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DEVELOPMENT AND VALIDATION OF AN UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR QUANTIFYING CURCUMIN IN SIMULATED TEAR FLUID

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ABSTRACT

The objective of this research is to provide a quick, easy, and definitive method, i.e. UV-visible Spectrophotometry procedure for calculating the precise amount of the pure type of curcumin in simulated tear fluid (STF). To date, no confirmed methodology has been established for estimating the presence of curcumin within simulated tear fluid. Curcumin exhibits strong biological effects like anti-cancer, antiangiogenic, anti-inflammatory, antitumor, anti-depressant, antifungal, and antidiabetic, anti-bacterial. Curcumin was determined utilizing a UV-Visible spectrophotometer with two beams at an absorption maximum of 430 nm in a solvent system with simulated tear fluid (pH 7.4). The International Conference on Harmonisations (ICH) recommendations were followed while determining analytical parameters including linearity, precision, and accuracy. Simulated tear fluid was chosen as the solvent system for the approach because it satisfied the ideal requirements for the penetration of drugs into the eye cavities and high-quality peak at the chosen wavelength. According to Beer's law, the devised method demonstrated a linear response with a 2-10 µg/ml curcumin dosage range, and a correlation value of 0.9992 was discovered. The accuracy was found between 99.00 and 101.01%. The limit of detection (LOD) and limit of quantification (LOQ) for the method's sensitivity were found to be 0.0308 µg/ml and 0.9334 µg/ml, respectively, while the percent RSD value was less than 2. According to the study, calculating curcumin is a linear, accurate, exact, and cost-effective
method that may also be used to assess drug compositions.

KEYWORDS: UV spectrophotometer, Curcumin, Stimulated tear fluid (STF), Parameters.

1. INTRODUCTION

Curcumin is a bioactive polyphenolic substance that is yellow in colour and which can be discovered in the perennial plants rhizome i.e. Curcuma longa, most popularly known as turmeric, a plant having the status of belonging to the ginger family (Zingiberaceae)(1). Due to its exceptional therapeutic potential, curcumin has advanced significantly during the recent decades as an anticancer, antioxidant (2),anti-diabetic, anti-angiogenic, anti-fungal, anti-aging, anti-inflammatory (3), antifungal, and antibacterial agent, (4), (5), (6),(7) along with this numerous in vivo, in vitro and clinical investigations proved that curcumin also helps in the treatment of wound healing, arthritis, and Alzheimer's disease, (8), (9). In 1815, Vogel and Pelletier successfully extracted curcumin first from the Curcuma longa plant's rhizomes, and Vogel Jr. purified curcumin in 1842. Melabedzka et al. discover curcumin's composition a few decades later in 1910 as diferuloylmethane or 1, 6-heptane-3, 5-dione-1,7-bis (4-hydroxy-3-methoxyphenyl)-(1E,6E) (10) (Figure 1). Lampe along with Melabedzka published a method for manufacturing curcumin three years later in 1913. Srinivasan published a study on the chromatographic separation and quantification of curcumin modules in the year 1953 (4). In addition to its multiple biological properties, curcumin is exploited within the context of the food sector as a coloring, and flavoring agent (due to the existence of oleoresins and essential oil), and as a preservative agent.

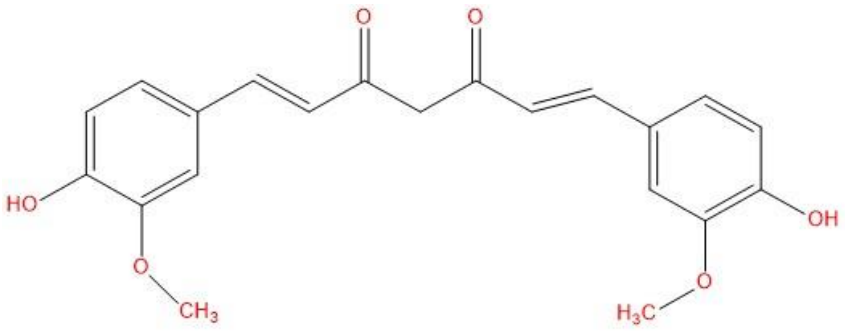

Figure 1: Chemical Structure of Curcumin.

