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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION AND PERCENTAGE RECOVERY OF NATURAL ACTIVE CONSTITUENTS (CURCUMIN AND PIPERINE) IN MARKETED PREPARATION BY UV METHOD

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ABSTRACT

This study establishes a validated UV spectrophotometric method for the simultaneous estimation of Curcumin and Piperine, supported by extensive pre-formulation characterization. Organoleptic properties, solubility profiling, pH measurement, melting point analysis, and FTIR spectroscopy confirmed the identity, purity, and stability of both compounds. UV analysis identified λmax values of 424 nm for Curcumin and 345 nm for Piperine, with an overlay peak at 367 nm suitable for simultaneous detection. The method demonstrated strong linearity ($R^2 > 0.99$), good sensitivity with low LOD and LOQ values, and acceptable precision in intra-day, inter-day, and repeatability studies (%RSD < 2%). Recovery values (Curcumin: 96.0%, Piperine: 98.10%) confirmed method accuracy. Although high %RSD in certain calibration and robustness evaluations suggests a need for better control of sample preparation and temperature, the method remains simple, reliable, and suitable for routine quality control of herbal and pharmaceutical formulations. Overall, the study provides a robust analytical foundation for future formulation development involving Curcumin and Piperine.

KEYWORDS: Curcumin, Piperine, UV spectrophotometry, method validation, ICH guidelines, linearity, precision, recovery, LOD, LOQ, quality control.

1. INTRODUCTION

Analytical method validation, as recommended by the International Council for Harmonisation (ICH) guidelines, is essential to confirm the reliability and suitability of the developed method for routine use **(Peris-Vicente *et al.*, 2015).** Key validation parameters include specificity, linearity, precision, accuracy, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ). Percentage recovery studies are particularly important for determining the accuracy of the method by evaluating how effectively Curcumin can be extracted and quantified in the presence of formulation excipients **(Majumder *et al.*, 2020).**

UV-visible spectrophotometry is one of the most widely utilized analytical techniques due to its simplicity, affordability, rapid operation, and good sensitivity. Unlike more advanced techniques such as HPLC, LC–MS, or GC–MS, UV spectrophotometry requires minimal infrastructure, making it especially valuable for regions with limited analytical resources **(Subash *et al.*, 2025).** Many pharmaceutical and herbal constituents possess distinctive absorbance maxima (λmax) within the UV-visible range, enabling the development of simultaneous estimation methods through absorbance ratio (Q-analysis), simultaneous equation methods, or derivative spectrophotometry **(Mandour *et al.*, 2020).** Developing a UV spectrophotometric method for simultaneous estimation demands careful selection of

several parameters, including solvent choice, λmax determination, spectrum scanning, and proper method design to resolve overlapping absorbance signals. The goal is to ensure that each analyte can be measured accurately without interference from other components present in the formulation. These considerations are particularly important for herbal formulations, where multiple phytochemicals and excipients may contribute to spectral overlap **(Kaushik and Kaushik 2024).**

Curcumin is the principal bioactive polyphenolic compound extracted from the rhizomes of *Curcuma longa* (turmeric), a medicinal plant widely used in traditional systems of medicine such as Ayurveda, Siddha, and Traditional Chinese Medicine **(Fuloria *et al.*, 2022).** Ensuring the quality, safety, and efficacy of herbal products containing Curcumin requires reliable analytical methods capable of identifying and quantifying the compound in the presence of excipients and other natural constituents **(Lalchandani *et al.*, 2025).** With the rapid expansion of the herbal and nutraceutical industry, regulatory bodies increasingly emphasize the need for validated analytical methods that meet international quality standards. Among the various analytical tools available, UV-visible spectrophotometry has gained significant attention due to its simplicity, affordability, high sensitivity, and suitability for routine quality control in both academic and industrial laboratories **(Singhal *et al.*, 2024).**

