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SPECTROPHOTOMETRIC ANALYSIS OF QUERCETIN IN FORMULATIONS

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ABSTRACT

In present study, the spectrophotometric analysis of quercetin in two marketed formulations using single point standardization and the calibration curve method. Ethanol was used as the solvent, and the absorbance was measured at 372 nm. The analysis was conducted over a concentration range of 2 to 10 µg/ml to determine the quercetin content in the formulations. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve method allowed for a more accurate quantification of quercetin in the formulations with good r2 (>0.999). The results showed that Formulation 2 exhibited absorbance values that were closer to those of the standard quercetin solution, indicating a more consistent quercetin concentration. These findings underscore the utility of UV spectrophotometry for the quantification and quality control of quercetin in pharmaceutical formulations. This analysis highlights the importance of using reliable methods like UV spectroscopy for ensuring the quality and therapeutic efficacy of quercetin-containing products.

KEYWORDS: Quercetin, UV -Visible spectrophotometry, pharmaceutical formulations.

INTRODUCTION

Quercetin, chemically known as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one, is a naturally occurring flavonoid found in various fruits, vegetables, and plants. It is known for its antioxidant, anti-inflammatory, and anticancer properties. Quercetin exerts its effects by scavenging free radicals, modulating inflammatory pathways, and inhibiting enzymes involved in oxidative stress. It also interacts with cell signaling molecules and regulates gene

expression, which contributes to its protective role in cardiovascular health, immune function, and neuroprotection. Due to these properties, quercetin is widely used in pharmaceutical, nutraceutical, and cosmetic formulations for its therapeutic benefits. Various formulations, includes oral dosage forms like tablets, capsules, and syrups, topical preparations such as creams, ointments, and serums for skincare. Additionally, it is incorporated into nanoparticle-based systems and combined with other compounds like vitamin C to enhance bioavailability and therapeutic effects.

Ultraviolet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200-400 nm.The main principle behind UV-Visible spectrophotometry is Beer-Lambert's law.The quantitative analysis by UV-Visible spectrophotometry is governed by the Beer-Lambert's law, it states that, "When a beam of monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, reduction of intensity of the light may occurs; the rate of reduction in intensity with the thickness of the medium is proportional to the intensity of the light and the concentration of the absorbing substance.

$$A = \log I_o / I_t = abc$$

Transmittance (T): It is the ratio of intensity of transmitted light to that of incident light.

$$T = I_t / I_o$$

Absorbance (A): It is the negative algorithm of transmittance to the base 10.

$$A = - \log_{10} T = \log_{10} I_o / I_t$$

$$A = abc$$

Absorptivity (a) is given by,

$$a = A/bc$$

Where, A = absorbance, b = pathlength, c = concentration in gm/100ml

Molar absorptivity: When concentration "c" in equation A =abc is expressed in mol/lit and cell length in cm, the absorptivity is called as molar absorptivity.

$$\text{Ɛ} = A/bc$$

MATERIAL AND METHODS

Chemicals and Reagents

1. Quercetin Sigma- Aldrich, Bangalore
2. Ethanol Labogen's Fine Chem Industry, Mumbai
3. Marketed Formulations

Instuments Used

1. Electronic Balance (ishtaa ITA 610)
2. UV-Visible Spectrophotometer (Systronics2202-TS)
3. Ultrasonicator

METHOD USED

Single Point Standardization involves measuring the absorbance of the sample at a single concentration of the standard quercetin solution, and this was used to estimate the concentration of quercetin in the formulation.

$$C\ Sample = C\ Standard \times A\ Sample / A\ standard$$

Calibration Curve method is a graphical representation of the relationship between the concentration of an analyte and the response of an analytical instrument or method.

EXPERIMENTALWORK

Selection of solvent

The solubility of drug was performed with different solvents. Quercetin was freely soluble in ethanol. The drug showed good absorbance spectrum and stability, hence ethanol was selected as solvent.

Preparation of standard stock solution

An accurately weighed quantity of quercetin (0.1g) were transferred to a 100ml of Volumetric flask. Ethanol is used to dissolve the drug, and the volume was made up to the mark with ethanol to get the solution having a concentration of 1000µg/ml. The solution is used as the **Stock A**, from that further dilution carried out.

Preparation of sample solutions of formulation 1 and formulation 2

Take 20 capsules and separate the powder and weighed. The quantity of powder equivalent to 100 mg was taken and transferred into a 100 ml volumetric flask, dissolved and volume made with ethanol. From the above solution, 10 µg/ml solution was prepared by diluting with ethanol and measured under UV-Visible spectrophotometer at 372 nm. From this concentration the amount of drug present in the formulation can be found by,

$$\text{Amount} = \text{Concentration} \times \text{Dilution Factor} \times \text{Average Weight/ Weight Taken}$$

METHODOLOGY

The working standard solutions of quercetin were scanned in UV from the range of 200-400 nm and it shows 372nm as the wavelength having maximum absorbance and these wavelengths are selected for the quantitative estimation of quercetin.

