SIMULTANEOUS ESTIMATION OF CURCUMIN AND ERLOTINIB BY OF UV-VISIBLE SPECTROPHOTOMETRIC TECNIQUE: DEVELOPMENT AND VALIDATION

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Abstract:
The objective of this research is to create and assess a UV spectrometric method for concurrent measurement of Curcumin and Erlotinib in tablet form. The current approach uses the absorbance ratio of Curcumin (425 nm) and Erlotinib (335 nm) at these two wavelengths to solve a simultaneous equation. In accordance with the ICH requirements, the mathematical properties of the suggested solution were evaluated for linearity, accuracy, precision, repeatability, roughness, and robustness. Beer-Lambert law concentration ranges of 200–1000 g/ml were achieved with this method, and concentrations of 50–450 g/ml were achieved with this method for Curcumin and Erlotinib, respectively. Because of the high R2 values, the calibration plot was quite linear. Accuracy and precision of 2% were confirmed for the linked fraction of RSD and for all validation metrics.

Keywords: Curcumin, Erlotinib, Simultaneous estimation, λmax, Validation.

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1. Introduction:

1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione is the chemical formula for curcumin. Curcumin Longa Linn roots produce a phenolic compound. Turmeric, the well-known Indian spice, belongs to the ginger family (Zingiberaceae) and contains the primary curcuminoid (diarylheptanoid)[1]. Turmeric gets its distinctive yellow hue from a compound called curcumin, which also provides many of the spice's therapeutic benefits[2, 3]. These are some of the qualities that it has are wound-healing, Antioxidant, anti-inflammatory, anticoagulant, anticarcinogenic, antispasmodic, hypocholesterolemic, anticancer, and hepatoprotective are only few of the many roles they play[4]. Although it is insoluble in water, it dissolves well in other solvents such acetone, dimethyl sulfoxide, ethanol, and oils. UV Complete absorption occurs at 425 nm[5]. Figure 1 depicts the molecular curcumin structure.

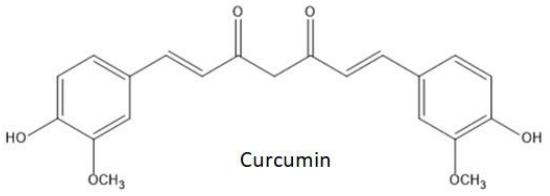

Figure 1: Curcumin structure

To identify non-small cell lung cancer (NSCLC) or pancreatic with EPF alterations, doctors use erlotinib, a tiny drug taken orally. Side effects include rash, diarrhoea, muscle soreness, knee discomfort, and cough. Respiratory issues, kidney issues, kidney failure, facial stitches, heart attacks, corneal ulcers, and other eye problems are among the more severe adverse effects. The infant will suffer harm during pregnancy. The epidermal growth factor receptor (EGFR) is the target of this tyrosine kinase inhibitor[6]. Drug approval package: tarceva (erlotinib) nda approved erlotinib for therapeutic use in the United States. It's one of the best and most effective medications, thus it's on the World Health Organization's list of essential medicines (WHO, 2019). In Fig. 2 we see the molecular structure of the drug erlotinib.

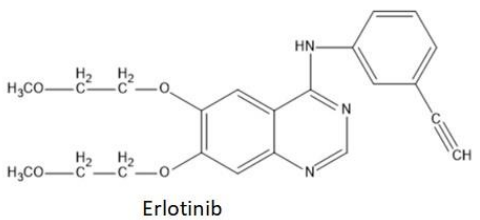

Figure 2: Chemical structure of Erlotinib

Therefore, there is no established method for determining the correct Curcumin and Erlotinib dosage when used together. Studies done so far have shown that there is no way to compare the effectiveness of tablet forms of Curcumin and Erlotinib. Because of its low cost, high specificity, and relative ease of use, spectrophotography has become the method of choice for identifying drugs[7]. As stated on page 228[8]. Objectives of study were to develop a rapid, simple, selective, accurate, sensitive, robust, reproducible, and cost-effective method for the simultaneous measurement of Curcumin and Erlotinib in tablet doses. The existing system has been validated to ICH Issue Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, 1996 and ICH Issue Q 2 (R1) Validation of Analytical Procedures: Methodology, 2020.