The main phytocomponents of Curcuma longa are the curcuminoids, which usually make up around 1-6 % by weight. There are three main curcuminoids such as curcumin (75-80%), desmethoxycurcumin (15-20%), bis-desmethoxycurcumin (3-5%), and other phytoconstituents are shown (figure 2) (11). In addition to curcumin's various benefits, researchers have also reported its potential downsides, i.e., its disadvantages, which encompass its poor pharmacokinetic/pharmacodynamic properties such as its low solubility, low bioavailability, low potency, and toxic effects under certain test conditions.

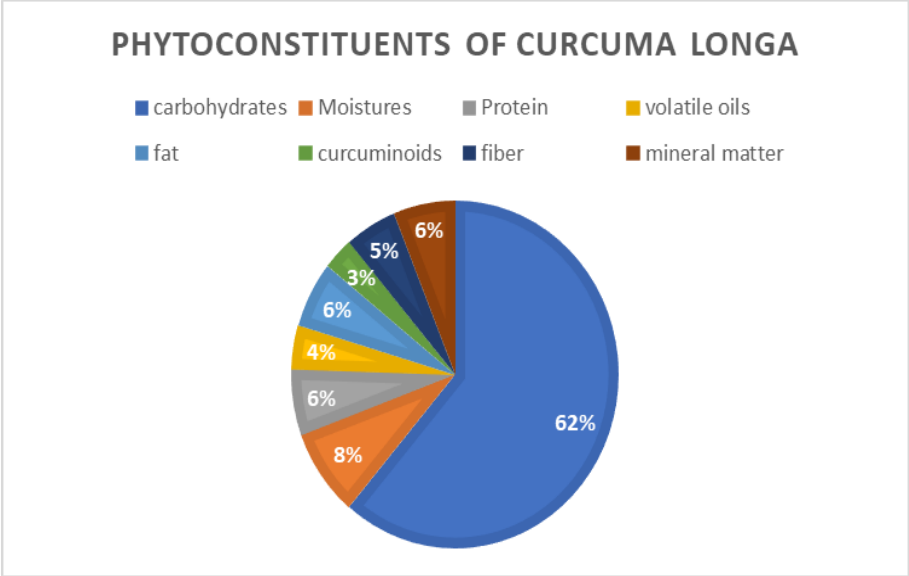

Figure 2: Major Phytoconstituents of Curcuma longa

Due to its biological properties, curcumin can be adopted as a preventive agent and therapeutic tool for multiple disorders. By obstructing the NF-B signaling network, COX-2, in addition to inflammation-promoting cytokines such as interleukin (IL)-1, IL-6, IL-18, and also the tumor necrosis factors (TNF), curcumin exerts anti-inflammatory properties. (12),(13). The NF-B pathway, which primarily controls the manifestation of prostaglandin E2, IL-1, IL-6, TNF-, and COX-2 is the most significant mediator of inflammatory responses (PGE2) (6). By controlling the transcription and expression rates of anti-oxidant enzymes as well as improving mitochondrial function, curcumin activates the body's anti-oxidant defense mechanisms. (14),(6) Curcumin has anti-cancer properties by inhibiting the bioactivity of carcinogens through the inhibition of particular cytochrome P450 isoenzymes and the activity or expression of phase 1 enzymes for induces the detoxification of carcinogens (15). As curcumin has outstanding anti-oxidant, anti-inflammatory, and anti-angiogenic qualities, curcumin grabs an essential involvement to treat severe eye diseases that is dry eye, anterior uveitis, ptergium, corneal neovascularization, glaucoma, age-related macular degeneration, cataract, corneal wound healing, conjunctivitis, and diabetic retinopathy. (16). Although curcumin belongs to BCS class 4 because of that it has poor solubility and poor permeability. To overcome this, we intend to encapsulate the curcumin in any nanoparticle system to enhance its properties. Several different solvents have high solubility for it such as ethanol, methanol, oils, and gland glacial acetic acid, but is insoluble in water, ethers, and buffers. Curcumin can be identified using several analytical techniques, including HPLC,

