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Simultaneous Equation Technique For The Estimation Of Quercetin And Curcumin By Uv- Visible Spectrophotometry

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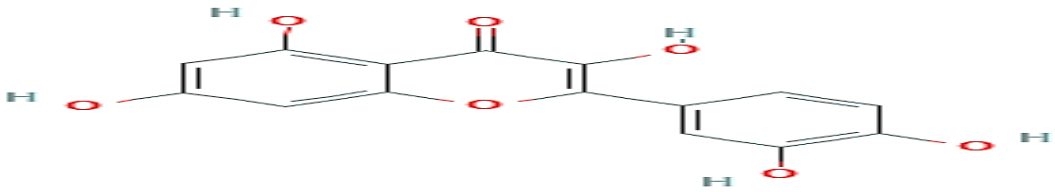
Abstract:
A simple and repeatable U.V. spectrophotometric approach was created and validated for the quantitative measurement of Curcumin (Cr) and Quercetin (Qr). According to the ICH criteria, by utilizing the parameters, namely accuracy, Limit Of Quantification (LOQ), linearity, Limit Of Detection (LOD), and recovery, the technique was validated. Now, by utilizing a quick, sensitive, exact, and affordable spectrophotometric approach in the UV area, Quercetin and curcumin concentrations in formulations might be determined. By utilizing methanol and techniques that adhered to Beers-law Lambert's at the concentration levels used for analysis, the samples were made. Therefore, for accurately measuring the amount of an active marker component in raw drugs and formulations, the suggested approach may be wielded. At 379 and 416.5 nm, the most absorbance was shown by the Quercetin and Curcumin, correspondingly. Bear Lambert's law is obeyed by each ingredient within the concentration of 2-10 µg mL-1 for Quercetin and Curcumin. For Quercetin, the LOD and LOQ values attained are.38 µg mL^{-1} and 4.20 µg mL^{-1}. Also, the LOD and LOQ values attained for Curcumin are 0.300 µg $mL^{-1}$and 0.9102 µg mL^{-1}, correspondingly.
Keywords: *Quercetin, Curcumin, UV- Spectrophotometry, Simultaneous estimation.*

INTRODUCTION

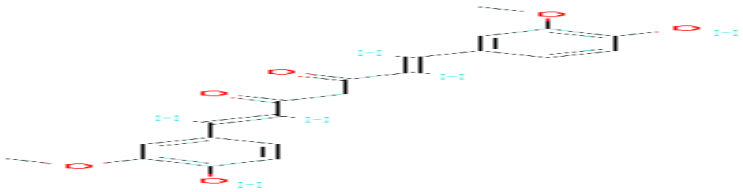
Quercetin is a bitter-tasting substance that is added to foods, drinks, and dietary supplements. It may protect people from cancer and heart disease. Indian spice turmeric which belongs to ginger family(Zingiberaceae). yellow colour of turmeric is due to presence ofnatural phenol that is curcuminoids.

Quercetin (Qr)



Quercetin, which belongs to the polyphenolic flavonoid family, is a plant flavonol. It is widely found in a variety of vegetables, seeds, fruits, leaves, and grains; also, kale, red onions, and capers are popular foods, which have considerable levels of it. Quercetin, which has an anti-inflammatory as well as antihistamine impact, could stabilize the body's histamine-releasing cells. Quercetin supplements should not be used if any of the following drugs are taken without consulting the doctor. The effects of anticoagulants (blood thinners) might be boosted by Quercetin, thus raising the risk of bleeding. Quercetin is pentahydroxy flavone that has the 5 group teams placed at the 3-, 3'-, 4'-, 5-, and 7-positions. [1]

Curcumin (Cr)



As per research, Curcumin might assist in the treatment of (1) metabolic syndrome, (2) arthritis, (3) hyperlipidemia, (4) oxidative and inflammatory diseases, (5) anxiety, etcetera. Moreover, it could also control the inflammation and muscular soreness caused by exercise, thus enhancing recovery along with following performance in physically active people. Only 2-9% of curcuminoids are contained in turmeric, of which 75% are active curcuminoids; thus, curcumin is named the "star" of turmeric. Since curcumin or turmeric supplements could enrich the blood-thinning effects, possibly to hazardous levels, these supplements are not advised to be taken by people who take blood-thinning medicine like warfarin (Coumadin), clopidogrel (Plavix), or else aspirin. Curcumin is (1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione. [2] As per the literature review, several analytical approaches are there for the study of Quercetin, either separately or in combination. Only a few approaches are used for estimating quercetin and curcumin concurrently. In light of reports on analytical techniques, including UV-VIS, HPLC, along

with HPTLC for the measurement of Qr and Cr, it was found that these techniques were not very cost-efficient concerning solvent consumption; thus, the following research is executed. The literature reviews also exposed that there are no well-established cost-effective approaches for the same. Therefore, it is absolutely crucial for developing an alternative UV analytical technique that might be utilized with accuracy for the simultaneous measurement of quercetin and curcumin [3].

