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Simultaneous Estimation of Curcumin and Gentamicin by UV-VIS Spectrometric Methods or Derivative Spectroscopic Techniques

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A simultaneous study is very important analytical parameter which helps to assess compatibility of mixture of drugs. UV spectrophotometer is one of the simplest and efficient methods to assess simultaneous parameters. Due to its economical approach, we have used UV spectrophotometer. Curcumin and Gentamicin sulphate have zero crossing points of 420 nm and 244 nm, respectively, in first-order derivative spectroscopy. The solvent for the spectrophotometric process was 0.1 N NaOH. Curcumin and Gentamicin sulphate linearity was established over range of concentrations of 2–12 g/ml, with correlation coefficients of 0.995 and 0.993. The mean percent recoveries Curcumin and Gentamicin sulphate were found to be in the range of 98.88 percent and 98.54 percent, respectively. The approach has been found to be repeatable in both inter day and intraday testing. The approach was proven to be both precise and reliable. According to the recovery investigation, the approach was effectively applied to pharmaceutical formulation with no interference from excipients. The results of the analysis were statistically evaluated, as well as by recovery trials. The LOD and LOQ for Gentamicin sulphate in phosphate buffer were found to be 0.024μg/ml and 0.045μg/ml, respectively and values for Curcumin were 0.024μg/ml and 0.037 μg/ml, respectively which indicates adequate sensitivity of method.

Keywords: Curcumin; Burn Wound Infections; Gentamicin sulphate; Simultaneous equation method; Validation.

Unlike other types of wound injuries, burn wound injuries results into remarkable mortality rate due to which millions of people get affected and seek medical attention. A number of antibiotics are utilized with an aim to drop down the threat of microbial infection in burn wound patient prior to be 0 encounters. [1] Few of them routed through dermal layers and others through oral or injectable route. There are a number of antibiotic therapies available for treatment of burn wound infections but these treatments face a number of challenges like antibiotic resistance, delayed release, delivery of optimum concentration of drug at site of action.[2] Gentamicin (GEN) and Curcumin (CUR) is the potent antibiotic against bacterial infections produced by Gram positive and gram-negative bacteria respectively. As per the literature, In burn injury, a combination of Curcumin and Gentamicin

revealed synergism against P. aeruginosa. So here we have selected combination of these two drugs to make an effective formulation.[3] the Curcumin is the natural compound (Phenolic), extracted from turmeric, one of the popular Indian spices from plant of *Curcuma Longa Linn*. This compound has antioxidant, anti-inflammatory as well as antimicrobial actions.[4]

It's already been suggested by a number of studies that Curcumin have poor absorption through gut and it also conquers Cytochrome P450 isoenzyme and metabolized by glucuronidation. [5]

Gentamicin sulfate is basically isolated from the Micro mono spore's purpurea that is an actinimycete. The Derived antibiotics is a water soluble antibiotic belongs to a group of aminoglycosides[6, 7.]

Various Curcumin and Gentamicin sulphate formulations include nanoparticles, liposomes phytosomes, improves bioavailability solubility, dissolution rate.The binary mixture must be more effective in controlling diseases. Analytical techniques tend to provide information of the constituents of particular formulation mentioned in the label.[8] For separation and estimation of multi-components of a formulation, various techniques, like chromatographic and Spectrophotometric, have been reported [9,10] .Chromatographic techniques like HPLC being quite more complicated , time-consuming, and require high consumption of organic solvents [11] while in HPTLC, plate length is limited so separation can take place up to certain length. Earlier methods reported for simultaneous estimation of Curcumin and Gentamicin sulphate in nanoformulations. UV Visible Spectrophotometer has advantages than other analytical techniques as it is one of simple, rapid, method for quantitative determination. UV Spectrophotometric method are reported in numerous mixture of drugs such as Cefixime and Ofloxacin[12],Cefixime and Lisinopril[13],Rosuvastatin Calcium and Glimepride[14],Nimesulide and Diclofenac Sodium[15],Metformine Hydrochloride and Pioglitazine[16],Tizanidine and Aceclofenac[17] , Levosulpride and Esomeprazole [18],Diazepam and Propranolol [19]in bulk and Tablet dosage form and alone,Triamcealone[20],Ziprasidone[21] in tablet dosage form. Present work emphasizes onvalidating of UV Visible Spectrophotometric analytical method for Curcumin and Gentamicin sulphate, in combination.

MATERIALS AND METHODS

Chemicals and Reagents

Curcumin was procured from Lobachemie, Mumbai while Gentamicin sulphate from Himedia Ltd., Mumbai, India and analytical grade of Methanol was used from M/s Merck Ltd., Mumbai, India.