Linearity and Range: Different dilutions of concentration 2,4,6,8,10 µg/ml of quercetin were prepared. The calibration curve was plotted and interpreted in terms of correlation coefficient and equation of line.

Method precision (Repeatability): The precision of the instrument was checked by repeated scanning and absorbance of solution of (n = 6) quercetin (6 µg/ml) without changing the parameters of the developed methods.

Reproducibility: The intraday and interday precision was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solution of quercetin (4,6,8µg/ml). Relative standard deviation (% RSD) was used to report the results.

Accuracy (% Recovery): Accuracy can be reported in terms of % recovery. The percentage accuracy levels are 80,100 and 120%, About 6µg/ml of quercetin were used for the study.

Limit of detection and Limit of quantification (LOD & LOQ): The LOD and LOQ were calculated by the equation method.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

RESULTS AND DISCUSSION

UV spectrophotometric method was performed by dissolving 0.1g of quercetin in 100ml of ethanol (stock A). It was further diluted 100ml using 10ml of stock A and ethanol (stock B), further stock C was prepared by diluting 10ml of stock B. Pipetted out (1,2,3,4,5)ml of stock C. The absorbance was measured at 372 nm using solvent blank and graph was plotted between absorbance and concentration of solutions. The Beer-Lambert's law was obeyed with concentration range of 2-10µg/ml at 372nm. Beer-Lambert's law was obeyed with the concentration range 2-10µg/ml at 372nm.

Linearity: Different dilutions of concentration 2,4,6,8,10 µg/ml were used to record the absorbance of each solution at its respective wavelength (372 nm) and the calibration curve was recorded.

LOD and LOQ: According to ICH guideline there are several methods for the determination of LOD and LOQ
In the present study the LOD and LOQ were calculated by equation.
LOD and LOQ of quercetin was found to be 0.476& 1.443 respectively
LOD and LOQ of formulation 1 as 0.0515 and 0.1560 and formulation 2 as 0.0411 and 0.1244 respectively
Precision (Repeatability): Here the % RSD is less than 2 indicate the method is repeatable.
Reproducibility (Intermediate Precision): Here the % RSD was found to be below 2% indicates the reproducibility method.

Accuracy: Here the recovery result indicates the accuracy of the proposed method. The accuracy was calculated by recovery studies in various levels.

Based on the spectrophotometric analysis at 372 nm, Formulation 2 exhibited a closer correlation with the standard quercetin solution, suggesting that it contains a quercetin concentration more similar to the standard. This indicates that Formulation 2 is more accurate in its quercetin content compared to Formulation 1.

Quercetin (λ_{max}=372nm)

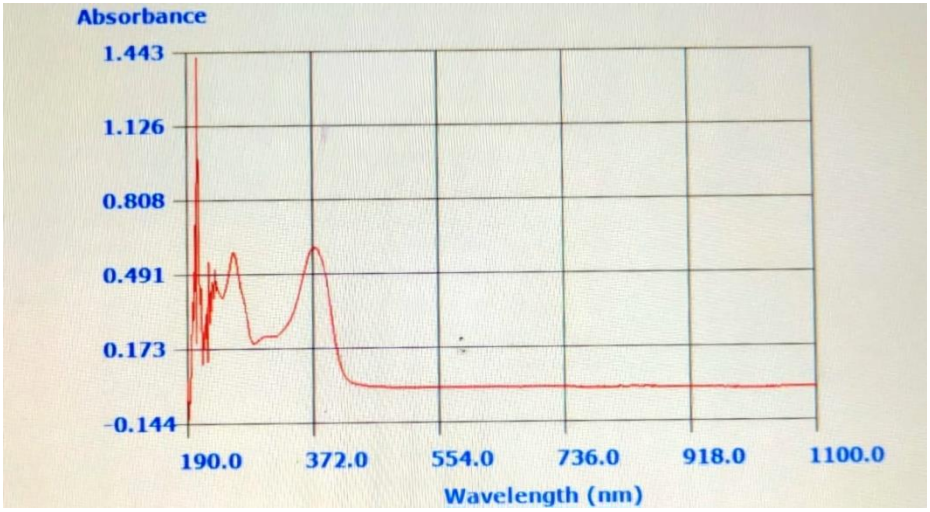

Figure 1: Absorption spectra of pure quercetin drug at 372.

Table 1: Regression analysis data and summary of validation parameters from the calibration plot.

Parameter	Quercetin	Formulation 1	Formulation 2
Absorption maximum	372 nm	372nm	372nm
Linearity range(µg/ml)	2-10	2-10	2-10
Correlation coefficient	0.9996	0.9993	0.9995
Regression equation	Y=0.0497x+ 0.0049	Y=0.0491x+ 0.0052	Y=0.0491x+ 0.007
Slope	0.0497	0.0491	0.0491
Y intercept	0.0049	0.0052	0.007

Precision Analysis

Table 2: Result of Precision study.