MATERIALS AND METHODS:

Instrument:

This study utilized Shimadzu UV-1800 240V UV/VIS Spectro-photometer that has 2 matched 1cmmatchedquartzcells.

Chemicals and Reagents:

All reagents as well as solvents are of analytical grade. From the Millipore, milli-Q (Bedford, mA, USA) purification system, higher purity, deionized water is acquired. Quercetin and Curcumin samples are obtained from Pawar provider, Karad, geographical region, Asian nation 415110. Alcohol and acetonitrile (Merck Chemicals) utilized are of analyticalgrade.

Preparation of stock solutions

By dissolving precisely 10 mg of every single drug in an adequate quantity of ethanol, the stock solution (100µg/mL) of Qr and Cr is made. After that, the volume is regulated to 100mL with ethanol. With ethanol, a series of dilutions are also made. By precisely dissolving 10mg of every single drug adequately with ethanol, the Qr as well as Cr's stock solution (100µg/mL) is made. Afterward, the volume is regulated to 100mL with ethanol. With this, further dilutions are prepared. The Qr along with Cr's stock solution (100µg/mL) is made by precisely dissolving 10mg of every single drug adequately with ethanol. Afterward, the volume is regulated to 100mL with ethanol. With ethanol, further dilutions are done. By dissolving precisely 10 mg of every single drug in 20ml of ethanol utilizing sonication, the stock solutions (of Quercetin and Curcumin) were prepared. Then, for getting a 100µg/ml concentration, the volume was adjusted to 100ml with ethanol. Also, with ethanol, a series of dilutions are done.

Quercetin and Curcumin calibration curve.

For attaining 2, 4, 6, 8, and 10µg / ml concentrations for Quercetin, appropriate aliquots of Quercetin stock solution are taken in a series of 10 ml calibrated volumetric flasks, which is further diluted up to the mark with alcohol. For all the flasks, the absorbance is computed at 379 nm against a reagent blank prepared similarly devoid of Qr. For the stock solution of Curcumin also, the same procedure is applied. Here, for all the flasks, the absorbance was measuredat416.5nm [4,5,6]. Here, a series of calibrated 10mL volumetric flasks are considered; also, the proper aliquots of the Qr's working standard solution are taken and diluted with ethanol up to 10mL. At absorption maxima 256 nm, the absorbance is computed against a reagent blank prepared similarly devoid of Qr. For Cr also, a similar procedure is employed and is measured at 263 nm against a reagent blank prepared similarly devoid of Cr. Beer's law limit as well as absorption maxima were noted. Also, the data, which proved the linearity and obeyed Beer's law limit were recorded. The linear correlation betwixt concentrations (x-axis) and absorbance (y-axis) of calibrated 10mL volumetric flask is considered; then, proper aliquots of the working standard solution of Qr are taken and diluted up to 10mL with ethanol. At 256 nm (absorption maxima), the absorbance is calculated against the reagent blank made alike devoid of Qr. Similarly, at 263 nm, the absorbance is calculated for Cr also devoid of Cr. Beer's law limit as well as absorption maxima were noted. Moreover, the data, which proved the linearity and obeyed Beer's law limit were recorded. Then, the linear correlation betwixt calibrated 10mL volumetric flask's concentrations (x-axis) and absorbance (y-axis) is regarded.

Selection of analytical Wavelength and Determination:

To determine the absorption maxima in the range (200 to 600 nm) against alcohol as blank, a 2-µg/ml sample of Curcumin and Quercetin separately is scanned. At 379 and 416.5 nm, the Qr and Cr's maximum absorption (λ_{max}) is established. At 379 and 416.5 nm, the absorbance of each drug is noted. The molar physical property(ε) for each medication is computed using,

ε =A/C Wherein, the absorbance is specified as A; the analyte's concentration is exemplified as C.

Simultaneous Equation Technique

It is feasible to determine both drugs using the concurrent equation approach if a sample includes 2 absorbing drugs in which each drug absorbs at the maxima of the other using the system of concurrent equation. 379nm and 416.5 nm are the wavelengths chosen for the equation's growth. At 379 and 416.5 nm, 38.54 (ax1) and 69.96 (ax2) are the physical property values for Qr, whereas 59.90(ay1) and 28.18 (ay2) are the values for Cr. These values are the average of 6 approximations. Table 1 displays the Absorptivity data. In equations 1 as well as 2, the absorbance and absorptivity atthesewavelengthsare substituted for getting the concentration of every single drug [4,5,6].