UV-visible spectroscopy, and HPTLC. UV spectrophotometry is the quickest, most accurate, and most dependable of this technique. It is still a widely used technique for quantifying the number of medicines (17). In order to analyze curcumin, the current work intends to provide a precise, focused, repeatable, valid, and stability-indicating approach employing an UV-Vis spectrophotometer in STF (pH 7.4).

2. MATERIALS AND METHOD

2.1 Materials

Central Drug House Products Ltd. in Delhi provided samples of curcumin. We bought sodium chloride, sodium bicarbonate, and calcium chloride dihydrate from Chemigens Research & Fine Chemicals, Sd Fine-Chem Limited in Mumbai, and Central Drug House Products Ltd. in Delhi, in that order. Analytical-grade materials were used for the rest of the investigation's chemicals and reagents. There was also the use of distilled water.

2.2 Instrumentation

Shimadzu's UV-visible spectrophotometer with two beams (model 1800) along with a slice thickness of 10mm was used to accomplish the spectrophotometric analytical determination of curcumin, together with quartz cuvette cells that had been modified for the task.

2.3 Method development

2.3.1 Preparation of simulated tear fluid (STF)

A solution of 0.670g NaCl, 0.20g $NaHCO_3$, and 8 mg (0.008gm) $CaCl_2$ created a simulated tear fluid by dispersing it in 1000 ml distilled water. The pH of the solution was brought up to 7.4 with the use of a solution that included sodium hydroxide (NaOH). (18)

2.3.2 Standard stock solution preparation

Stock solution 1: By dissolving 10 mg (0.01 g) of pure curcumin in a 10 ml volumetric flask, the material was converted into a primary standard stock solution with a concentration of 10 mg/ml (1000 µg/ml). The volumetric flask was filled until the mark was reached with ethanol and the concentration of the solution that was produced after this process was 1000 µg/ml. Sonicating the liquid with a bath sonicator resulted in the formation of the transparent solution.

Stock solution 2: To produce a secondary stock solution that has a concentration of 100 µg/ml a 1 ml aliquot of the prepared main standard stock solution was moved to a 100 ml volumetric flask and made up of that volumetric flask with simulated tear fluid. After that, STF (simulated tear fluid) was added to the volume of 10 ml volumetric flasks after aliquots of various test solutions (2–10 g/ml in concentration) had been transferred there.

2.4 Optimization of the method

2.4.1Selection and optimization of solvent

It is necessary to keep in mind that the solvent may affect the features and shape of the peak. Estimating the drug's presence in the simulated tear fluid is necessary for ocular delivery. Due to the medicine's poor solubility in STF, STF was employed in conjunction with a solvent, such as ethanol, to dissolve the drug. As a result, STF with ethanol satisfied all requirements for Peak quality at the designated wavelength. (19)

2.4.2 Determination of maximum wavelength (λmax):

Using STF against the blank, a 100 µg/ml Curcumin test sample was scanned under a UV spectrophotometer in the 400–800 nm spectral range. The highest value that could be accomplished in order to measure the prepared solution and the absorbance maximum was utilized to create the calibration curve.

2.4.3 Preparation of standard calibration curve:

STF was used to create the curcumin calibration curve. A curcumin solution's absorbance in the (2-10 µg/ml) concentration range in STF was measured to obtain the curcumin standard calibration curve. In triplicate, the operation was carried out, and the mean absorbance was recorded (Figure 3). After this step, the calibration curve for curcumin was generated, with the concentration of curcumin plotted along the x-axis and absorbance plotted along the y-axis.

