

# Development and Validation of UV-visible Spectrophotometry Method for Simultaneous Estimation of Rutin and Curcumin in Bulk Pharmaceuticals

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## ABSTRACT

**Background:** The primary objective of this study is to pioneer a novel UV spectrophotometric method, designed with simplicity and high sensitivity to accurately measure both Rutin and Curcumin simultaneously. Rutin has a therapeutic impact on treating chronic venous insufficiency and Curcumin's pharmacological activities include anti-inflammatory, anti-hyperpigmentation, etc. An effort has been made to quantify Rutin and Curcumin in bulk powder form within a topical formulation to treat Stasis dermatitis. **Purpose:** To develop and validate a simple, accurate, reliable, sensitive and robust UV spectrophotometer technique for the simultaneous measurement of rutin and curcumin in accordance with ICH Guidelines. **Materials and Methods:** To estimate Rutin and Curcumin simultaneously, a simple double-beam UV spectrophotometer method was developed. Methanol was used as the solvent to create the ideal conditions for the drug's analysis. **Results:** The optimal absorption wavelengths were identified at 257 nm for Rutin and 422 nm for Curcumin, representing their respective maximum absorbance peaks. The method showed a linear response over the concentration ranges of 4-20 µg/mL for Rutin and 1-5 µg/mL for Curcumin. Both analytes demonstrated a linear regression coefficient of 0.991, indicating a strong correlation between concentration and absorbance. The method was validated according to ICH Q2(R1) guidelines, including various parameters such as Linearity, Precision, Repeatability, Limit of Detection (LOD), Limit of Quantification (LOQ), Robustness, and Ruggedness. All validation results fell within the acceptance criteria, affirming the reliability and suitability of the method for quantitative analysis of Rutin and Curcumin. Thus, the study concludes that the developed method is novel, simple, selective, specific and precise for the simultaneous determination of Rutin and Curcumin in bulk powder samples. **Conclusion:** The method demonstrates selectivity, specificity, precision, accuracy, ruggedness and robustness, facilitating its utility for the routine assessment of Rutin and Curcumin in both bulk substances and pharmaceutical formulations.

**Keywords:** Rutin, Curcumin, Anti-inflammatory, Anti-hyperpigmentation, ICH guidelines, UV-spectrophotometry

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## INTRODUCTION

Rutin is a flavanol that is widely distributed in plants, including apples, buckwheat, tea and passion flowers. Its chemical name is 3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside, illustrated in Figure 1, possesses a molecular formula of  $C_{27}H_{30}O_{16}$  and a

molecular mass of 610.5 g/mol. It's a glycoside made up of the flavonoid aglycone quercetin and the disaccharide rutinose, which has pharmacological activities such as anticancer, antidiabetic, antimicrobial, etc.<sup>1</sup> The effectiveness of Rutin in treating chronic venous insufficiency has been studied in patients in combination with other herbal active moieties. Rutin, classified as a bioflavonoid, enhances collagen synthesis, leading to decreased vascular permeability and enhancement of vascular endothelial barrier function. Prolonged administration of high doses of flavonoid glycosides such as rutoside and rutin found in citrus fruits has led to clinical improvements and, in certain



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instances, resolution of symptoms.<sup>2</sup> Curcumin, a hydrophobic aromatic phytoextract, was initially extracted from the aromatic rhizome of the Indian plant turmeric (*Curcuma longa* L.), which is a member of the ginger family (Zingiberaceae). Chemically identified as (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, as depicted in Figure 2, it possesses a molecular formula of  $C_{21}H_{20}O_6$  and a molecular weight of 368.4 g/mol. Its pharmacological activities include antioxidant, anti-inflammatory, anti-cancer, anti-diabetic and antiviral properties.<sup>3</sup> The healing process in stasis dermatitis is impacted by the overexpression of Matrix Metalloproteinases (MMPs), which are involved in tissue repair and cause hyperpigmentation.<sup>4,5</sup> Curcumin inhibits MMP expression and downregulates NF-kappa B, which speeds up healing and prevents hyperpigmentation.<sup>6</sup> The skin conditions brought on by stasis dermatitis are not currently treatable. This necessitates the creation of a suitable formulation containing rutin and curcumin for the topical treatment of stasis dermatitis.