Instrumentation

The projected effort was carried on Shimadzu UV-1800; UV spectrophotometer which have double beam double detector pattern with a1 cm quartz coordinated cell. Calibrated analytical balance was opted for weighing of contents.

Selection of Solvents

On the basis of solubility study methanol was selected as the solvent for dissolving Curcumin and Gentamicin sulphate.

Preparation of binary mixture

The binary mixtures Curcumin and Gentamicin sulphate in a 1:1 proportion by weight were prepared and mechanically homogenized with a mortar and pestle

Preparation of standard stock solution of Gentamicin sulphate and Curcumin

Curcumin Stock Solution

Curcumin (10 mg) was properly weighed and dissolved in methanol (20 ml) using ultrasonication for approximately 10 minutes in a 100 ml volumetric flask. Curcumin standard stock solution (100/ml) was then prepared up to the required volume using methanol.

Curcumin Working Standard Solution

Curcumin Standard Solution for Work Standard stock solution of Curcumin To make a working standard solution, 5 ml of methanol was diluted to 50 ml a concentration of ten grammes per milliliter

Gentamicin sulphate Stock Solution

Stock Solution of Gentamicin sulphate In a 100 ml volumetric flask, a properly weighed quantity of Gentamicin sulphate (10 mg) was dissolved in water (20 ml) using ultrasonication for around 10 minutes. The Gentamicin sulphate standard stock solution (100/ml) was then brought up to the required volume using water.

Gentamicin sulphate working Standard Solution

Working Standard Solution of Gentamicin sulphate Standard stock solution of Gentamicin sulphate to make a working standard solution, 5 ml of phosphate buffer pH 6.8 was diluted to 50 ml. 100 g per ml.

Determination of ë Max of Individual Component

For Curcumin and Gentamicin sulphate 2 g/ml dilution, an adequate aliquot fraction of Gentamicin sulphate and Curcumin (2 ml) was transferred to two separate 10 ml volumetric flasks, and the volume was brought up to the mark using methanol and water. Between 200 nm and 800 nm, drug solutions were scanned sequentially. Gentamicin sulphate has a maximum at 244 nm, while Curcumin has a peak at 420 nm. Before analysis, the solutions were filtered employing Wattmann filter paper

Linearity Study for Curcumin

Six separate 10 ml volumetric flasks were loaded with an accurately specified quantity of Curcumin working standard solution. To get concentrations (2-12g/ml), the volume was brought up to the mark with methanol. These solutions' absorbance was measured at 420 nm the absorbance Vs concentration calibration curve was constructed as illustrated in (fig. 1).

Linearity Study for Gentamicin sulphate

Six separate 10 ml volumetric flasks were filled with accurately measured aliquot amounts of Gentamicin sulphate working standard solution. To acquire concentrations (2-12g/ml), the volume was brought up to the mark using distilled water. These solutions' absorbance was measured at 244nm. The absorbance Vs concentration calibration curve was plotted as depicted in (Fig. 2).

Overlay Spectra of Gentamicin Sulphate and Curcumin

The overlain spectrum of both drugs (2 µg/ml) was recorded (Fig.5) and two wavelengths 420.0 nm (ë max of Curcumin) and 244 nm (ë max of Gentamicin sulphate) were selected for further study.

Linear regression analysis of the data attained 2-12 µg/ml which depicts highly linear relationship as the correlation coefficient (R^2) value were around 0.995for Curcumin and 0.993 for Gentamicin sulphate. Calibration Curve equation, Y= 0.0015x ±001 for Gentamicin sulphate and Y=0 .139x±002 for Curcumin at $ë_{max}$244nm for Gentamicin sulphate and 420 for Curcumin

Simultaneous Equation method

Curcumin and Gentamicin sulphate mixtures were weighed appropriately at 25mg each. The Gentamicin sulphate is first dissolved in water (5 ml), then transferred to a volumetric flask with a capacity of 50 ml, where the Curcumin is dissolved in a small amount of methanol (20 ml) using ultrasonication for about 10 minutes. The two solutions were then combined in a 50 ml volumetric flask, and the volume was increased to the desired level with methanol to create a mixed standard stock solution (100 mg/ml). To obtain a working standard solution of 100 g / ml, 5 ml of standard stock solution was diluted to 50 ml in methanol. In 10 ml volumetric flasks, an adequate amount of Gentamicin sulfate and Curcumin (2 ml) were transferred, and the volume was made up to the mark using the simultaneous equation approach (Vierodt's method), the concentrations of each component were calculated**.**

$A1 = ax_1bc_x + ay_1 bc_y$ _____ (at λ1–244nm)

$A2 = ax_2bc_y + ay_2bc_y$ ———————— (at λ2–420 nm)