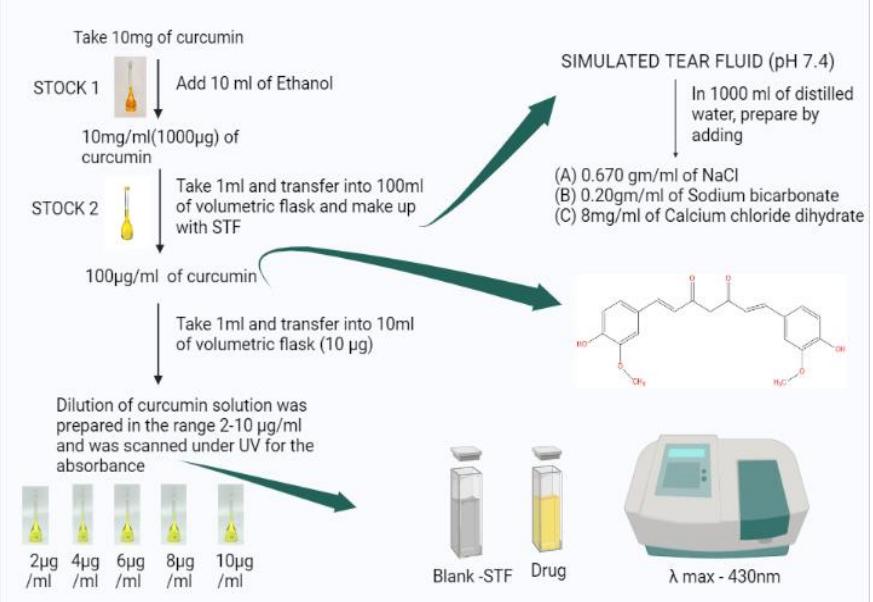

Figure 3: Preparation of curcumin test solutions (2-10 µg/ml) in STF (pH 7.4)

2.5 Analytical method Validation

A high degree of activity that consistently generates a desired outcome or a product that complies with specified requirements and quality qualities is known as validation, based on the International Conference on Harmonization (ICH). Following ICH criteria, the developed approach was validated. (20)

2.5.1 Linearity and range: A method is said to be linear if its observed concentration results for the samples it is analyzing are directly correlated with the theoretical sample's concentration of the analyte is measuring, or if it does so indirectly through a suitable mathematical transformation. abs. vs. conc. The result of the independent variable (concentration X) being on the X-axis, the dependent variable (absorbance Y) was taken into consideration while plotting the calibration curve. (21)

2.5.2 Accuracy: If test outcomes are more closely aligned with the theoretical value, an analytical way procedure is considered to be accurate % the test responses are analyzed using the recommended techniques, and the back-calculation of measured concentrations from the produced calibration curve using the regression model and compared to the theoretical concentration levels. Studies on analyte recovery are conducted at levels 80, 100, and 120 %**.** (22)

2.5.3 Precision: When the developed method's accuracy is used to confirm its dependability, reproducibility, and repeatability, and the individual test results are consistent whenever the method is repeatedly applied to multiple samples of the same homogeneous sample. The same analysis technique is repeatedly applied to the exact sample under standard experimental conditions. The variances in the results were reported as a percentage RSD, and the inter-day and intra-day precision of the sample precision was measured. Low, High-quality control concentrations were employed. (23)

2.5.4 Limit of Quantification (LOQ) & Limit of Detection (LOD): LOD can always be described as the lowest possible concentration of the analyte that can yet be accurately and precisely determined within a sample under a specified experimental set of conditions. Under specific situations and for particular experimental conditions, the results are particular to the LOD for a given sample. The outcomes of this quantitative research technique were impacted by modifications to the instrument, methodology, etc. The LOQ is almost ten times greater than the value of the blank and is the least analytical concentration of a tested sample among a set of samples that may be quantified. The following equations were used to calculate LOD and LOQ using the response's standard deviation and its slope: LOD is 3.3(σ/S) and LOQ is 10 (σ/S). Where S is the calibration curve's slope and σ is the absorbance sample's standard deviation. (24),

2.5.5 Ruggedness: By assessing 6 µg/ml concentration solutions in STF six times by 2 completely separate analysts at 430nm, ruggedness was ascertained. The results were given as %RSD.