Currently, there is limited available data on the simultaneous estimation of Rutin and Curcumin using the UV method development and validation parameters. Therefore, this study aimed to establish and validate a straightforward, accurate and highly sensitive UV-spectroscopy technique for the simultaneous estimation of Rutin and Curcumin in bulk and pharmaceutical formulations.

## MATERIALS AND METHODS

### Drug samples

Rutin was procured from Fine-Chem Ltd., located in Uran Islampur, Maharashtra, while Curcumin was acquired from the storage facility of KLE College of Pharmacy in Belagavi.

### Reagents and chemicals

The analytical quality chemicals and reagents used in the experiment were procured from the storehouse of KLE College of Pharmacy, Belagavi. HPLC-grade methanol was employed.

### Instruments and apparatus

The UV-Spectrophotometers Shimadzu UV-1900 with Lab Solutions software and Shimadzu UV-1800 with UV probe were utilized for simultaneous estimation of Rutin and Curcumin.

### Method Development

Different solvents were tested for the solubility of both samples, like methanol, water, acetonitrile, 0.1N HCl and 1N NaOH, out of which methanol showed better solubility and was selected as the solvent. Both samples were scanned with a UV spectrophotometer in the range of 800-200 nm, with methanol serving as the solvent. Rutin exhibited maximum absorption at 257 nm, while Curcumin showed its peak absorption at 422 nm.

## Preparation of standard stock solution

Rutin (10 mg) was carefully weighed and then added to a volumetric flask (10 mL). In a similar way, a separate 10 mL volumetric flask was filled with 10 mg of Curcumin, which was precisely weighed. Methanol was used to adjust the volume in both flasks to the appropriate level, resulting in a concentration of 1000  $\mu\text{g/mL}$  for each, which formed the primary stock solutions. 1 mL of Rutin and 1 mL of Curcumin were individually drawn into 10 mL volumetric flasks from the above-mentioned primary stock solution and adjusted to the mark with methanol. This process resulted in the formation of a secondary stock solution with a concentration of 100  $\mu\text{g/mL}$  for each drug.

## Method Validation

The UV-Spectrophotometric method for Rutin and Curcumin was developed and validated following the ICH guidelines to evaluate parameters such as linearity, specificity and selectivity, precision, ruggedness, stability, LOD and LOQ.<sup>7,8</sup>

## Specificity and Selectivity

The UV spectra of the rutin and curcumin solution and the blank methanol solvent were scanned from 800 nm to 200 nm in wavelength. This was done to check for solvent-induced interference at the wavelength at which each analyte has its greatest absorbance.

## Linear range response

Linearity ranges of 4-20  $\mu\text{g/mL}$  for Rutin and 1-5  $\mu\text{g/mL}$  for Curcumin were tested in triplicate using the stock solution and measuring absorbance at 257 nm and 422 nm. Using absorbance versus concentration, the calibration curve was plotted and a linear regression equation was generated.

## Precision

Precision was performed:

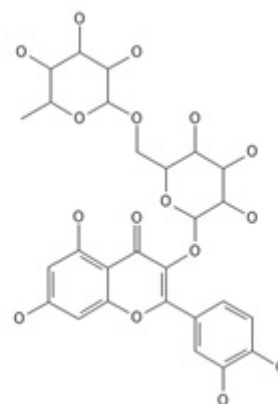


Figure 1: Molecular structure of Rutin.<sup>5</sup>

### System Precision

From the above-mentioned stock solution, six replicates of concentrations of 12 µg/mL and 3 µg/mL of rutin and curcumin, respectively, were prepared, analyzed and the percentage RSD was computed.

### Intraday precision

On the same day, the concentrations of 1, 3 and 5 µg/mL of Curcumin and 4, 12 and 20 µg/mL of Rutin in triplicate were made from the above-mentioned stock solutions, analyzed and the percentage RSD was computed at various intervals.

### Interday precision

On three consecutive days, the concentrations of 1, 3 and 5 µg/mL of Curcumin and 4, 12 and 20 µg/mL of Rutin in triplicate were made from the above-mentioned stock solutions, analyzed and the percentage RSD was computed.