Piperine is the major bioactive alkaloid found in *Piper nigrum* (black pepper) and *Piper longum* (long pepper), plants that have been widely used in traditional medicine and culinary practices for centuries. Its unique ability to enhance the absorption of several drugs and phytochemicals—including Curcumin—has led to its increasing incorporation into herbal formulations, nutraceuticals, and functional foods **(Takooree *et al.*, 2019).** Consequently, ensuring the accurate quantification of Piperine in marketed preparations has become essential for maintaining product quality and therapeutic reliability. However, quantifying Piperine in commercial formulations can be challenging because of the presence of multiple plant-derived components, excipients, and additives that may interfere with analysis. Furthermore, Piperine's limited water solubility, sensitivity to light, and tendency to degrade under certain conditions highlight the importance of selecting an appropriate analytical approach **(Quijia and Chorilli 2020).**

This study aims to develop a UV spectrophotometric method capable of accurately estimating Curcumin and Piperine simultaneously in marketed preparations. The method is validated by evaluating key parameters such as specificity, linearity, precision, accuracy, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ). Additionally, percentage recovery studies are conducted to assess the accuracy of the method in the presence of formulation excipients.

2. MATERIALS AND METHODS

2.1 Chemicals

Methyl paraben, Propylene glycol, Acetonitrile and Ethanol were obtained from Merck, a reputable supplier of analytical reagents. Loba chemie provided the Triethanolamine. Sigma eldrich provided the PVA and Ethyl cellulose. Distilled water were obtained from Vizag Chemicals. Piperine were obtained from Panacea Phytoextracts Pvt. Ltd while Bioprex Labs provided the Curcumin.

2.2 Pre-formulation studies of selected Drug

Pre-formulation studies of Curcumin and Piperine involve evaluating their physicochemical properties to guide the development of an effective formulation. These studies typically assess parameters such as solubility, stability, melting point, and compatibility with excipients. Curcumin, known for its poor water solubility and low bioavailability, is often studied alongside Piperine, which can enhance its absorption. Compatibility tests, such as FTIR, are conducted to ensure that the two compounds do not interact negatively when combined. These preliminary evaluations are essential for selecting suitable formulation strategies and ensuring the stability and efficacy of the final product **(Ahirwar and Shukla 2023).**

2.2.1 Organoleptic Properties

Organoleptic properties of Curcumin and Piperine refer to the sensory characteristics of a drug that can be assessed using the senses, such as color, odor, taste, texture, and overall appearance **(Díaz-Guerrero *et al.*, 2025).**

2.2.2 Solubility study

A solubility study of Curcumin and Piperine was conducted using the visual inspection method to assess their solubility in various solvents. Precisely 1 mg of each compound was added to individual test tubes containing 1 mL of different solvents, including distilled water, ethanol, methanol, acetone, and others. The mixtures were then thoroughly shaken or stirred using a magnetic stirrer for approximately 15–30 minutes at room temperature. After settling, each sample was visually examined for clarity, undissolved material, or sediment formation. A transparent solution was considered indicative of good solubility, while cloudiness or visible residue indicated limited or poor solubility. These observations were documented to determine the most appropriate solvents for future formulation work **(Alshehri *et al.*, 2018).**

2.2.3 Melting Point

The melting points of Curcumin and Piperine were determined using a melting point apparatus to evaluate their purity and confirm their identity **(Wdowiak *et al.*, 2023).**

2.2.4 pH determination

The pH of Curcumin and Piperine solutions was measured using a digital pH meter to evaluate their acidic or basic nature **(Murti *et al.*, 2019).**

2.2.5 Spectroscopic Analysis and Calibration of Curcumin and Piperine

- **Lambda (λ) max analysis**

Stock solutions of Curcumin and Piperine were individually prepared at a concentration of 1 mg/mL using methanol as the solvent. These stock solutions were then diluted appropriately with the same solvent to obtain working standard solutions of 100 µg/mL. Each solution was scanned across the UV range of 200–800 nm using a Shimadzu 1700 double beam spectrophotometer to record their absorbance spectra and determine the wavelength of maximum absorbance (λmax) for both Curcumin and Piperine **(Martins *et al.*, 2015).**

- **Standard calibration curve analysis**

To prepare the calibration curves for Curcumin and Piperine, 100 mg of each compound was accurately weighed and dissolved separately in methanol in a 100 mL volumetric flask. The solutions were then diluted to the mark with methanol to obtain the stock solutions. From these, 1 mL of each stock solution was transferred into a 10 mL volumetric flask and diluted with methanol to prepare the working standard solutions. The absorbance of these solutions was measured using a UV spectrophotometer over the wavelength range of 200 to 800 nm to determine the λmax for each compound. Subsequently, a series of Curcumin and Piperine solutions with different concentrations were prepared from the stock solutions, and their absorbance values at the respective λmax were recorded to construct the calibration curves **(Nandiyanto *et al.*, 2023).**