2. Materials & methods

2.1. Apparatus and instruments

Japanese UV-1800 UV-Visible Double Beam Shimadzu Spectroradiometer

Absorption of the resultant solutions was measured using a pair of 1 cm quartz cells, a spectral bandwidth of 1 nm, and a wavelength precision of 0.5 nm. The automatic balance of weights was used to accurately measure all of the materials.

2.2. Chemicals and reagents:

The curcumin came from a free sample provided by the Green Chemical Laboratory in Bangalore, India. A free trial of erlotinib was generously provided by Medreich, Bangalore, India. The rest of the chemicals and reagents were analytical grade. In this experiment, only water that has been twice distilled was used.

2.3. Selection of a solvent:

Solvents like ethanol, water, and methanol have been used to investigate the effects of curcumin and Erlotinib. These solutions have been observed to include the UV spectra of both medications. Ethanol was as effective as other solvents in facilitating the absorption of both medications. Double-filtered water was used to dilute the solution further.

2.4. Determination of Iso-bestic Point and absorption maxima

Curcumin and Erlotinib Standard stock solutions were diluted in distilled water to the appropriate concentrations for wavelengths between 190 and 800 nm. It has been calculated that each of these have a similar overall absorption[9].

2.5. Curcumin and Erlotinib Calibration plots

The calibration plots for Curcumin and Erlotinib at their respective maximal absorption levels (max) were created using dual filtered water to guarantee linearity of the drugs.

2.6. Development of simultaneous equation

To ensure optimal effectiveness, guidelines for both medications' absorption and absorption is established at their maximal absorption (λmax). Sample solution drug concentrations determined with Eq.1 and 2:

A1= ax1CA+ ay1CC At 425 nm (1)

A2= ax2CA+ ay2CC At 335 nm (2)

Where Curcumin and Erlotinib concentration were CA and CC in µg / ml is, 425 nm and 335 nm were A1 and A2 respectively. Curcumin and Erlotinib absorption values at 425 nm and 335 nm are Ax1, ax2 and ay1, and2, respectively[10].

2.7. Proposed method Validation.

Linearity, accuracy, precision, repeatability, durability, resilience, sensitivity, and forced deterioration are all verified through the method's validation.

2.7.1. Linearity

Several aliquots of Curcumin and Erlotinib were made, with the former ranging from 200 to 1000 g/ml and the latter from 50 to 450 g/ml. A UV-Visible (190-800 nm) spectrophotometer was used to analyse the solutions. The spectrum was analysed at two different wavelengths, 425 nm and 335 nm. This concentration data was supposed to be absorbed by the calibration plot[11].

2.7.2. Studies of Recovery (accuracy)

To evaluate the efficacy of the suggested strategy, a recovery study was conducted using the canonical addition technique. Curcumin (500 g/ml) and Erlotinib (20 g/ml) were spiked at 50, 100, and 150% in advance of analysis. A percentage recovery was calculated from the data we gathered[12].

2.7.3. Precision

When talking about analytical procedures, precision refers to how easily they can be repeated under controlled laboratory circumstances. Over the full linearity range, three separate quality assurance measurements were taken at varied concentration rates to determine the degree of precision. Intermediate precision (interday) and Repeatability (intraday) along with a replicate measurements are recorded statistically significant number of as RSD percent to determine the system's accuracy. The test was measured over the course of three days, yielding results of medium precision, which are presented as % RSD deviation and standard[13].

2.7.4. Robustness

Analytical method robustness is determined by demonstrating the technique's efficiency under typical conditions of use and remaining unaffected by small but deliberate changes in the method parameters. Maximum (425 nm) and minimum (335 nm) detecting wavelengths are used to determine robustness[14]. Validation of robustness has been achieved.

2.7.5. Limit of detection (LOD) and Limit of quantification (LOQ)

The limits of detection (LOD) and quantitation (LOQ) represent the lowest detectable concentration (DCC) that can be established by a given analytical method. During the development and verification of the analytical process, these are two crucial factors to examine. The lowest analyte quantity in a sample that may be detected under defined experimental conditions is known as the detection limit (LOD), however it is not typically quantified. The quantification limit (LOQ) is the minimal detectable concentration in a sample for quantitative analysis. For the LOD and LOQ, use the standard formulas.

