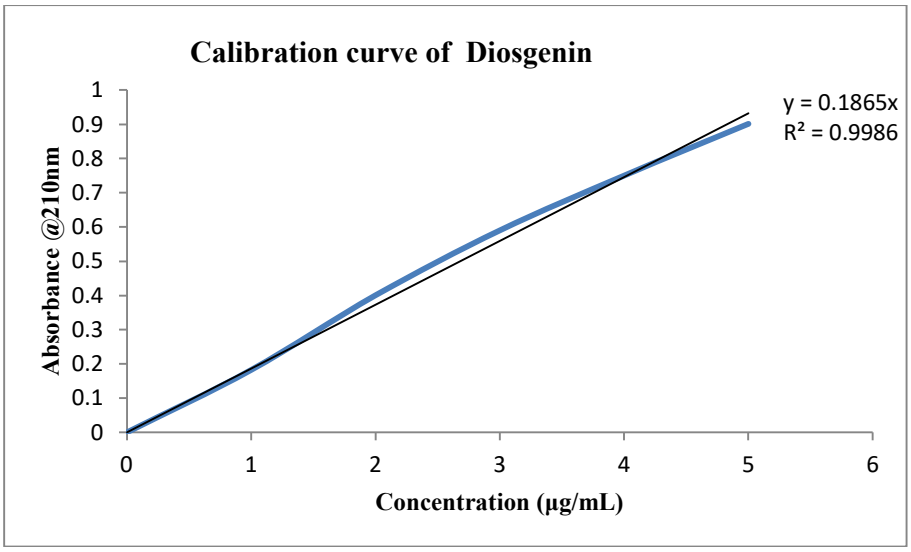

Fig 3: Calibration curve of diosgenin

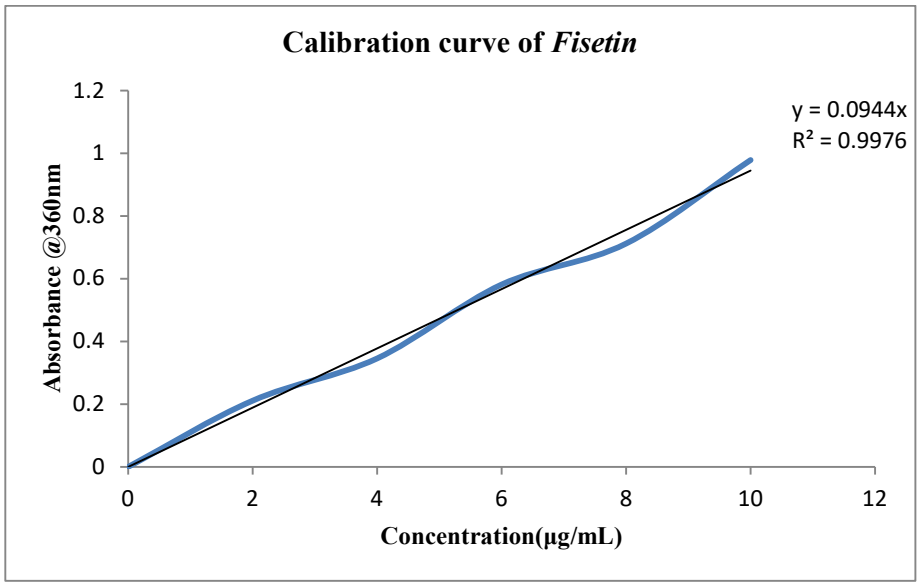

Fig 4: Calibration curve of fisetin

Validation

The linear regression data for the calibration curves showed good linear relationship over the concentration range 1–5 and 2-10µg/ml for diosgenin and fisetin respectively [Figure3& 4]. Linear regression equation was found to be y = 0.1865x (r2 = 0.9954) and y = 0.0944x (r2 = 0.9924). The result is presented in Table 2.

Table 2: Results of linearity, LOD and LOQ of diosgenin and fisetin

Parameter	Diosgenin	Fisetin
Wavelength (nm)	210	360
Concentration Range (µg/mL)	1-5	2-10
Linear Regression Equation*	y = 0.1865x + 0.0016	y = 0.0944x + 0.0092
Correlation Coefficient (r^2)	0.9954	0.9924
Slope	0.1798	0.0956
Intercept	0.0016	0.0092
Standard Error of Intercept	0.0084	0.0063
LOD	0.154 µg/mL	0.217 µg/mL
LOQ	0.467 µg/mL	0.659 µg/mL

**(Y= mx + c)*

Accuracy

The solutions were reanalyzed by the proposed method; results of recovery studies are reported in Table 3 which showed that the % amount found was between 99.0.5and 101.3 % with % RSD > 2and the % amount found was between 99.0and 101.0 % with % RSD > 2 for diosgenin and fisetin respectively.