2.2.6 Preparation of calibration curve

To construct calibration curves for Curcumin and Piperine, individual stock solutions of each compound were initially prepared and subsequently diluted using the selected solvent to produce a series of working standards. For Curcumin, concentrations of 2, 4, 6, 8, and 10µg/mL were prepared, while for Piperine, the working concentrations were 2, 4, 6, 8, and 10µg/mL. The absorbance of each solution was recorded using a UV-visible spectrophotometer, with the solvent serving as the blank to calibrate the instrument. Absorbance readings were then plotted against their respective concentrations, with concentration placed on the X-axis and absorbance on the Y-axis. The resulting standard curves displayed a linear correlation, consistent with the Beer-Lambert law, within the examined concentration ranges **(Desta and Amare 2017).**

2.2.7 Fourier Transform Infrared (FT-IR) Spectroscopy of Curcumin and Piperine

FT-IR analysis was conducted for Curcumin, Piperine, and their physical mixtures with formulation excipients to evaluate potential interactions between the active compounds and other components. The infrared spectra were recorded in the range of 4000–400 cm^{-1} using the potassium bromide (KBr) pellet method. For each sample, 1 mg of Curcumin, Piperine, or their respective blends with excipients was finely mixed with 100 mg of dry, spectroscopic-grade KBr. The mixture was dried under an infrared lamp to eliminate residual moisture, then compressed into a clear pellet using a hydraulic press. These KBr discs were placed in the sample holder of the FT-IR spectrophotometer, and spectra were obtained to identify the characteristic functional groups and evaluate the compatibility of the drug with excipients **(Chadha and Bhandari 2014).**

2.3. UV-Vis Spectroscopy Method

2.3.1 Selection of Solvent for Method Development Based on Solubility Study

Based on the findings of the solubility study, methanol was identified as the most suitable solvent for the method development of Curcumin and Piperine. Both compounds demonstrated superior solubility in methanol compared to other solvents evaluated, resulting in clear solutions and stable absorbance values during UV-Visible spectrophotometric analysis. The use of methanol also enabled straightforward and accurate preparation of standard solutions, supporting the generation of consistent and reproducible results. Therefore, methanol was selected as the preferred solvent for the development of the analytical method involving Curcumin and Piperine **(Mondal *et al.*, 2016).**

(A) Analysis of Oral Dosage Forms

An accurately weighed quantity of finely powdered material equivalent to 10 mg of Curcumin and Piperine was individually transferred into separate 10 mL volumetric flasks. Each compound was dissolved in methanol and sonicated for five minutes to ensure complete dissolution. The volume in each flask was then adjusted to the mark with methanol, resulting in stock solutions with concentrations of 1 mg/mL for both Curcumin and Piperine. These stock solutions were subsequently diluted with methanol to prepare working solutions in the concentration range of 20–120 µg/mL for Curcumin and 5–30 µg/mL for Piperine. Methanol was used as the blank for all spectrophotometric measurements. The prepared solutions were scanned over the wavelength range of 200 to 800 nm using a UV-Visible spectrophotometer to obtain their absorbance spectra **(Setyaningsih *et al.*, 2021).**

2.3.2 Determination of Wavelength of Maximum Absorption (λmax) for Curcumin and Piperine

To determine the λmax of Curcumin and Piperine, individual standard solutions of each compound were prepared in methanol and scanned using a UV-Visible spectrophotometer across the wavelength range of 200 to 400 nm. This scanning process generated the UV absorption spectra for each compound, from which the wavelength corresponding to maximum absorbance

(λmax) was identified. The λmax represents the wavelength at which the compound exhibits peak absorbance and is considered optimal for accurate and sensitive measurements. These λmax values were then utilized in the subsequent development and validation of the UV spectrophotometric method for Curcumin and Piperine **(Bhairy, 2022).**

