Journal of Pharmaceutical and Biomedical Analysis Letters
CODEN (USA): JPBAC9 | ISSN: 2347-4742
Journal Home Page: www.pharmaresearchlibrary.com/jpbmal

Research Article

Development and Validation of UV-VIS Spectrophotometer Method for Estimation of Flavonoid in *Tagetes Erecta*

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Abstract

For the determination of Quercetin in the *Tagetes Erecta* extract, a rapid, clear, selective and accurate UV-Visible Spectrophotometric method has been developed. The detection was carried out using ethyl acetate as a solvent at an absorption maximum of 369 nm. As per ICH guidelines, the procedure was validated. Quercetin was found to be 0.82±0.020w/w present in the *Tagetes Erecta* extract. The techniques obey the law of Beer's-Lamberts in concentration ranges used for evaluation. The outcome of the study was statistically confirmed. The approach proposed can therefore be used to accurately measure the active marker compound in the crude drug.

Keywords: *Tagetes Erecta*, Beer's-Lamberts Law, ICH guidelines, UV-visible spectrophotometry

Article Info

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Article History: Received 28 March 2023, **Accepted** 30 April 2023, **Available Online** 05 May 2023

Citation: *V. Amaravathi, et al. Development and Validation of UV-VIS Spectrophotometer Method for Estimation of Flavonoid in Tagetes Erecta. J. Pharm, Biomed. A. Lett., 2023, 11(1): 08-12.*

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1. Introduction

Tagetes erecta is an annual herb that has been commercialized worldwide as an ornamental plant and as a source of natural pigments from its yellow/orange flowers. This species persists after areas where it has been planted are abandoned, and it has also successfully escaped from cultivation. Various parts of this herb, including flowers, are used to treat different diseases in folk medicines[1]. Within the genus *Tagetes* L., flavonoids are the primary components and can to some degree have the sense in chemosystematics interpretations. A total of forty-nine flavonoids from the genus Tagetes L have been described. In the free or glycoside type, flavonoids occur within this genus[2]. Several laboratory studies indicate that quercetin can have anti- inflammatory effects and a wide variety of possible health benefits are being investigated[3]. 2-(3,4-dihydroxy phenyl)- 3,5,7-trihydroxy-4H-chromen-4-on Quercetin, a flavanols, is a flavonoid extracted from plants present in fruits, flowers, herbs, leaves and grains. Other clinical applications include gout

therapy, pancreatitis, prostatitis, and under inflammatory conditions. Literature research shows that several techniques such as U.V., HPLC, HPTLC and quercetin electrochemical determination have been documented for Quercetin estimation [4].

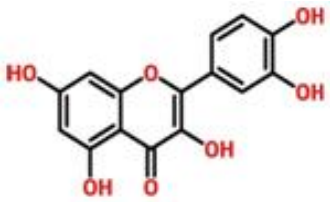

Fig: 1 Structure of Quercetin

2. Materials and Methods

Plant Material

In the month of October, the fresh flower plant *Tagetes erecta* was collected from local place, India and authenticated the plant with a voucher specimen with voucher specimen sample No.2023/431. BSI/ WRC/ Tech./ 2023/431.

Apparatus

The instrument used was a SHIMADZU model 1800 (Japan) UV Visible spectrophotometer with a spectral width of 2 nm, a wavelength precision of 0.5 nm and a pair of 10 mm matched quartz cells to test the absorption of all the solutions. For weighing the sample, an electronic analytical balance was used.

Reagents and Materials

A. R. Grade's the chemicals and solvents were used. Standard Quercetin was procured as gift sample from Yucca enterprises, Mumbai.

Preparation of crude extracts

For 20 days, the fresh flower section of the plant was dried in the shade. By continuous hot percolation using Soxhlet apparatus, approximately a large amount of dry flower petals was extracted with methanol (40-600C).The extract of methanol was refined and condensed using vacuum distillation into a dry mass. A deep reddish brown viscous residue with a potent spicy odour has been obtained. The solvents were further evaporated to dryness. For formulation, the dried extractthus obtained was used.