### Ruggedness

Ruggedness was achieved by altering both the instrument and the analyst. The concentrations of 4, 12 and 20 µg/mL of Rutin and 1, 3 and 5 µg/mL of Curcumin in triplicate of each were prepared from the above stock solutions and measured absorption at 257 nm and 422 nm, respectively and measured by different analysts and with a different instrument (UV Spectrophotometer: UV-1800), the same concentration as previously mentioned was measured, examined and the percentage RSD was computed.

### Robustness

Robustness parameter was achieved by a change in the wavelength. The concentrations of 4, 12 and 20 µg/mL of Rutin and 1, 3 and 5 µg/mL of Curcumin in triplicate of each were prepared from the above stock solutions and measured absorption at 255 nm, 259 nm of Rutin and 420 nm, 424 nm of Curcumin and analyzed and %RSD was computed.

### Solvent and standard stock solution stability

The stability of the solvent and standard stock solution was demonstrated by making a fresh stock solution. Analyses were carried out on dilutions of 2, 6 and 10 µg/mL containing both analytes after a fresh stock solution was prepared. The absorbance was computed as a percentage of RSD and compared to that of the old stock dilutions.

### LOD and LOQ

The linear curve was used to calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ) by statistical methods utilizing the following formulas.<sup>9-12</sup>

$$LOD = \frac{3.3 \times \text{standard deviation of } y - \text{intercept}}{\text{Slope of the calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard deviation of } y - \text{intercept}}{\text{Slope of the calibration curve}}$$

## RESULTS

The maximum absorption of Rutin and Curcumin showed  $\lambda_{\max}$  at 257 nm and 422 nm and the spectra of both drugs are illustrated in Figures 3 and 4 and overlay spectra of different linearity ranges are shown in Figure 5.

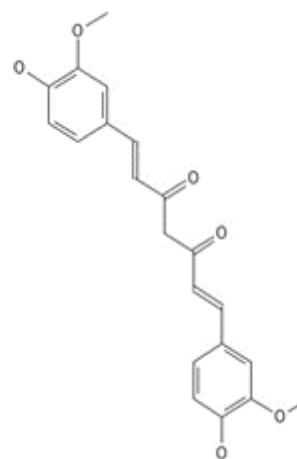


Figure 2: Molecular structure of Curcumin.<sup>7</sup>

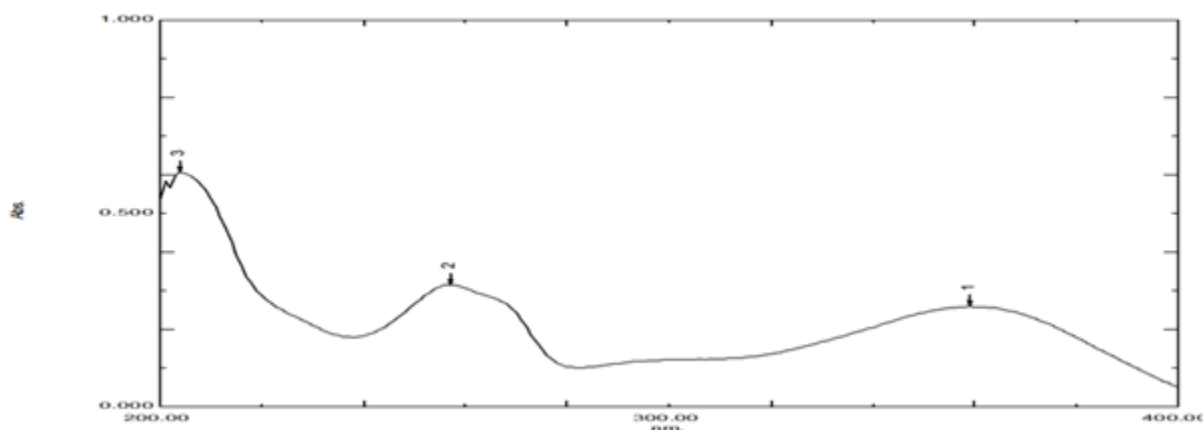


Figure 3: UV-spectrum of Rutin.