2.4 Validation of the Method

This study aimed to develop a simple, affordable, and reliable UV spectrophotometric method for quantifying Curcumin and Piperine. The method was validated following ICH guidelines by evaluating linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ to ensure its suitability for routine quality control of these compounds in pharmaceutical products **(Patil and Deore 2024).**

2.4.1 Specificity

The method's specificity was evaluated to ensure Curcumin and Piperine could be accurately measured without interference from excipients or impurities. Their individual spectra showed no overlapping peaks at the selected λ max values, confirming clear and selective detection **(Ramaswamy *et al.*, 2021).**

2.4.2 Linearity

Linearity was determined by preparing standard solutions of both compounds at different concentrations and plotting absorbance versus concentration. Regression analysis showed strong linearity within 20–120 µg/mL for Curcumin and 5–30 µg/mL for Piperine, in accordance with Beer–Lambert's law **(Abdalla *et al.*, 2018).**

2.4.3 Precision

Precision was assessed through repeatability, intermediate precision, and reproducibility by repeatedly analyzing samples and calculating %RSD. Results confirmed consistent performance of the method under varying conditions **(McAlinden *et al.*, 2015).**

- **Intraday Precision**

Intraday precision was assessed by analyzing 30 µg/mL Curcumin and Piperine solutions three times in one day. The %RSD of absorbance values indicated good consistency, confirming the method's reliability for same-day analysis **(Miraghaei *et al.*, 2017).**

- **Interday Precision**

Interday precision was evaluated by analyzing 30 µg/mL solutions over three consecutive days. Low % RSD values demonstrated stable results across days, proving the method's reproducibility for routine testing **(Bonifas and Li 2024).**

2.4.4 Repeatability

Repeatability was tested by analyzing 30 µg/mL solutions six times under the same conditions. % RSD values below the USP limit of 2% confirmed excellent precision and consistent performance **(da Silva *et al.*, 2017).**

2.4.5 Accuracy (Ruggedness Section Correction)

Accuracy was determined through recovery studies at 80%, 100%, and 120% levels. Recovery values between 98–102% confirmed that the method accurately measures Curcumin and Piperine without interference **(Kroon *et al.*, 2023).**

2.4.6 Robustness

Robustness was checked by making small, intentional changes to parameters like wavelength and solvent volume. Minimal variation in results confirmed that the method remains stable under slight experimental changes **(Ferreira *et al.*, 2017).**

2.4.7 Limit of Detection (LOD)

LOD was calculated using the ICH formula:

$$LOD = 3.3 \times (N / S)$$

Where *N* is the standard deviation of peak areas and *S* is the slope of the calibration curve.

2.4.8 Limit of Quantification (LOQ)

LOQ was calculated using:

$$LOQ = 10 \times (N / S)$$

Following ICH guidelines.

2.5 Simultaneous method Curcumin and Piperine

Standard solutions of Curcumin and Piperine, each at a concentration of 6 µg/mL, were individually prepared in methanol and scanned against a methanol blank across the full UV range to determine their respective λ max values. Distinct absorption peaks were observed at 424.0 nm for Curcumin and 345.0 nm for Piperine. These wavelengths were selected as the λ max for each compound. Subsequently, standard solutions of Curcumin (2–10 µg/mL) and Piperine (2–10 µg/mL) were prepared in methanol, and their absorbance values were recorded at 424.0 nm and 345.0 nm, respectively. Calibration curves were constructed to confirm adherence to Beer's law, and the specific absorptivity values were calculated at the selected wavelengths for both compounds **(Murti *et al.*, 2019).**

UV-visible spectroscopy, a simple, rapid, precise and highly accurate method for quantitative estimation is in great use now a day. The basic principle behind this technique is that the amount of light absorbed is proportional to the concentration of analyte.

By using the below equations, the concentrations in the samples were obtained

$CX = A1ay2 – A2ay1 / ax1ay2 – ax2ay1$ Eq. 1

$CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1$ Eq. 2

Where A1 and A2 are absorbance's of mixture at 424.0 nm and 345.0 nm respectively, ax1 and ax2 are absorptivity's of Curcumin at λ1 and λ2 respectively, ay1 and ay2 are absorptivity's of Piperine at λ1 and λ2

respectively, Cx and Cy are concentrations of Curcumin and Piperine respectively.