Preparation of standard stock solution of Quercetin: The stock solution (100µg/ml) of Quercetin were prepared by dissolving accurately about 10mg of standard Quercetinin sufficient quantity of ethyl acetate and then volume was adjusted to 100ml with ethyl acetate. From standard stock solution, aliquots portion were suitably taken and diluted to different concentration using ethyl acetate to get final concentration of 4 µg/ml, 8 µg/ml, 12 µg/ml respectively and were scanned in the wavelength range of 200- 800 nm to determine λ max[5].

Calibration curve of Quercetin

A series of standardized 10ml volumetric flasks were taken andsufficient dilutions (2 to 12 µg/ml) were extracted and diluted with ethyl acetate in a working standard solution of Quercetin up to 10ml. The absorbance was measured at an absorption maximum of 369 nm against the blank reagent prepared without Quercetin in a similar manner. Absorption maxima and the limit of Beer's law were reported and data proving the linearity and obeying the law of Beer were recorded; limits were noted. For the linear equation (y=mx-b), the linear correlation between these concentrations (X-axis) and absorption (Y-axis) was graphically presented and slope (m), intercept (b) and coefficient of correlation (R2) were determined by regression for the linear equation (y=mx-b)[4, 6].

Estimation of Quercetin in Tagetes Erecta extract: In 50 ml of distilled water, a total of 0.1 gm of methanolic extract was dissolved and 25 ml of ethyl acetate was added and shaken several times. Filter via the filter paper of Whatman no.41. The process of ethyl acetate was split and the volume was changed to 25ml. Transfer it to 100 ml of volumetric flask from the resulting solution pipette out of 2.5 ml and make up the amount with ethyl acetate to get 100 µg/ml final concentration. Using the Quercetin calibration curve, the corresponding concentration (6µg/ml) of Quercetin against the respective absorbance value was calculated. A statistical analysis was carried out to verify the uniformity. [6,7].

Validation of Developed Method [8, 9]

Linearity and range

The standard stock solution containing 100µg/ml of Quercetin each was further diluted in order to achieve a 2-12µg/ml linearity concentration for Quercetin. In triplicates, each concentration was analysed. The calibration curve was plotted using x-axis concentration and y-axis absorbance. The relationship between the drug and its absorption was represented by the y= mx-b equation, where m= slope, and b= intercept.

Limit of detection and limit of quantitation

The drug's LOD and LOQ were obtained by measuring the signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using the following ICH guideline-designated equation. To calculate LOD and LOQ, the residual standard deviation of theregression line or standard deviation of the y intercept of the regression lines was used. [10].

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

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

Where, D=Standard deviation of y intercept of regression lines & S =Slope of calibration curve.

Accuracy

The accuracy of an analytical method reflects the closeness of agreement between the value that is accepted as either a traditional true value or a reference value accepted and the value found. In recovery trials, 80, 100 and 120 per cent of the test concentration according to ICH guidelines were carried outto check the accuracy of the proposed method. The recovery analysis was performed three times at each stage. Normal

concentrations (1µg/ml) of Quercetin in ethyl acetate were prepared using the standard analysis method from different stock solutions and their strengths were measured using the standard curve.[11, 12].

Precision

The precision of the system was calculated by repeatability (intra-day) and intermediate precision (inter-day). Analysis of 6µg/ml of Quercetin three times a day and measurement of the average percentage of RSD were used to determine the intra- day precision. By assessing the same solution concentration for three days and calculating an average percentage of RSD, the inter-day accuracy was determined [13,14].

Table 1. Table I: Data for standard curve of Quercetin

Concentration (µg/ml)	Absorbance (369nm)
2	0.2123
4	0.4257
6	0.6494
8	0.8517
10	1.1001
12	1.2808