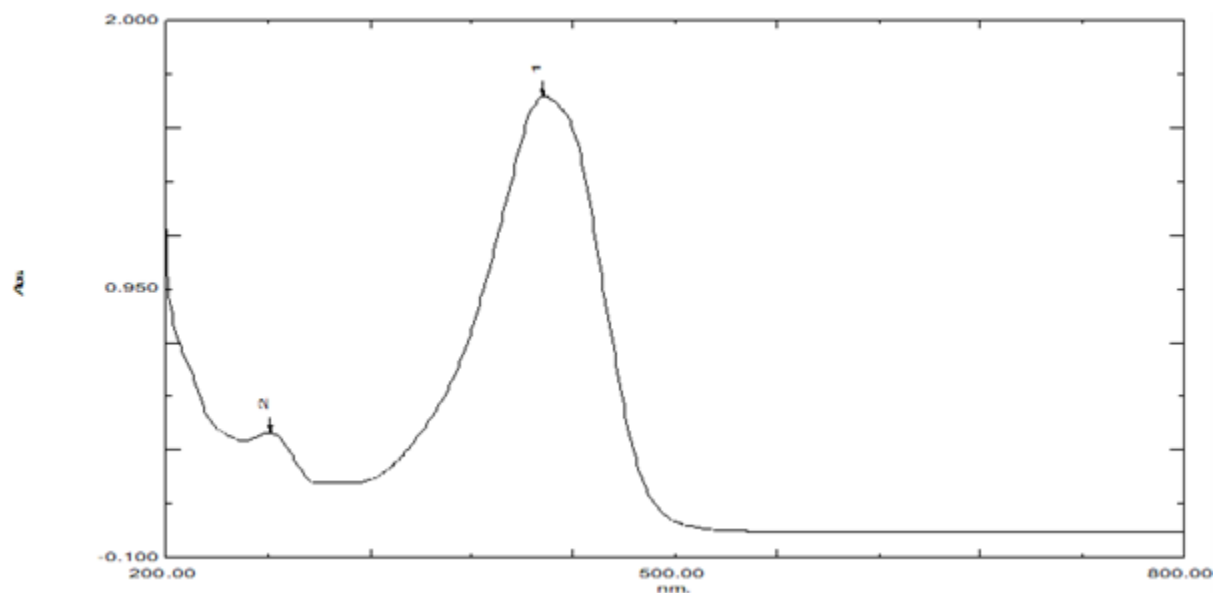


Figure 4: UV-spectrum of Curcumin.

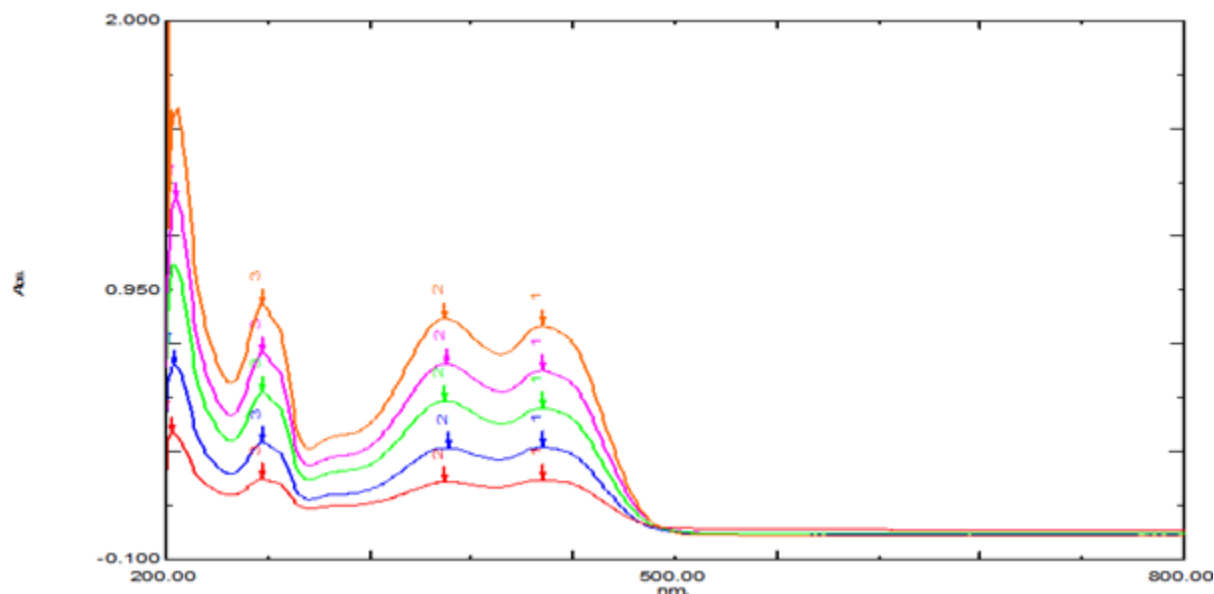


Figure 5: Overlay Spectra.