Eqn mentioned 3 and 4[15].

$LOD = 3.3\times\delta / S$ (3)

$LOQ=10\times\delta / S$ (4)

Where δ = Lowest standard concentration Standard deviation. S = standard curve Slope.

2.8. Curcumin and Erlotinib Assay

A mixture of Erlotinib (100mg) and Curcumin (500mg) was prepared by crushing ten tablets (250 mg each), then adding them to 100 ml of ethanol and crushing them again. After soning, filtering, and appropriately diluting the solution, we tested its quality using a double UV spectrophotometer at 425 nm and 335 nm using the simultaneous equation approach. There have been three separate analyses of each sample[16].

3. Discussion of results

Estimation of more than one active component quantitatively in the same pharmaceutical dosage form has shown to be more laborious in the field of pharmaceutical analysis. Time and money savings when compared to chromatography motivated the development and validation of spectrophotometric UV visible methods for the stability indicator UV Tablet-based Curcumin and Erlotinib quantifica-

tion using a visible spectrophotometric approach. The current method relies on a parallel system of equations developed from absorption observations at 425 nm and 335 nm for Curcumin and Erlotinib, respectively. In accordance with guidelines from the International Conference on Harmonization (ICH), statistical tests were conducted to ensure the system's linearity, accuracy, repeatability, robustness, and resilience.

3.1. Determination of Absorption Maxima (λmax) and Iso-bestic Point

The spectral regions of both medicines with the highest rates of absorption were identified. Absorption maximum (alone max) was measured at 425 nm and 335 nm for the typical stock solutions of curcumin and erlotinib, respectively. Wavelength was selected in order to construct both the measurement plot and for additional drug analysis due to its appearance in both the Curcumin and Erlotinib overlay spectra as shown in Fig.3.

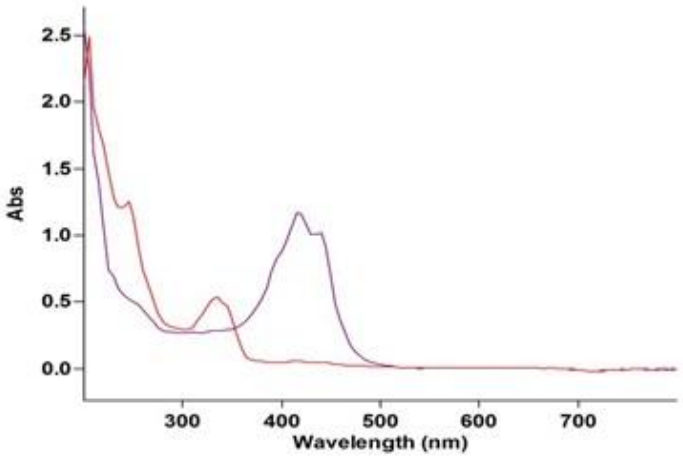

Figure 3: Erlotinib and Curcumin Overlay spectra.

3.2. Erlotinib and Curcumin Calibration plots

Figures 4 and 5 show the corresponding calibration curves for Curcumin and Erlotinobi, respectively, over the concentration range of 20-1000 g/ml and maximum absorption (total) of 50-450 g/mL, respectively, at 425 nm and 335 nm, respectively. The 425 nm and 335 nm wavelengths of curcumin were calibrated, and a regression equation was found to be Y=0.014X+0.037, R2=0.985, and Y=0.009X+0.045, R2=0.974. Similarly, a 335 nm and 425 nm Erlotinib calibration curve was created, and the regression equation was found to be Y=0.048X+0.153, R2=0.995 for 335 nm, and Y=0.008X-0.005, R2=0.991 for 425 nm. In this equation, Y is the absorbent at a given wavelength, X is the association coefficient in gm-2, and R2 is the concentration of the solute in the solution.