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad \text{Equationno-1.}$$

$$Cy = \frac{A1ay2 - A2ay1}{ax2ay1 - ax1ay2} \quad \text{Equationno-2.}$$

Wherein, the Qr and Cr concentrations individually in µg mL^{-1} are specified as Cx and Cy. At379nmand416.5nmindividually, the mixture's absorbances are exemplified as A1 and A2.

Method Validation

According to the ICH guidelines' analytical procedure for precision, LOQ, linearity, LOD, and accuracy for the analyte, the technique was developed as well as examined [7,8].

Linearity

By examining a series of various concentrations of Qr& Cr (1 to 10 µg ml^{-1}), the proposed technique's linearity was assessed and repeated. Better linear relationships were shown by the calibration graphs acquired under the experiential conditions defined. By plotting the calibration curve forQrandCr as displayed in Figures 1and 2, the linearity of the relationship betwrrn absorbance and concentration is established.

Limit of Detection and limit of quantitation

By computing the signal-to-noise ratio (S/N, that is, 3.3 for LOD, whereas 10 for LOQ) utilizing the equations according to International Conference on Harmonization (ICH) guidelines, the drug's LOD, as well as LOQ, is derived.

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

Wherein, the standard deviation of the response is specified as σ; the calibration curve's slope is signified as S. LOD, which would be detected but not quantitatively, is the analyte's lowest quantity. LOQ, which would be determined quantitatively with proper precision as well as accuracy, is the analyte's lowest quantity in a sample [9].

Precision

By evaluating a 7 µgmL^{-1} solution of Qr and Cr, repeatability measurements were executed 6 times; also, percentage Relative Standard Deviation (% RSD) was computed. By assessing various Qr and Cr's replicate samples, the model's repeatability was determined. By carrying out inter-day as well as intraday variations, precision was executed. The samples were evaluated on 3 following days in inter-day variation. By utilizing 7µg, the inter-day, as well as intraday precision, was conducted. The absorbance was assessed thrice per day in intra-day variation.

Intra day precision

For 3,5, and 7mg/mL standard solutions of Qr and Cr, the Intra-day preciseness was determined. It was repeated 3 times per day, and % RSD was considered.

Interday Precision

For 2, 6, along with 10mg/ml standard solution of Qr and Cr, it was conducted3 times on various days. Here, %RSD was considered.

Accuracy (%recovery)

By utilizing the standard addition technique, the accuracy study was executed. The Qr and Cr's pre-quantified 2 µg mL^{-1} sample resolution was spiked with 50, 100, along with 150 of standard Qr as well as Cr. At 379nmand 416.5nm(λ max of Qr and Cr, correspondingly), absorbance was computed. Then, the concentration of every single drug was measured. The suggested technique assessed these mixtures. The experiment was executed six times. At every single concentration level, the samples' percentage recovery, percentage RSD, along with percentage was computed [10,11,12,13].As per the aforementioned factors, the proposed technique is accurate, sensitive, simple, precise, and cost-effective; also, it could be effectively applied for the estimation of Quercetin and Curcumin in bulk drug formulation

RESULTS AND DISCUSSION

Table1:Absorptivity Data for Qr and Cr

Sr. No.	conc. (µg$ml^{-1)}$	Qr				conc.(µg $ml^{-1)}$	Cr			
		ax1	ax2	^{x1}	x2		ay1	ay2	y1	y2
1	1	0.060	0.096	60	96	1	0.061	0.025	61	25
2	2	0.056	0.086	28	43	2	0.143	0.058	71.5	29
3	3	0.090	0.139	30	46.33	3	0.189	0.076	63	25.33
4	4	0.130	0.201	32.52	50.25	4	0.296	0.119	74	29.75
5	5	0.187	0.290	37.54	58	5	0.343	0.137	68.6	27.56
6	6	0.242	0.374	40.33	62.33	6	0.453	0.181	75.5	30.31
7	7	0.279	0.432	39.92	61.71	7	0.521	0.209	74.42	29.88
8	8	0.330	0.511	41.32	63.87	8	0.569	0.228	71.12	28.55
9	9	0.335	0.519	37.3	57.66	9	0.635	0.254	70.55	28.1
Average				**38.54**	**59.90**		**Average**		**69.96**	28.18

Table2:Linearity study for Qr and Cr

Sr. No.	conc. (µg ml^{-1})	absorbance of Qr at 416.5 nm	conc. (µgml^{-1})	absorbance ofCrat379nm
1	2	0.082	2	0.135
2	4	0.234	4	0.28
3	6	0.389	6	0.444
4	8	0.469	8	0.573