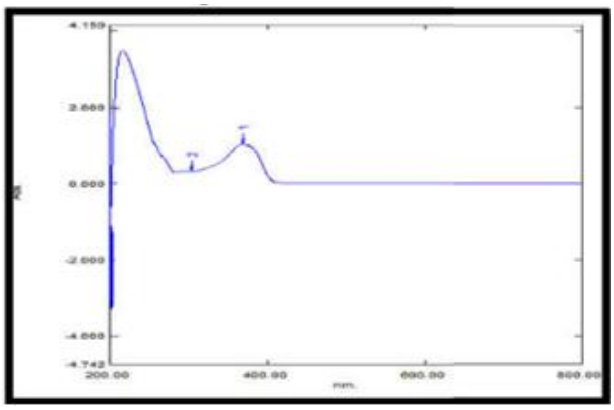

Fig.2 Maxima absorption of Quercetin on U.V. Spectrophotometer

3. Result and Discussion

The procedure was validated according to ICH guidelines. The method discussed in this paper provides a convenient way to simultaneously test Quercetin. (Fig.1). Quercetin in the 2- 12µg/ml concentration range at λ max 369 nm complies with the Beer Lambert law. (Table I) (Fig. 2) (Fig. 3).Estimation of Quercetin was carried out in *Tagetes Erecta* extract. It was found that the concentration of Quercetin present in the extract was 0.82 ± 0.020 w/w (Table II).The validated parameters of Quercetin, with a correlation coefficient (R2) value of 0.999, were discussed, suggesting a strong linearity between concentration and absorption. For Quercetin, the percent relative standard deviation (percent RSD) value was found to be 0.38±0.020 interday accuracy and 0.33±0.015 intraday accuracy. The low standard deviation value has shown that the approach is precise (Table IV). It is evident from the data that the current UV Spectrophotometric method was found to be quick, sensitive, precise, reliable, economical and rapid for the routine estimation of Quercetin in *Tagetes Erecta* extract.

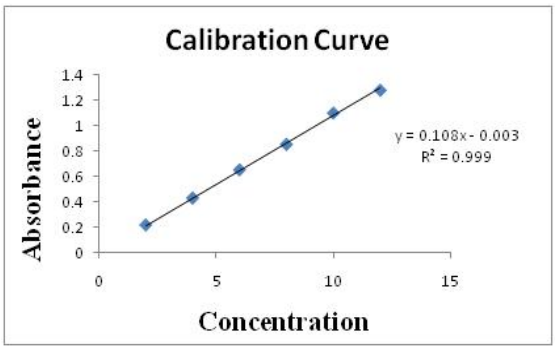

Fig.3: Calibration curve of Quercetin on UV Spectrophotometer

Table II Content of Quercetin with SD in *Tagetes erecta* extract

Extract	Standard	Content % w/w
Tagetes erecta	Quercetin	0.82 ± 0.020

Table III Results of Accuracy

Level of Recovery	Sample Conc. (µg/ml)	% Recovery	Mean % Recovery	SD	%RSD
80%	8	99	99.003		
80%	8	99.51			
80%	8	98.5			
100%	10	99.18	99.175		
100%	10	99.12			
100%	10	99.23			
120%	12	99.5	99.2		
120%	12	99.8		0.440211	0.44%
120%	12	98.5			

Table IV: Results of intra-Day & inter-Day

Con. taken (µg/mL)	Observed Conc. of Quercetin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.33	Mean = 0.33	0.37	Mean = 0.38
10	0.34	SD = 0.006429	0.39	SD = 0.001
10	0.342	%RSD = 1.91	0.38	%RSD = 2.63

Table V Validated parameters of Quercetin

Validation Parameters	Results Obtained
Drug	Quercetin
Wavelength (nm)	369
Beer's law limit (µg/ml)	12-Feb
Regression equation	
y= mx-b (m= slope, b= intercept)	y= 0.108x - 0.003
slope (m)	m=0.108
intercept (b)	b= -0.003
correlation coefficient (r2)	r^2 = 0.999
LOD (µg/ml)	0.12163
LOQ (µg/ml)	0.11866

Recovery was calculated with ±SD, where I, II, III are amount drug taken i.e. 80, 100 and 120% respectively

4. Conclusion

The UV Visible Spectrophotometer analytical method developed was simple, effective, precise and reproducible. The key characteristics of this method are low cost, faster speed, adequate accuracy and good specificity to unambiguously test the analyte in the presence of components that might be assumed to be present. As per ICH guidelines, the method has been successfully validated and can be conveniently used for routine quality control analysis of Quercetin in extract without any intervention from any phytoconstituent. Active marker compound UV spectrophotometric estimation highlights assurance of batch uniformity and purity of the produced product. For quantitative estimation of target molecules in herbal products, UV analysis is most helpful. UV identification of such a compound is key screening by chromatographic technique for further study of it.

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