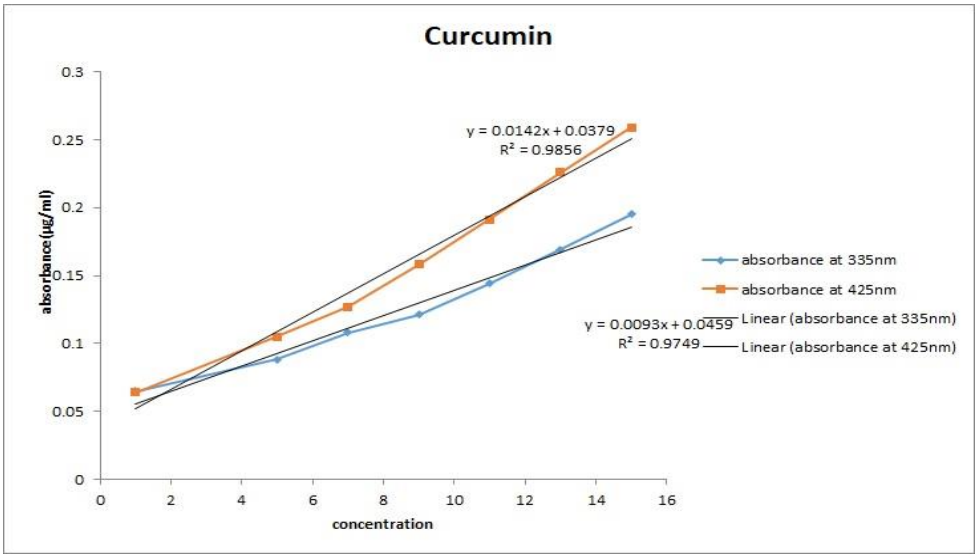

Figure 4: Curcumin Calibration plot

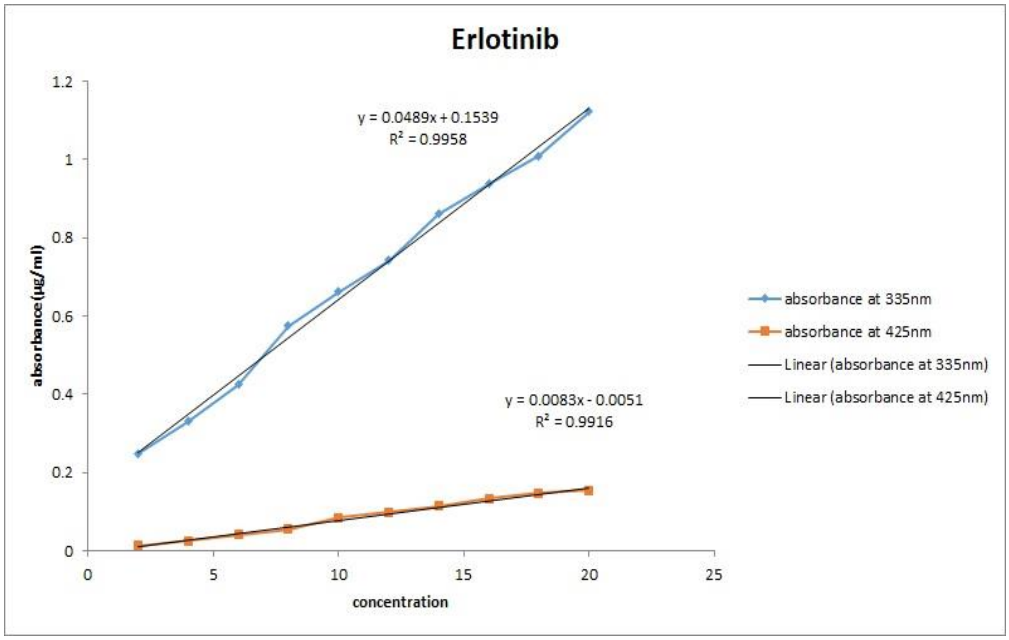

Figure 5: Erlotinib Calibration plot

3.3. Simultaneous equation Development

For Curcumin and Erlotinib, the absorbability values were calculated by ratio of absorbance ands concentration. From what can be seen in Tables 1 and 2, it appears that Curcumin and Erlotinib have similar absorption values across both concentration ranges. The following equation was derived for the Curcumin and Erlotinib simultaneous estimation in a combination tablet form by substituting ax1, ax2, ay1, and ay2 values from Tables 1 and 2 into Eqn. 4 and 5.