2.5.6 Robustness: The method's robustness was assessed by using a working concentration of 6 g/ml of curcumin-test solution at two different concentrations and changing the wavelength to 430 nm. (25)
2.5.7 Repeatability: Six repetitions (6 g/ml) of the same experimental concentration were used to determine the curcumin test solution.

3. RESULTS AND DISCUSSION : The suggested methodology offers a straightforward, precise, cost-effective, and practical way to analyze curcumin using UV spectrophotometry. For the interday and intraday categories, it was discovered that the approach had less than 2% RSD. Due to the RSD values being under two, the method was also determined to be robust and rugged. The suggested method is sensitive, as evidenced by the detection limit and quantification limit of the proposed approach, which were 0.0308 µg/ml and 0.9334 µg/ml, respectively. The resulting test findings were in excellent accord with the label data, indicating that there is no excipient interference.

3.1 Determination of the UV absorption maxima

The absorption maximum of the curcumin test solution was measured at 430nm which was previously unknown in STF and Figure 3 demonstrates this.

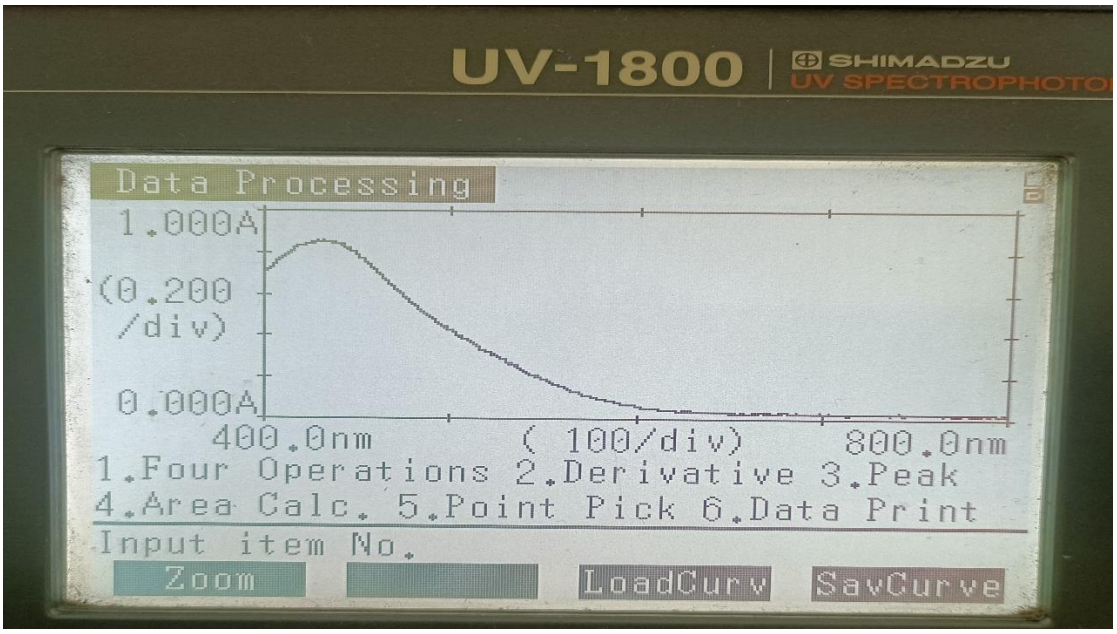

Figure 3 : Curcumin's UV spectrum in simulated tear fluid (pH 7.4)

3.2 Preparation of calibration curve

After scanning the test solutions at varying concentrations of the curcumin solution, the calibration curve for the substance was discovered to be linear and have a correlation coefficient of 0.9992 as seen in figure 4.