Concentration Quercetin (6µg/ml); n=6	Quercetin Absorbance	Formulation 1 Absorbance	Formulation 2 Absorbance
1	0.303	0.301	0.302
2	0.301	0.302	0.304
3	0.303	0.301	0.303
4	0.302	0.302	0.301
5	0.305	0.303	0.302
6	0.302	0.301	0.305
MEAN	0.302	0.301	0.302
SD	0.0013 %	0.0008	0.0014
RSD%	0.451	0.2706	0.48

Accuracy

Table 3: Accuracy study of quercetin.

Drug	Accuracy Level %	Amount			% Recovery	MEAN±SD	% RSD
		Actual (µg)	Added (µg)	Found (µg)			
QE	80%	6	4.8	10.75	99.53	99.65± 0.1201	0.1205
	100%	6	6	11.96	99.66		
	120%	6	7.2	13.17	99.77		

Single point standardization

Table 4: Quercetin data.

Sl. No:	Concentration of standard (µg/ml)	Absorbance of standard	Absorbance of sample	Concentration of sample (µg/ml)
1	6	0.303	0.304	6.01

Table 5: Formulation 1 data.

Sl. No:	Concentration of standard (µg/ml)	Absorbance of standard	Absorbance of sample	Concentration of sample (µg/ml)
1	6	0.301	0.299	5.96

Table 6: Formulation 2 data.

Sl. No:	Concentration of standard(µg/ml)	Absorbance of standard	Absorbance of sample	Concentration of sample (µg/ml)
1	6	0.302	0.302	5.98

Calibration curve

Table 7: Calibration data of quercetin.

Sl. No	Concentration (µg/ml)	Quercetin Absorbance	Formulation 1 Absorbance	Formulation 2 Absorbance
1	2	0.105	0.101	0.104
2	4	0.204	0.202	0.203
3	6	0.303	0.301	0.302
4	8	0.397	0.404	0.405
5	10	0.505	0.491	0.494

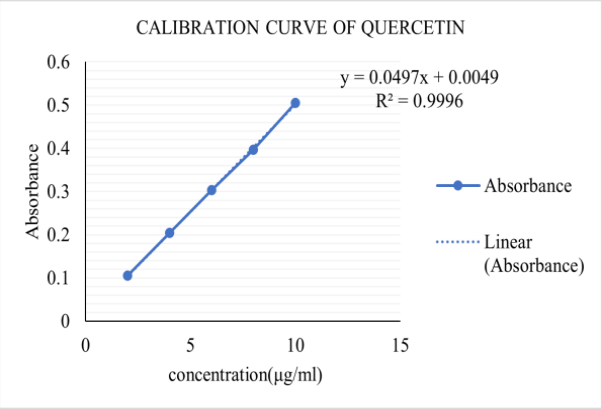

Figure 2: Calibration curve of quercetin.

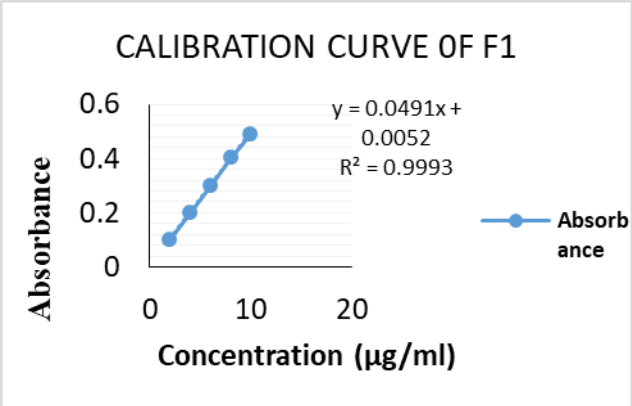

Figure 3: Calibration curve of F1.

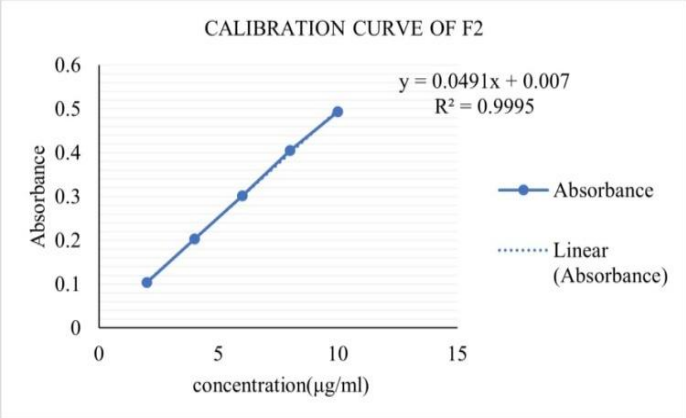

Figure 4: Calibration curve of F2.

CONCLUSIONS

The aim of study was to establish a reliable and efficient UV-Visible spectrophotometric method for routine analysis of quercetin in pharmaceutical formulation and to identify the formulation that is more similar to the standard quercetin drug using spectrophotometric method.

Ethanol was used as solvent.

The absorption maxima of quercetin was found to be 372nm.

Quercetin shows linearity from2-10 µg/ml. Formulation 2 is more accurate to quercetin drug in its quercetin content compared to Formulation 1.

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