A1=0.0030CA +0.0002CC (4) at 425 nm

A2= 0.00030CA + 0.00032CC (5) at 335 nm.

A1 is 425 nm and A2 is 335 nm when CA and CC are Curcumin and Erlotinib, respectively, in g / ml. This eqn has been answered. It is possible to quantify the concentrations of both curcumin and erlotinib in a single tablet dose, as shown in Examples 4 and 5.

Table 1: Curcumin Absorptivity and absorbance

S. No	concµg/ml	Absorbance at 335nm±SD	absorbance at 425nm±SD	Absorptivity at335nm	Absorptivity at 425nm
1	50	0.149±0.003	0.014±0.003	0.0030	0.00028
2	100	0.270±0.004	0.026±0.004	0.0027	0.00026
3	150	0.425±0.003	0.042±0.003	0.0028	0.00028
4	200	0.575±0.002	0.055±0.002	0.0029	0.00028
5	250	0.762±0.001	0.084±0.001	0.0030	0.00034
6	300	0.942±0.003	0.098±0.003	0.0031	0.00033
7	350	1.090±0.002	0.114±0.002	0.0031	0.00033
8	400	1.238±0.002	0.133±0.002	0.0031	0.00033
9	450	1.380±0.002	0.147±0.002	0.0031	0.00033
				average aX1=0.0030	aX2=0.00030

Table 2: Erlotinib Absorptivity and absorbance

S. No	concµg/ml	absorbance at 335nm±SD	absorbance at 425nm±SD	Absorptivity at335nm	absorptivity at 425nm
1	200	0.065±0.001	0.064±0.001	0.00033	0.00032
2	300	0.077±0.004	0.095±0.004	0.00026	0.00032
3	400	0.098±0.003	0.127±0.003	0.00025	0.00032
4	500	0.121±0.002	0.158±0.002	0.00024	0.00032
5	600	0.144±0.002	0.191±0.002	0.00024	0.00032
6	700	0.169±0.003	0.226±0.003	0.00024	0.00032
7	800	0.195±0.002	0.259±0.002	0.00024	0.00032
8	900	0.221±0.002	0.295±0.002	0.00025	0.00033
9	1000	0.269±0.001	0.329±0.001	0.00027	0.00033
				Average ay1=0.00026	ay2=0.00032

3.4. Proposed method validation

3.4.1. Linearity

If a method is linear, its results will be proportionate to the concentration of the analyte over a specific concentration range. In the example given, the range is the difference between the highest and lowest levels predicted by the disclosed method's accurate and linear analysts. The dose ranges of 20-1000 g/ml for curcumin and 50-450 g/ml for erlotinib are chosen for linearity testing. The regression equation for the calibration curve was derived by plotting the concentration of the standard solution against the amount of absorption. The least squares approach was employed for the estimate of the Slope, Intercept, and Correlation Coefficient (R2). As can be shown in Fig., curcumin showed linearity between 2 and 20 g/ml, while erlotinib showed linearity between 5 and 15 g/ml. Within the prescribed concentration range, as seen in Figures 4 and 5, the substance is in accordance with the law of Beer-Lambert. Calibration curve linearity for both medications is indicated by strong R2 values[17].

Accuracy (Percentage recovery) (Percentage recovery)

Researchers use % Recovery trials to prove that their new technique works. Using the standard additive method, we measured at three different concentrations (50, 100, and 150%) by adding the prescribed amount of medicines to the sample solution, validating the precise usage of the strategy proposed. Recovery percentages for curcumin varied from 99.302.34% to 99.961.38%, with a mean recovery of 99.840.36% and a relative standard deviation (RSD) of 0.36%. Similar results are found in Table 3 for Erlotinib, which exhibits a range of 99.201.38% to 99.801.94%, with 99.440.32% mean percent recovery of and an 0.32 percent of RSD value. Good accuracy of the devised approach was shown by the fact that the measured percentage of RSD values fell within the acceptability of 2% limit. 99.0 percent Mean accuracy, demonstrated the excellent precision of the proposed method[18].