3. RESULT AND DISCUSSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 1: **Organoleptic properties of Curcumin and Piperine.**

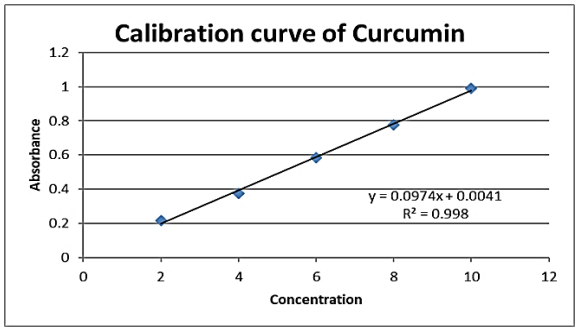
Drug	Organoleptic properties	Observation of Curcumin	Observation of Piperine
Curcumin and Piperine	Color	Bright yellow	Pale yellow to yellow
	Odor	Mildly aromatic odor	Pungent and sharp
	Appearance	Yellow crystalline powder	Crystalline powder
	State	Solid powder	Solid powder

3.1.2 Melting point, pH determination of Curcumin and Piperine

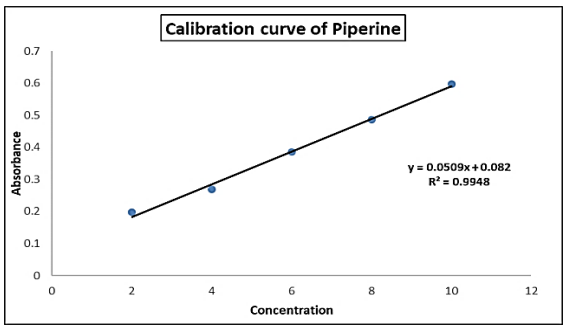
Table 2: Melting point, pH of Curcumin and Piperine.

Drugs	Reference range (Melting point)	Observed (Melting point)	Reference range (pH)	Observed (pH)
Curcumin	***180 to 185°C***	183 °C	7.8	7.5 to 8.5
Piperine	***128 to 130 °C***	129 °C	6.5	6.4 to 7.5

3.1.3 Calibration curve

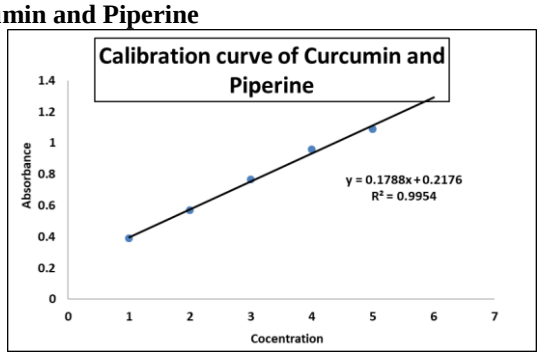


Graph 1: Calibration curve of Curcumin.



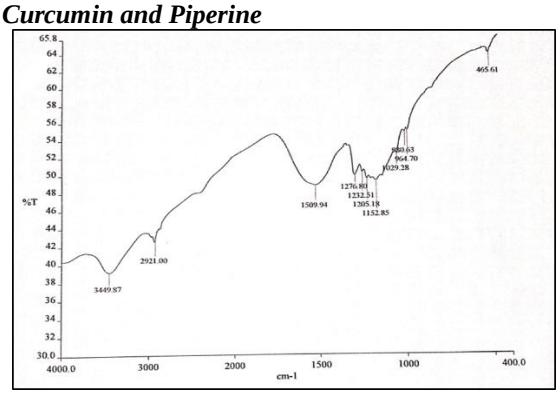
Graph 2: Calibration curve of Piperine.

3.1.4 Calibration curve of Curcumin and Piperine

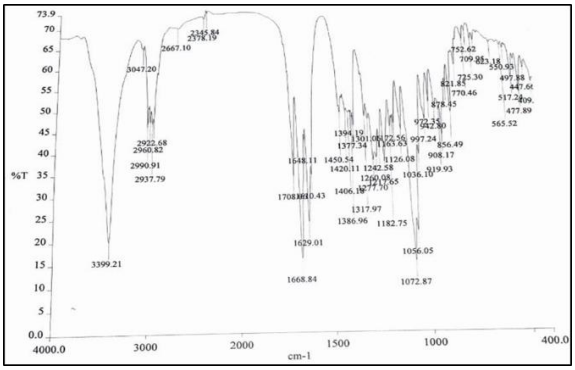


Graph 3: Calibration curve of Curcumin and Piperine.