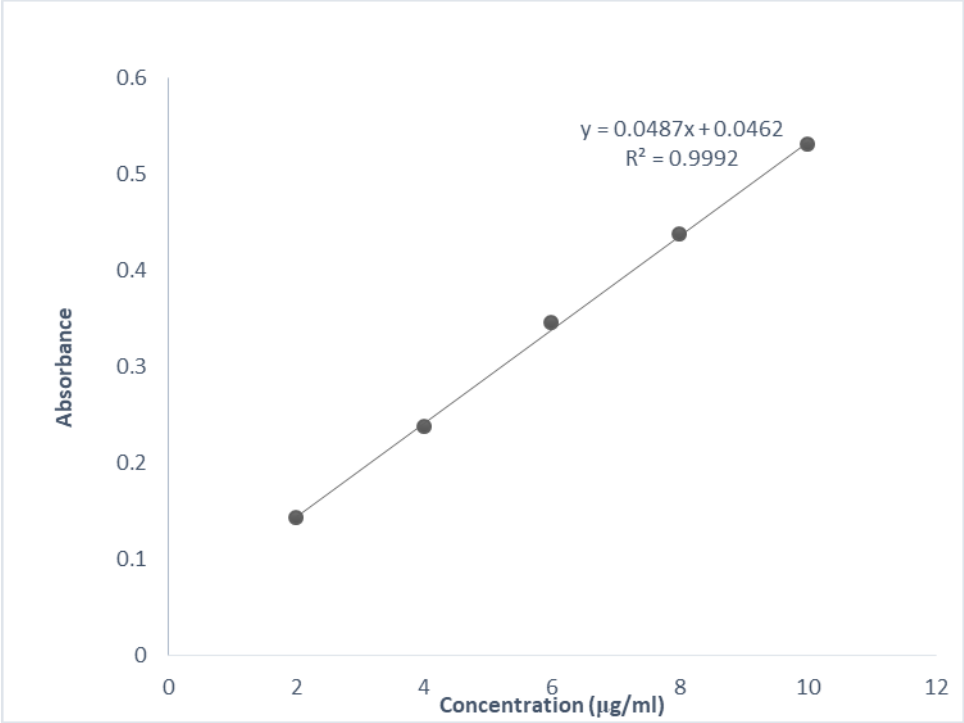

Figure 4: Calibration curve of curcumin in simulated tear fluid (STF) at 430 nm

3.3 Analytical method validation

Linearity

The results collected for curcumin solutions' linear regression within the range (2–10) µg/ml demonstrate strong linearity across the board range of (2–10) µg/ml with lower limits of standard deviation, indicating good consistency of the solutions. The derived linear regression equation is represented in Fig. 4 and Table 1 as y= 0.00487x + 0.0462 and R^2= 0.9992.

Table 1:- Table of curcumin linearity

Concentration (µg/ml)	Absorbance	
2	0.143	Y= 0.0487x + 0.0462 R^2=0.9992
4	0.237	
6	0.345	
8	0.437	
10	0.530	

3.4 Accuracy

As indicated in table 2, the recovery fell within the range of 99.00 to 101.01%. The typical addition proved the precision of the examined curcumin solutions created in STF and the precision of the intended technique.

Table 2: Table of Accuracy reading of curcumin

Labeled claimed	Level added	Added dosage of the drug	Average recovery %
6	80 %	80	100.23±0.27
6	100 %	100	99.20±0.18
6	120 %	120	100.30±0.25

3.5 Precision

The established approach is best for estimating at all concentration levels because the % RSD outcomes for within-day and between-day variations are often minimal which 2% of the absolute value is. Tables 4 and 5 display the intra-day and inter-day precision results. Additionally, the relative variance proportion was estimated.