Fig :1 Calibration curve of Quercetin

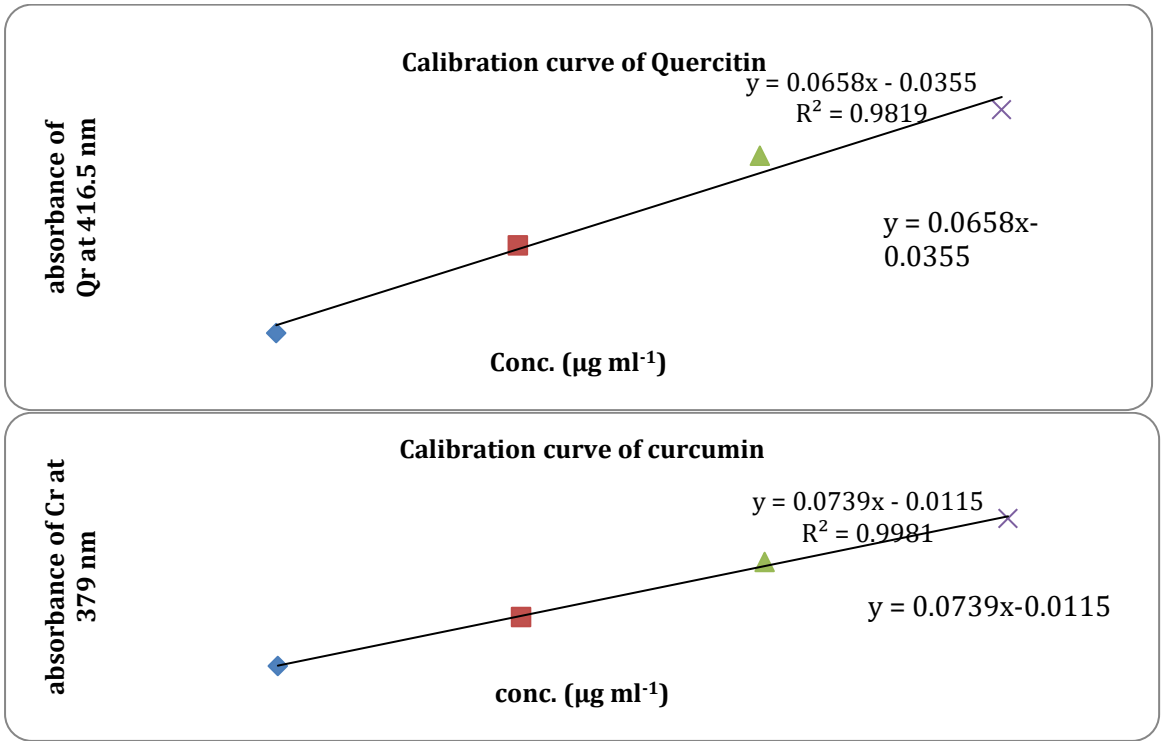


Fig :2 Calibrationcurve of curcumin

Table3:-Linear regression analysis of calibration curves

Particulars	CURCUMIN	QUERCETIN
Range(mg/ml)	2-10	2-10
Wavelength(nm)	416.50	379
CorrelationCoefficient(r^2)	0.9969	0.9826
RegressionEquation	y=0.0739x-0.0115	y=0.0658x-0.0355
LOD	0.300	1.38
LOQ	0.9102	4.20

Table4: -Intraday precision:-

Theoretical concentration (µg/ml)	Curcumin			Quercetin		
	Mean absorbance	Standard deviation	%RSD	Mean Absorbance	Standard deviation	%RSD
3	0.1826	0.000583	0.0001861	0.134	0.003872	2.88
5	0.4836	0.000583	0.0001861	0.296	0.003162	1.047
7	0.673	0.003	0.4457	0.427	0.003162	1.047

Table5:-Interday precision: -

Theoretical absorbance(µg/ml)	Curcumin			Quercetin		
	Mean absorbance	Standard deviation	%RSD	Mean absorbance	Standard deviation	%RSD
3	0.183	0.002236	1.22	0.133	0.003162	1.047
5	0.485	0.003872	2.88	0.294	0.003162	1.047
7	0.670	0.0080	1.20	0.426	0.0080	1.20

Table6:-linear regression analysis of calibration curves(n =6)

Particulars	Qr	Cr
Range (mg/ml)	1-10	1-10
$\boldsymbol{\lambda max}$ (nm)	416.5	379
Correlation coefficient (r2)	0.9712	0.9883
Regression equation	y = 0.0621x-0.0613 (r2 = 0.9712)	y=0.1027x-0.0556 (r2 = 0.9883)
LOD	1.38	0.300
LOQ	4.20	0.9102

Conflict of Interest; Authors declare that there are no conflicts of interest

CONCLUSION:

As per the aforementioned factors, the proposed technique is accurate, sensitive, simple, precise, and cost-effective; also, it could be effectively applied for the estimation of Quercetin and Curcumin in bulk drug formulation.

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