## Method development

In order to carry out simultaneous estimation, a single solvent must dissolve both analytes. For this approach, analytical-grade methanol was chosen as the solvent since both of the analytes, Rutin and Curcumin, are fully soluble in it. Method development parameters are illustrated in Table 1.

## Method validation

### Specificity and Selectivity

The solvent methanol was scanned and the UV spectra showed the absence of any solvent interference, as shown in Figure 6.

## Linear range response

Linearity response for Rutin and Curcumin solutions were obtained by analyzing 4-20  $\mu\text{g/mL}$  and 1-5  $\mu\text{g/mL}$  in triplicate and a graph was plotted of drug concentration against absorbance, which resulted in a regression coefficient of 0.9991 for both drugs. The linearity data are shown in Table 2. The standard calibration curves of Rutin and Curcumin are displayed in Figures 7 and 8, respectively.

## LOD and LOQ

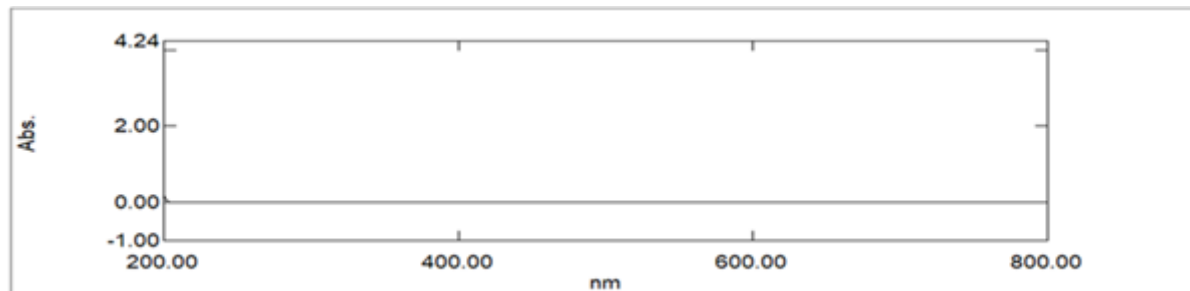
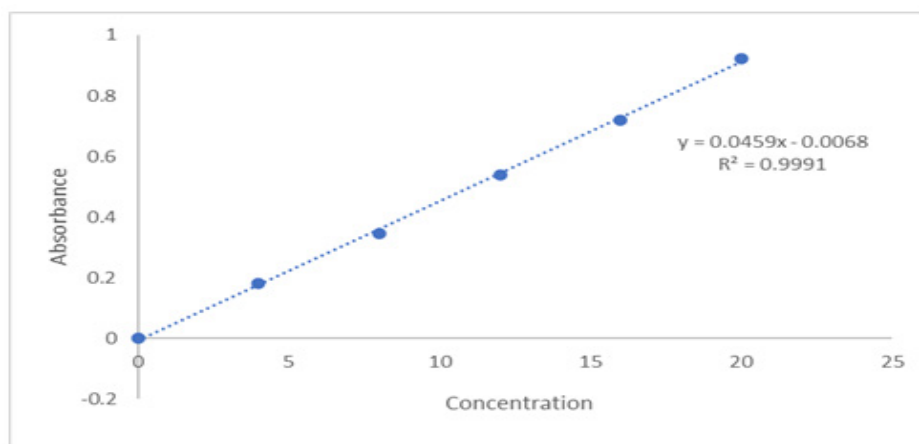
Rutin has a Limit of Detection (LOD) of 0.59  $\mu\text{g/mL}$  and a Limit of Quantification (LOQ) of 1.81  $\mu\text{g/mL}$ . Similarly, the LOD and LOQ of Curcumin were computed and found to be 0.15  $\mu\text{g/mL}$  and 0.45  $\mu\text{g/mL}$ .