3.1.5 Curcumin and Piperine



Graph 4: FTIR study of Curcumin.



Graph 5: FTIR study of Piperine.

Table 3: Interpretation of IR spectrum of Curcumin.

Peak obtained	Reference peak	Functional group	Name of functional group
3449.87	3500- 3400	N-H stretching	primary amine
2921.00	3000-2800	N-H stretching	amine salt
1276.80	1275-1200	C-Ostretching	Alkyl aryl ether
1152.85	1205-1124	C-Ostretching	Tertiary alcohol
1029.28	1050-1040	CO-O-COstretching	Anhydride

Table 4: Interpretation of IR spectrum of Piperine.

Peak obtained	Reference peak	Functional group	Name of functional group
3399.21	3500- 3400	N-H stretching	Primary amine
2937.79	3000-2800	N-H stretching	Amine salt
2345.84	2600-2550	S-H stretching	Thiol
1668.84	1675-1665	C=C stretching	alkene
1450.54	1465	C-H bending	alkene
1386.96	1390-1310	O-H bending	phenol
1260.08	1275-1200	C-O stretching	alkyl aryl ether
1182.75	1205-1124	C-O stretching	tertiary alcohol

3.2 Method Validation via UV spectroscopy Curcumin and Piperine

3.2.1 Precision study of Curcumin

(A) Intraday Precision

Table 5: Result of Intraday Precision (three times on the same day) Curcumin and Piperine.

Concentration (µg/ml)	Day 1 Absorbance (1) at 424.0 nm (Curcumin)	Day 1 Absorbance (2) at 424.0 nm (Curcumin)	Day 1 Absorbance (3) at 424.0 nm (Curcumin)	Day 1 Absorbance (1) at 345.0 (Piperine)	Day 1 Absorbance (2) at 345.0 nm (Piperine)		Day 1 Absorbance (3) at 345.0 nm (Piperine)
06	0.586	0.585	0.581	0.385	0.381		0.386
06	0.585	0.587	0.584	0.388	0.380		0.386
06	0.588	0.583	0.584	0.387	0.383		0.384
Mean	0.586333333	0.585	0.583	0.386666667	0.381333333		0.385333333
SD	0.001527525	0.002	0.001732051	0.001527525	0.001527525		0.001154701
%RSD	0.1706	0.3418	0.1715	0.2590	0.2624		0.2597
AVG % R.S. D	0.2273			0.2603			

(B) Interday Precision

Table 6: Result of Interday Precision (Three times on the different day) of Curcumin and Piperine.

Concentration (µg/ml)	Day 1 Absorbance at 424.0 nm (Curcumin)	Day 2 Absorbance at 424.0 nm (Curcumin)	Day 3 Absorbance at 424.0 nm (Curcumin)	Day 1 Absorbance at 345.0 (Piperine)	Day 2 Absorbance at 345.0 nm (Piperine)	Day 3 Absorbance at 345.0 nm (Piperine)
06	0.586	0.584	0.582	0.385	0.382	0.381
06	0.586	0.581	0.585	0.382	0.387	0.385
06	0.588	0.583	0.587	0.386	0.385	0.384
Mean	0.586666667	0.582666667	0.584666667	0.384333333	0.384666667	0.383333333
SD	0.001154701	0.001527525	0.002516611	0.002081666	0.002516611	0.002081666
%RSD	0.1706	0.1718	0.3424	0.5208	0.5208	0.522
AVG % R.S. D	0.2282			0.5212		

(C) Repeatability

Table 7: Result of repeatability of Curcumin and Piperine.

Concentration (µg/ml)	Absorbance		Statistical analysis		
	Curcumin	Piperine		Curcumin	Piperine
06	0.586	0.385	**Mean**	0.583833333	0.383666667
06	0.581	0.387	**SD**	0.002316607	0.002160247
06	0.583	0.382	**% RSD**	0.3430	0.5221
06	0.584	0.381			
06	0.582	0.384			
06	0.587	0.383			

(D) Ruggedness

Table 8: Result of ruggedness of Curcumin and Piperine.