Table 3: Percentage recovery statistical data of accuracy

Drug	amount of drug taken(µg/ml)	amount of drug added(µg/ml)	Conc. actually found(µg/ml)	% recovery(*n=3)±S.D
Curcumin	200	100	299	99.30±2.34
	200	200	401	99.96±1.38
	200	300	498	99.54±1.34
			Mean:99.84±0.36	%ageRSD=0.36
Erlotinib	500	250	745	99.33±2.34
	500	500	998	99.80±1.94
	500	750	1240	99.20±1.38
			Mean:99.44±0.32	%age RSD=0.32

3.4.2. Precision

The accuracy was measured by twice-daily and once-a-week inspections of a test sample at their designated places. On the same day, three replicate measurements were taken at different times and concentrations to assess the linearity range of the medications. The network's standard operating procedure included three days of continuous inter-day monitoring. The UV-Visible spectro-photometer was used to conduct interday precision experiments on Curcumin and Erlotinib, and the results showed a high degree of accuracy, with mean values of 98.921.59% and 98.920.38%, respectively, and RSDs of 1.612% and 0.391%, as shown in Table 4. Table 5 shows that the mean value of Curcumin and Erlotinib inter-day precision studies is 99.681.03% and 100.011.29%, respectively, with respective RSD values of 1,031 and 1,391. There is little room for error in the developed approach, since 97.5 percent of the measured RSD values fall below the 2% acceptance threshold[19].

Table 4: curcumin and erlotinib intraday precision

S. No	conc taken(µg/ml)	absorbance(*n=3)		%age conc found	
		335nm	425nm	erlotinib	curcumin
1	200	0.640	0.122	98.00	98.50
2	300	0.958	0.183	98.00	99.00
3	400	1.312	0.248	100.75	99.25
		Mean±S.D		98.92±1.59	98.92±0.38
		%RSD		1.61	0.39

Table 5: curcumin and erlotinib inter-dayprecision Statistical data

S. No	conc taken(µg/ml)	absorbance(*n=3)		%age conc found	
		335nm	425nm	erlotinib	curcumin
1	200	0.644	0.124	98.50	101.50
2	300	0.980	0.186	100.30	99.67
3	400	1.306	0.247	100.25	99.00
		Mean±S.D		99.68±1.03	100.01±1.29
		%RSD		1.03	1.39

3.4.3. Repeatability

Erlotinib 100 ng/ml and Curcumin 500 ng/ml were absorbed in six separate samples to verify the device's consistency. According to Table 6, the concentration of curcumin was 201.700.82 g/ml and that of Erlotinib was 398.330.65 g/ml. It was determined that the proposed approach is reproducible within the required limitations (2 %), with RSD values of 0.65 and 0.40 for Erlotinib and Curcumin, respectively. Based on research[20].

Table 6: Repeatability studies of curcumin and erlotinib statistical data

S. No	conc taken(µg/ml)of curcumin	conc taken (µg/ml)of erlotinib	absorbanceat 335nm(*n=3)	absorbanceat 425nm(*n=3)	conc found (µg/ml)of Curcumin	conc found (µg/ml)of Erlotinib
1	200	400	0.71	0.187	202	395
2	200	400	0.707	0.189	201	402
3	200	400	0.708	0.188	201	399
4	200	400	0.713	0.189	203	400
5	200	400	0.705	0.187	201	396
6	200	400	0.712	0.188	202	398
				Mean	201.7	398.33
				SD	0.82	2.58
				%RSD	0.4	0.65

3.4.4. Robustness

If you're studying a technique, you should think about how you're going to evaluate its robustness as it's being developed. Analyses should be shown to be reliable in light of known and known-to-be-inaccurate fluctuations in process parameters. This study measured absorbance at 425 nm and 335 nm to evaluate the stability of the developed system. No significant outliers were found during the course of the investigation, demonstrating the reliability of the technique. Table 7 displays that, given a reliable method, the RSD values were found to be acceptable (2%)[21].