**Table 1: Method development parameters.**

Sl. No.	Parameters	Specifications
1	Analytes	Rutin and Curcumin
2	Solvent	Methanol
3	Maximum absorbance of Rutin	257 nm
4	Maximum absorbance of Curcumin	422 nm

**Table 2: Linearity data for Rutin and Curcumin.**

Sl. No.	Concentration ( $\mu\text{g/mL}$ ) Rutin	Absorbance (257 nm)	Concentration ( $\mu\text{g/mL}$ ) Curcumin	Absorbance (422 nm)
1	4	0.183	1	0.185
2	8	0.346	2	0.35
3	12	0.54	3	0.51
4	16	0.72	4	0.66
5	20	0.924	5	0.829
R <sup>2</sup>	0.9991		0.991	
Slope	0.0459		0.1637	

**Figure 6:** UV-spectrum of solvent (Methanol).**Figure 7:** Standard calibration curve of Rutin.

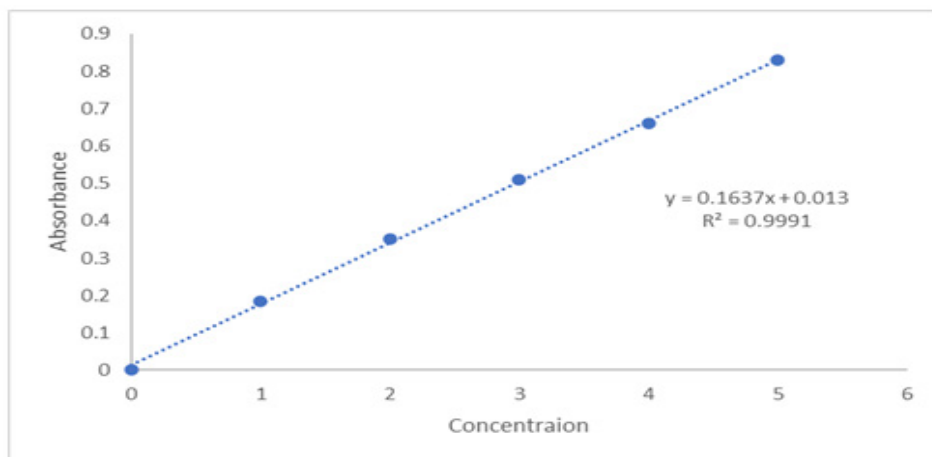


Figure 8: Standard calibration curve of Curcumin.

Table 3: System precision.

Sl. No.	Conc (µg/mL) Rutin: Curcumin	Absorbance at 257 nm	Absorbance at 422 nm
1	12:3	0.540	0.504
2	12:3	0.540	0.504
3	12:3	0.540	0.504
4	12:3	0.541	0.504
5	12:3	0.541	0.504
6	12:3	0.542	0.505
Avg		0.541	0.504
SD		0.004	0.0004
%RSD		0.75%	0.08%

Table 4: Intraday precision (Initial Hour, 1<sup>st</sup> hr and 5<sup>th</sup> hr) (\*n=3).

Concentration (µg/mL)	Rutin (257 nm)	Curcumin (422 nm)	Rutin (257 nm)	Curcumin (422 nm)	Rutin (257 nm)	Curcumin (422 nm)
	Initial Hour		1 <sup>st</sup> hr		5 <sup>th</sup> hr	
Rutin: Curcumin	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
4:1	0.193	0.172	0.193	0.173	0.193	0.173
SD	0.001	0.0005	0.002	0.002	0.001	0.0005
%RSD	0.79%	0.33%	1.07%	1.15%	0.79%	0.33%
12:3	0.531	0.506	0.536333	0.503	0.536	0.502
SD	0.001	0.001	0.002082	0.002	0.002	0.0005
%RSD	0.21%	0.34%	0.38%	0.39%	0.37%	0.11%
20:5	0.905	0.955	0.905333	0.953667	0.906	0.954
SD	0.0005	0.001	0.001155	0.001155	0.001	0.001
%RSD	0.06%	0.10%	0.12%	0.12%	0.11%	0.18%