Analyst-1			Analyst-2		
Concentration (µg/ml)	**Absorbance**		**Concentration (µg/ml)**	**Absorbance**	
	Curcumin	**Piperine**		**Curcumin**	**Piperine**
06	0.586	0.385	06	0.587	0.388
06	0.584	0.382	06	0.583	0.386
06	0.582	0.383	06	0.581	0.384
Mean	0.584	0.383333333	**Mean**	0.583666667	0.386
SD	0.002	0.001527525	**SD**	0.00305505	0.002
% RSD	0.3436	0.2610	**% RSD**	0.5145	0.5181

(E) Robustness

Table 9: Results showing robustness of Curcumin and Piperine.

Temperature 25^0C			Temp 30^0C		
Concentration (µg/ml)	**Absorbance**		**Concentration (µg/ml)**	**Absorbance**	
	Curcumin	**Piperine**		**Curcumin**	**Piperine**
06	0.586	0.385	06	0.589	0.388
06	0.584	0.388	06	0.587	0.381
06	0.587	0.390	06	0.585	0.383
Mean	0.585666667	0.387666667	**Mean**	0.587	0.384
SD	0.001527525	0.002516611	**SD**	0.002	0.003605551
% RSD	0.1709	0.5167	**% RSD**	0.3407	0.7812

(F) LOD and LOQ

Table 10: Results showing LOD and LOQ.

Drug name	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
Curcumin	424.0 nm	10.47	31.75
Piperine	345.0 nm	10.62	32.20

Table 11: Optical Characteristics and Validation Study of Formulation.

Parameters	Curcumin	Piperine
Wavelength λ max nm	424.0 nm	345.0 nm
Beer's law limit µg/ml	2-10	2-10
Correlation coefficient (R2)	0.998	0.9948
Slope	0.0974	0.0509
Intercept	0.0041	0.082
SD	0.308158563	0.161376
% RSD	52.380	41.559
Precision Intraday (% RSD) Interday (% RSD) Repeatability (% RSD)	 0.2273 0.2282 0.3430	 0.2603 0.5212 0.5221
Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	 0.3436 0.5145	 0.2610 0.5181
Robustness Temp.25^0C (% RSD) Temp.30^0C (% RSD)	 0.1709 0.3407	 0.5167 0.7812
LOD (µg/ml)	10.47	31.75
LOQ (µg/ml)	10.62	32.20
% Recovery	96.0	98.10

DISCUSSION

The optical characteristics and validation parameters for Curcumin and Piperine, confirm the reliability and suitability of the developed UV spectrophotometric method. Curcumin and Piperine showed λ max at 424.0 nm and 345.0 nm, respectively. The method obeyed Beer's law in the range of 2–10 µg/mL for Curcumin and 2–10µg/mL for Piperine, with high correlation coefficients (R^2 = 0.998 for Curcumin and 0.9948 for Piperine), indicating good linearity. The precision studies

showed %RSD values below 2% in most cases, demonstrating good intraday, interday, and repeatability performance for both compounds. Ruggedness and robustness data also showed %RSD values within acceptable limits, confirming the method's reliability across analysts and slight temperature variations. The LOD and LOQ values (10.47 µg/mL and 31.75 µg/mL for Curcumin; 10.62 µg/mL and 32.20 µg/mL for Piperine) indicate adequate sensitivity. Overall, these results validate the method as accurate, precise, robust, and suitable for routine analysis of Curcumin and Piperine in formulation.

4. CONCLUSION

The study demonstrates that UV-visible spectrophotometry is a valid, sensitive, and moderately precise method for the qualitative and quantitative estimation of Curcumin and Piperine, both individually and in combination.

- While linearity and sensitivity are excellent, the relatively high % RSD in calibration and robustness studies highlights the need for method refinement, especially in sample preparation, temperature control, and analyst training to improve precision.
- The method shows promise for routine analytical use in herbal formulations, but careful attention to experimental consistency will enhance its robustness and reproducibility.

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