Table 7: Robustness Data

S. No	conc taken(µg/ml		absorbance(*n=3)		conc found (µg/ml)		absorbance(*n=3)		conc found (µg/ml)	
	Curcumin	Erlotini	425nm	335nm	Curcumin	Erloti	425nm	335nm	Curcumin	Erloti
1	200	400	0.710	0.187	202	395	0.709	0.189	202	402
2	200	400	0.707	0.189	201	402	0.703	0.187	200	397
3	200	400	0.708	0.188	201	399	0.707	0.189	201	402
4	200	400	0.713	0.189	203	400	0.698	0.187	198	399
5	200	400	0.705	0.187	201	396	0.702	0.188	199	401
6	200	400	0.712	0.188	202	398	0.708	0.188	201	399
			mean		201.67	397.50	mean		200	400
							SD		1.47	2.00
							%RSD		0.74	0.50

3.4.5. Limit of quantification (LOQ) and Limit of detection (LOD)

ICH offered a number of methods for determining identification and quantification constraints. In particular, the ratio of signal-to-noise, a visual analysis, the implementation of Standard, deviation of response, and the course of the calibration curve. Lowest standard concentration Standard deviation and the pitch of the curve of is S; these values were used to calculate the LOD and LOQ for curcumin and erlotinib, respectively, in this work. The new approach is very sensitive, with limits of detection and quantitation of 2.00 and 6.67 micrograms per millilitre for erlotinib and 0.01 and 0.02 micrograms per millilitre for curcumin, respectively[22].

3.5. Assay in tablet formulation for Curcumin and Erlotinib.

The percentages of Curcumin and Erlotinib in the tablets were 98.80±0.91 and 95.80±0.01, respectively, for the assay, demonstrating the versatility of the established approach. Results in Table 10 indicated that the UV-Visible spectroscopic approach was suitable for simultaneous measurement of Curcumin and Erlotinib in tablet dose. Table 11, which summarises the parameters, demonstrates that the proposed spectrophotometric UV approach for both curcumin and erlotinib is rapid, selective, responsive, exact, reliable, robust, reproducible, and cost-effective.

Table 8: Results of Assay

S. No	Absorbance		Concentration		% Drug Found	
	425nm	335nm	CUR (mg)	ERLO (mg)	CUR	ERLO
1	0.155	1.503	200	500	99.8	95.8
2	0.153	1.482	200	500	98.6	95.8
3	0.152	1.474	200	500	98	95.8
				Mean ±SD	98.80±0.92	95.80±0.00
				RSD	0.93	0

Conclusion:

ICH recommendations were followed in development and validation of Curcumin and Erlotinib's concurrent assessment. The results demonstrated that the suggested UV-Visible spectrophotometric approach was quick, easy, selective, sensitive, exact, precise, robust, reproducible, and inexpensive when estimating Curcumin and Erlotinib concurrently. The established UV-Visible Spectrophotometric technique was demonstrated by testing a mixed dose tablet formulation. The method relies on not only the employment of complex, high-priced techniques like high-performance liquid chromatography (HPLC), reversed-phase high-performance thin-layer chromatography (RP-HPLC), and high-temperature thermocyclic voltammetry (HPTLC).

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Reference:

1. Jenita, J.L., Formulation and evaluation of microparticles containing curcumin for colorectal cancer. Journal of Drug Delivery and Therapeutics, 2012. **2**(3).
2. Sharma, R., G escher AJ, S teward WP. 2 005. C urcumin: the story so far. Eur. J. Cancer, 1955. **41**: p. 1955-1968.
3. BB, A., Curcumin: the Indian solid gold. Adv Exp Med Biol, 2007. **595**: p. 1.
4. Ashraf, K., et al., Validated HPTLC analysis method for quantification of variability in content of curcumin in Curcuma longa L (turmeric) collected from different geographical region of India. Asian Pacific Journal of Tropical Biomedicine, 2012. **2**(2): p. S584-S588.
5. Sharma, K., S. Agrawal, and M. Gupta, Development and validation of UV spectrophotometric method for the estimation of curcumin in bulk drug and pharmaceutical dosage forms. International Journal of Drug Development and Research, 2012. **4**(2): p. 0-0.
6. Reck, M., et al., Erlotinib in advanced non-small cell lung cancer: efficacy and safety findings of the global phase IV Tarceva Lung Cancer Survival Treatment study. Journal of Thoracic Oncology, 2010. **5**(10): p. 1616-1622.
7. Pundarikakshudu, K. and H.N. Dave, Simultaneous determination of curcumin and berberine in their pure form and from the combined extracts of Curcuma longa and Berberis aristata. International Journal of Applied Science and Engineering, 2010. **8**(1): p. 19-26.
8. Chaudhary, H., et al., A Novel Validated Spectrophotometric Method for Simultaneous Estimation of Diclofenac Diethylamine and Curcumin in Transdermal Gels. Analytical Chemistry Letters, 2011. **1**(3): p. 224-233.
9. Kumar, B.K., V.T. Rajan, and N.T. Begum, Analytical method development and validation of lidocaine in ointment formulation by U. V spectrophotometric method. International Journal of Pharmacy and Pharmaceutical Sciences, 2012. **4**(2): p. 610-4.
10. Beckett, A. and J. Stenlake, Practical Pharmaceutical Chemistry, part-II. CBS Publications and Distributors, New Delhi, 1997. **1**: p. 275-300.
11. Jain, P.S., et al., Development and validation of the UV-spectrophotometric method for

determination of terbinafine hydrochloride in bulk and in formulation. Pharmaceutical methods, 2011. **2**(3): p. 198-202.
12. Sethuraman, S. and K. Radhakrishnan, Analytical method development and validation of caffeine in tablet dosage form by using UV-spectroscopy. International Journal of Novel Trends in Pharmaceutical Sciences, 2013. **3**(4): p. 82-86.
13. Balasaheb, B.G., et al., Development and validation of UV spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form. Malaysian Journal of Analytical Sciences, 2015. **19**(6): p. 1156-1163.
14. Cazedey, E.C.L. and H.R.N. Salgado, Development and validation of UV spectrophotometric method for orbifloxacin assay and dissolution studies. Brazilian Journal of Pharmaceutical Sciences, 2014. **50**: p. 457-465.
15. Sharma, D., et al. Development and Validation Of Spectroscopic Method For Simultaneous Estimation of Salbutamol Sulphate, Ambroxol Hydrochloride And Cetirizine Hydrochloride In Combined Pharmaceutical Tablet Formulation: A Novel Technique For In-Vitro Dissolution Studies. in Conference on Harmonization guidelines (ICH). 2014.
16. Rasheed, S.H., et al., Comparison of different superdisintegrants in designing of fast dissolving tablets of salbutamol sulphate. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2011. **2**(2): p. 155-163.
17. Prasad, A. and B. Thireesha, UV-spectrophotometric method development and validation for the determination of lornoxicam in microsponges. Int J Appl Pharm, 2018. **10**: p. 74-8.
18. Gadiya, H., M. Maheshwari, and A. Dashora, UV-analytical method development and validation for simultaneous estimation of dapoxetine hydrochloride and sildenafil citrate in tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research, 2019. **12**(1): p. 328-31.
19. Mali, S., S. Ahmad, and V. Shastry, Development and validation of UV visible spectrophotometric method for estimation of aceclofenac and tramadol in bulk and dosage form. International Journal of Pharmaceutical Sciences and Research, 2018. **9**(9): p. 3852-3857.
20. Abdelwahab, N.S., B.A. El-Zeiny, and S.I. Tohamy, Two spectrophotometric methods for simultaneous determination of some antihyperlipidemic drugs. Journal of pharmaceutical analysis, 2012. **2**(4): p. 279-284.
21. Shaikh, J.S. and N.N. Rao, Simultaneous estimation and forced degradation studies of amiloride hydrochloride and furosemide in a pharmaceutical dosage form using reverse-phase high-performance liquid chromatography method. Asian J. Pharm. Clin. Res, 2018. **11**: p. 215-221.
22. Mondal, S., et al., Development and Validation of Few UV Spectrophotometric methods for the Determination of valganciclovir in bulk and pharmaceutical dosage form. Pharmaceutical Methods, 2018. **9**(2): p. 64-68.