**Table 5: Interday precision(\*n=3).**

Concentration (µg/mL)	Rutin (257 nm)	Curcumin (422 nm)	Rutin (257 nm)	Curcumin (422 nm)	Rutin (257 nm)	Curcumin (422 nm)
	Day 1		Day 2		Day 3	
Rutin: Curcumin	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
4:1	0.193	0.172	0.193	0.173	0.193	0.173
SD	0.001	0.0005	0.001	0.0005	0.002	0.002
%RSD	0.79%	0.33%	0.79%	0.33%	1.07%	1.15%
12:3	0.531	0.506	0.536	0.502	0.536	0.503
SD	0.001	0.001	0.002	0.0005	0.002	0.002
%RSD	0.21%	0.34%	0.37%	0.11%	0.38%	0.39%
20:5	0.90	0.955	0.906	0.954	0.905	0.953
SD	0.0005	0.001	0.001	0.001	0.001	0.001
%RSD	0.06%	0.10%	0.11%	0.18%	0.12%	0.12%

**Table 6: Ruggedness (Change in Analyst and Change in Instrument) (\*n=3).**

Concentration (µg/mL)	Rutin (257 nm)	Curcumin (422 nm)	Rutin (257 nm)	Curcumin (422 nm)
	Change in Analyst		Change in Instrument	
Rutin: Curcumin	Absorbance	Absorbance	Absorbance	Absorbance
4:1	0.194	0.172	0.193	0.172
SD	0.003	0.003	0.001	0.0005
%RSD	1.65%	1.74%	0.79%	0.33%
12:3	0.535	0.505	0.531	0.506
SD	0.0005	0.003	0.001	0.001
%RSD	0.10%	0.71%	0.21%	0.34%
20:5	0.907	0.953	0.905	0.955
SD	0.0005	0.002	0.0005	0.001
%RSD	0.06%	0.24%	0.06%	0.10%

**Table 7: Robustness (Change in Wavelength) (\*n=3).**

Concentration (µg/mL)	Rutin (255 nm)	Rutin (259 nm)	Curcumin (420 nm)	Curcumin (424 nm)
Rutin: Curcumin				
4:1	0.189	0.190	0.172	0.173
SD	0.002	0.001528	0.002	0.002
%RSD	1.09%	0.80%	1.53%	1.33%
12:3	0.531	0.534	0.502	0.503
SD	0.0005	0.0005	0.0005	0.0005
%RSD	0.10%	0.10%	0.11%	0.11%
20:5	0.909	0.914	0.953	0.955
SD	0.001	0.0005	0.001	0.0005
%RSD	0.16%	0.06%	0.10%	0.06%

## Precision

The approach resulted to be precise as the %RSD for both the analytes was calculated for each level, namely system precision, intraday precision and interday precision and % RSD was calculated from the absorbance and found to be less than 2%. The precision data are illustrated in Tables 3, 4 and 5.

## Ruggedness

The ruggedness parameter was carried on both the analytes by changing the analyst and the instrument and %RSD was found to be less than 2%. Table 6 illustrates the ruggedness statistics.

## Robustness

The robustness parameter was carried out by a change in the wavelength for both analytes and %RSD was calculated and found to be less than 2%, hence the method was found to be robust. The robustness data is illustrated in Table 7.

## Solvent and standard stock solution stability

After analyses of the fresh stock solution and the old standard stock solution for ten days, the %RSD for both analytes were determined to be less than 2%, indicating that the solvent and the

standard stock solution were stable. The solution stability data are illustrated in Tables 8 and 9.

## DISCUSSION

The development and validation of a UV method for the simultaneous estimation of Rutin and Curcumin present a significant advancement in analytical chemistry. UV spectroscopy offers a cost-effective and widely accessible technique for quantifying compounds with distinct absorbance profiles, such as rutin and curcumin. The selection of appropriate wavelengths, 257 nm for rutin and 422 nm for curcumin, reflects careful consideration of their absorption maxima, ensuring optimal sensitivity and specificity for their quantification. The linearity assessment across the concentration ranges of 4-20 µg/mL for rutin and 1-5 µg/mL for curcumin demonstrates the method's capability to accurately measure a wide range of analyte concentrations. The resulting linear regression equations indicate a strong correlation between concentration and absorbance, highlighting the method's reliability. Furthermore, the evaluation of precision through %RSD calculations underscores the method's reproducibility, essential for robust analytical procedures. This UV method's sensitivity, reflected in low detection and quantification limits, signifies its potential for detecting trace levels of rutin