Table 3: Results of Recovery studies

Spike Level	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery ± SD
		Diosgenin (%)		
100%	2.0	1.98	99.0	
100%	2.0	2.02	101.0	99.67 ± 1.15%
100%	2.0	1.99	99.5	
120%	2.4	2.38	99.2	
120%	2.4	2.41	100.4	100.13 ± 1.21%
120%	2.4	2.43	101.3	

Fisetin (%)				
100%	4.0	3.96	99.0	
100%	4.0	4.03	100.8	99.93 ± 0.90%
100%	4.0	4.00	100.0	
120%	4.8	4.79	99.8	
120%	4.8	4.82	100.4	100.37 ± 0.61%
120%	4.8	4.85	101.0	

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise for the determination of both the drugs [Ttable4].

Table 4: Results of precision studies

Drug	Concentration (µg/mL)	Intraday Precision n=3		Interday Precision n=3	
		Conc.found	%RSD	Conc.found	%RSD
Diosgenin	2	1.99 ± 0.015	0.75	1.99 ± 0.025	1.26
	4	3.99 ± 0.025	0.63	3.99 ± 0.035	0.88
	6	5.99 ± 0.040	0.67	5.99 ± 0.055	0.92
Fisetin	2	1.99 ± 0.025	1.26	1.99 ± 0.035	1.76
	4	4.00 ± 0.035	0.88	4.01 ± 0.040	1.00
	6	5.99 ± 0.050	0.83	6.00 ± 0.070	1.17

Repeatability

Repeatability was assessed by analyzing 6 µg/ml concentrations of diosgenin and fisetin solutions six times. The percentage amounts found were between% and% with100% RSD < 2 and% and100.16% with% RSD < 2 respectively [Table 5].

Table 5: Results of repeatability studies

No. of Estimations	Diosgenin (µg/mL)	Fisetin (µg/mL)
	Amount Found	Amount Found
1	5.98	5.96
2	6.02	6.03
3	5.97	5.98
4	6.04	6.05
5	5.99	5.99
6	6.01	6.02
Mean ± SD	**6.00 ± 0.028**	**6.01 ± 0.035**
% **found**	100	100.16
%RSD	**0.47**	**0.58**

Ruggedness

The method demonstrated good ruggedness with %RSD of 0.23% for both compounds when analyzed by different analysts. The results are in the acceptable range for both the drugs. The results are given in Table 6. The result showed that the % RSD was less than 2%.

Table 6:Results of ruggedness studies

Diosgenin (6 µg/mL)				
Parameter	**Analyst 1**	**Analyst 2**	**Mean ± SD**	**%RSD**
Mean Concentration (µg/mL)	6.00	5.98		
Standard Deviation	0.025	0.032	5.99 ± 0.014	0.23
%RSD	0.42	0.53		
Fisetin (6 µg/mL)				
Mean Concentration (µg/mL)	6.01	5.99		
Standard Deviation	0.031	0.028	6.00 ± 0.014	0.23
%RSD	0.52	0.47		

Application of the proposed method for Pharmaceutical formulation

The spectrum was recorded at 210 nm and 360nm for diosgenin and fisetin. The concentrations of the drug were calculated from the linear regression equation. The % amount found was between 99.7% and 103% (RSD: 1.75%) and between99.5% and 103.1 % (RSD: 1.83%) [Table 7].

Table 7: Estimation of diosgenin and fisetin in formulations

Concentration (µg/ml)	Amount Found (µg)	Amount found (%)
Diosgenin		
6	6.12	103.2
6	6.10	102.5
6	5.99	99.71
Mean		101.6
%RSD		1.75
Fisetin		
6	6.12	100.61
6	5.90	99.51
6	6.63	103.1
Mean	6.21	100.06
%RSD	1.85	1.83

The developed method offers several advantages over existing chromatographic methods [13]. The approach is environmentally friendly due to minimal solvent usage, making it a greener alternative to conventional methods.

CONCLUSION

A simple, accurate, and precise UV spectrophotometric method has been successfully developed and validated for the simultaneous determination of diosgenin and fisetin in bulk and pharmaceutical formulations. The method demonstrates good linearity, accuracy, precision, and sensitivity, meeting all ICH validation criteria (11). The developed method is suitable for routine quality control analysis in pharmaceutical industries and research laboratories. This analytical approach provides a reliable and cost-effective alternative to more complex chromatographic methods (14) for the determination of these bioactive compounds.

Declaration of Interest

The authors declare no conflicts of interest related to this work. The responsibility for the content and writing of this paper lies solely with the authors.

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