Amount of each drug was estimated using following equations,

$$Cx = A2 \times ay1 - A1 \times \frac{ay2}{ax2} ay1 - ax1\ ay2$$

$$Cy = A1 \times ax2 - A2 \times ax1 / \ ax2\ ay1 - ax1\ ay2$$

A1= absorbance value(244nm)

A2= absorbance value (420nm)

ax_1= Absorptivity of Gentamicin sulphate at 244nm

ay_1= Absorptivity of Curcumin at 420nm

ax_2= Absorptivity of Gentamicin sulphate at 420nmn

ay_2= Absorptivity of Curcumin at 244nm

C1= concentration of Gentamicin sulphate in ìg/ml

C2= concentration of Curcumin in ìg/ml

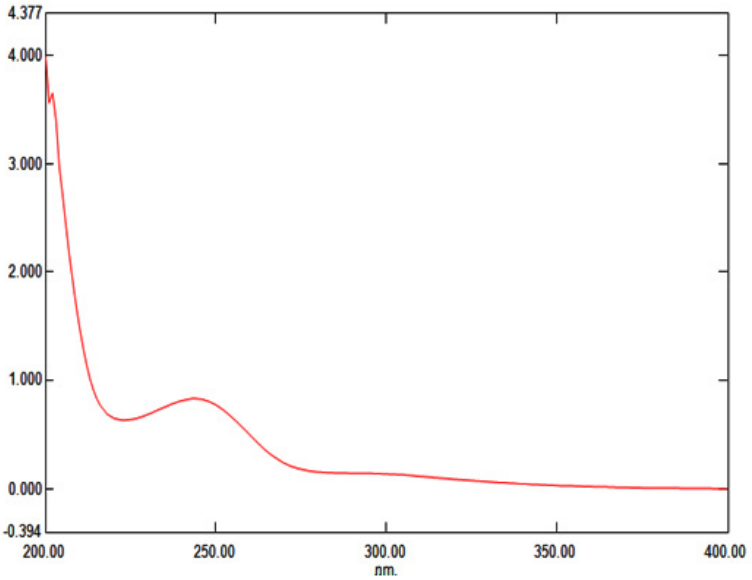
The Absorptivity values for simultaneous equation are enclosed in Table 2

Validation of Proposed Method

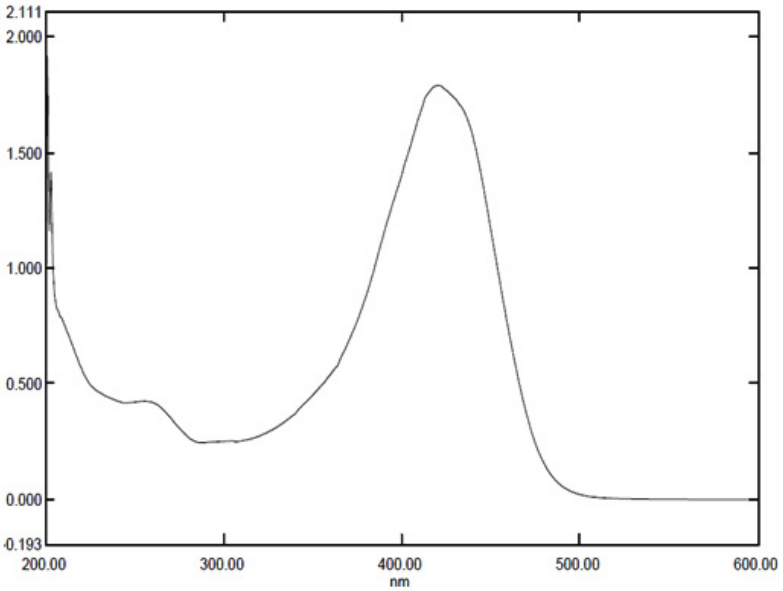
The Proposed method was validated as per the ICH guidelines

Accuracy

The closeness of agreement between the value regarded as a conventional true value and the value found is expressed by the accuracy of an analytical technique. It's a match between the value discovered and a previously agreed-upon reference value. [21] The method's accuracy was tested by making a 1 g/ml stock solution, then diluting it to 100 g/ml, then preparing 80 percent, 100 percent, and 120 percent dilutions and analyzing them at these varied levels. With a percent relative standard deviation (percent RSD) of less than 2.00 percent, it has outstanding reproducibility. Within the range stated in table no. 2, the accuracy was established.[22]



Graph 1. UV Spectra of Gentamicin Sulphate



Graph 2. UV Spectra of Curcumin

Precision

It is defined as the method's degree of repeatability under normal operating conditions. Three distinct concentrations of diluted solution (25 percent, 50 percent, and 75 percent) were evaluated at different time-points on the same day, and the study was repeated the next day. To validate the precision of the devised approach, SD and percent RSD values were computed. [24] Intermediate (inter-day) and repeatability (intra-day) investigations calculated it for three concentration levels (2, 4,6g/ml), encompassing the complete linearity range, and it was expressed as percent RSD. The results show that the procedure is extremely accurate. In Table No. 3, the precision values are tabulated.