**Table 8: Solution stability (\*n=3).**

Concentration (µg/mL)	Rutin (257)		Curcumin (422)	
	Fresh	Old	Fresh	Old
	Absorbance	Absorbance	Absorbance	Absorbance
4:1	0.195	0.201	0.174	0.184
SD	0.001	0.001	0.001	0.002
%RSD	0.78%	0.75%	0.87%	1.08%
12:3	0.537	0.545	0.504	0.513
SD	0.005	0.546	0.001	0.001
%RSD	0.10%	0.54%	0.22%	0.29%
20:5	0.907	0.915	0.956	0.966
SD	0.005	0.003	0.001	0.002
%RSD	0.63%	0.33%	0.10%	0.20%

**Table 9: An overview of validation parameters.**

Sl. No.	Validation parameters	Rutin		Curcumin	
1	Absorption maxima ( $\lambda_{max}$ )	257 nm		422 nm	
	Linearity range	4-20 µg/mL		1-5 µg/mL	
	Standard regression equation	Y=0.0459x-0.0068		Y=0.1637x+0.013	
	Slope (m)	0.0459		0.1637	
	Intercept	0.0068		0.013	
	Correlation co-efficient (R <sup>2</sup> )	0.9991		0.9991	
		Conc (µg/mL)	%RSD	Conc (µg/mL)	%RSD

Sl. No.	Validation parameters		Rutin		Curcumin		
2	Precision	System precision	12	0.75	3	0.08	
			Interday Day-1	4	0.79	1	0.33
				12	0.21	3	0.34
		20		0.06	5	0.10	
		Interday Day-2		4	0.79	1	0.33
			12	0.37	3	0.11	
			20	0.11	5	0.18	
		Interday Day-3	4	1.07	1	1.15	
			12	0.38	3	0.39	
			20	0.12	5	0.12	
		Intraday Initial hr	4	0.79	1	0.33	
			12	0.21	3	0.34	
			20	0.06	5	0.10	
		Intraday 1 <sup>st</sup> hr	4	1.07	1	1.15	
			12	0.38	3	0.39	
			20	0.12	5	0.12	
		Intraday 5 <sup>th</sup> hr	4	0.79	1	0.33	
			12	0.37	3	0.11	
			20	0.11	5	0.18	
		3	Ruggedness	Change in Analyst	4	1.65	1
12	0.10				3	0.71	
20	0.06				5	0.24	
Change in Instrument	4			0.79	1	0.33	
	12			0.21	3	0.34	
	20			0.06	5	0.10	
4	Robustness	Change in wavelength (255 nm and 420 nm).	4	1.09	1	1.53	
			12	0.10	3	0.11	
			20	0.16	5	0.10	
		Change in wavelength (259 nm and 424 nm).	4	0.80	1	1.33	
			12	0.10	3	0.11	
			20	0.06	5	0.06	
5	Solution stability	10 days at room temperature.	4	0.75	1	1.08	
			12	0.54	3	0.29	
			20	0.33	5	0.20	
6	LOD		0.59 µg/mL		0.15 µg/mL		
7	LOQ		1.81 µg/mL		0.45 µg/mL		

and curcumin in complex matrices. Overall, the developed UV method stands as a valuable industry for pharmaceutical industries, facilitating quality control and formulation studies with enhanced efficiency.

In the context of simultaneous estimation of Rutin and Curcumin, UV spectroscopy might be preferred for its quantitative accuracy and sensitivity, especially if the compounds have distinct UV absorbance spectra. However, Raman spectroscopy could offer

advantages in terms of chemical specificity and non-destructive analysis, which might be beneficial if there are potential interferences or if detailed structural information is required. It is worth noting that fluorescence spectroscopy also offers its own set of advantages, such as high sensitivity to trace amounts of analytes and the ability to provide additional structural and dynamic information about molecules. Ultimately, the choice between these techniques would depend on factors such as the specific requirements of the analysis, the nature of the samples and the instrumentation available.<sup>13</sup>

## CONCLUSION

An approach for simultaneously determining Rutin and Curcumin was developed and validated in accordance with the ICH guidelines. The statistical analysis results showed the current method for simultaneous quantification of Rutin and Curcumin in bulk and pharmaceutical formation to be simple, specific, selective, linear, precise, robust and reproducible.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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