Table 3: Precision outcomes show repeatability

Concentration (µg/ml)	Absorbance	Statistical Evaluation
6	0.340	
6	0.347	
6	0.351	MEAN- 0.3443 STANDARD DEVIATION- 0.004546 %RELATIVE STANDARD DEVIATION- 1.320
6	0.343	
6	0.339	
6	0.346	

Table 4: Intraday Precision

Concentration (µg/ml)	Absorbance A (10:00 am)	Absorbance B (12:00 pm)	Absorbance C (2:00 pm)	Average RSD %
6	0.340	0.342	0.340	1.3909
6	0.347	0.346	0.345	
6	0.351	0.355	0.352	
6	0.343	0.349	0.339	
6	0.339	0.351	0.350	
6	0.346	0.349	0.341	
% RSD	1.320	1.265	1.587	

Table 5: Interday precision

Concentration (µg/ml)	Absorbance A (DAY 1)	Absorbance B (DAY 2)	Absorbance C (DAY3)	Average RSD %
6	0.342	0.342	0.344	1.0778
6	0.353	0.345	0.350	
6	0.348	0.343	0.342	
6	0.346	0.347	0.344	
6	0.350	0.340	0.349	
6	0.342	0.350	0.347	
RSD %	1.268	1.050	0.913	

3.6 Limit of detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ were utilized to calculate the sensitivity of the suggested technique; their values were 0.030 µg/ml and 0.9334 µg/ml, respectively.

3.7 Ruggedness

Ruggedness was found by doing the test under the same conditions on entirely separate days, by completely different analysts, at completely different hours, and at completely different time points. The test outcomes ranged from 99 to 101.01%, as indicated in (table 6).

Table 6: Ruggedness results

Concentration (µg/ml) Analyst 1	Absorbance	Statistical Evaluation
6	0.340	MEAN- 0.3443 STANDARD DEVIATION- 0.004546 %RELATIVE STANDARD DEVIATION- 1.320
6	0.347	
6	0.351	
6	0.343	
6	0.339	
6	0.346	
Analyst 2		
6	0.347	MEAN- 0.3453 STANDARD DEVIATION- 0.004131 %RELATIVE STANDARD DEVIATION- 1.1962
6	0.345	
6	0.341	
6	0.349	
6	0.340	
6	0.350	

3.8 Robustness

By stopping the test during the alteration wavelength, robustness was firmly established. The acute RSD was discovered to be less than 2%, which fell under the permissible range as demonstrated in Table 7.

Table 7: Result of robustness

Concentration (µg/ml)	Absorbance	Statistical Evaluation
Temperature-10°C		MEAN- 0.3443 STANDARD DEVIATION- 0.004546 %RELATIVE STANDARD DEVIATION- 1.320
6	0.340	
6	0.347	
6	0.351	
6	0.343	
6	0.339	
6	0.346	
Temperature- 20°C		
6	0.347	MEAN- 0.3445 STANDARD DEVIATION- 0.003619 %RELATIVE STANDARD DEVIATION- 1.0506
6	0.342	
6	0.345	
6	0.343	
6	0.350	
6	0.340	

4. CONCLUSION

The provided approaches can locate the validation parameters within the chosen range. Table 8 displays the summary

Table 8- Observed characteristics

Parameters	Outcomes
Absorption peak	430nm

Accuracy	99-101.01 %
Range of bear law	2-10 µg/ml
Regression equation	0.0487x + 0.0462
Correlation coefficient	0.9992
Intercept	0.0462
slope	0.0487x
Precision	Intraday 1.3909, Interday 1.0778
LOQ µg/ml	0.9334 µg/ml
LOD µg/ml	µg/ml

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