Robustness

It is used as a parameter characterizing the stability of the method with respect to variations

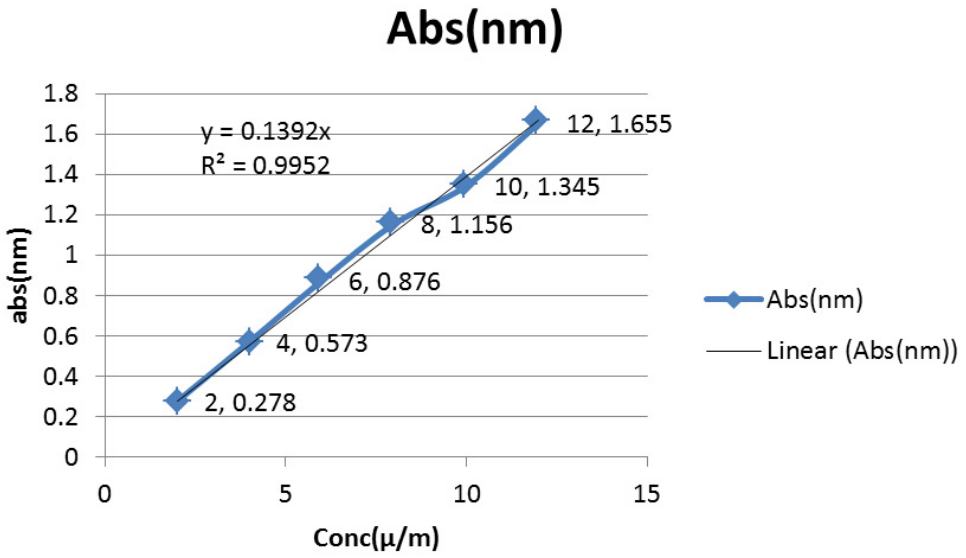


Fig. 1. Calibration curve of Curcumin

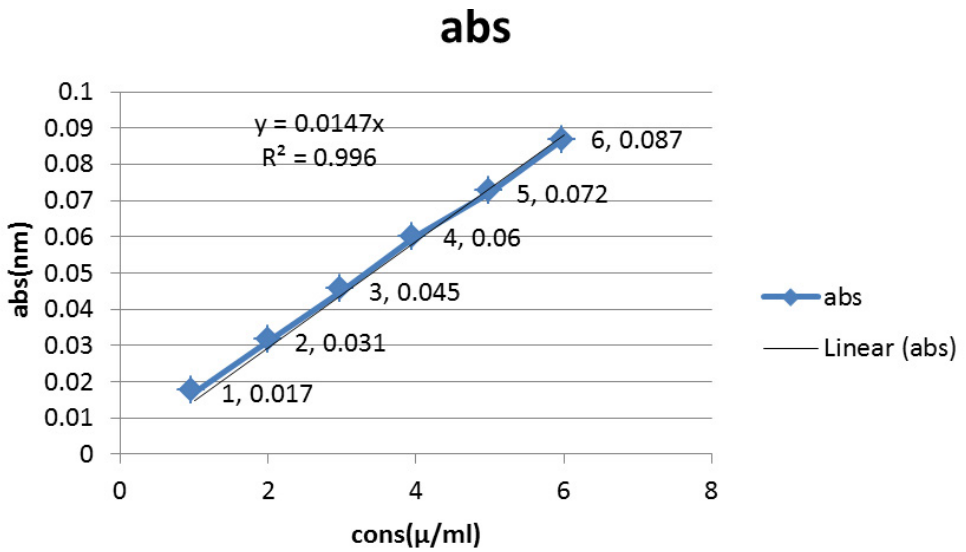


Fig. 2. Calibration curve of and Gentamicin sulphate

of the internal factors of the method.[25]It was examined by analyzing a drug concentration of 100µg/ml with minute changes in $\ddot{e}_{max}$ of both the drugs. It has ability to remain unaffected by changes in $\ddot{e}_{max}$ in analytical parameters. Conc. taken is 3µg/ml which was observed 3.01 in Gentamicin Sulphate and 3.09 in Curcumin by changing $\ddot{e}_{max.}$

Ruggedness

Spectrophotometric analysis of different concentrations equivalent to 25 %, 50 % and 75 % diluted solution was performed by two analysts assesses the ruggedness of the developed method. All analytical parameters are remaining unaffected even by performed by another analyst. As Conc. taken is 3µg/ml by both analyst and observed is 3.04 and 3.08

Sensitivity

LOD

The lowest concentration of analyte that can be recognized but not measured in a test sample. The lowest concentration of the standard

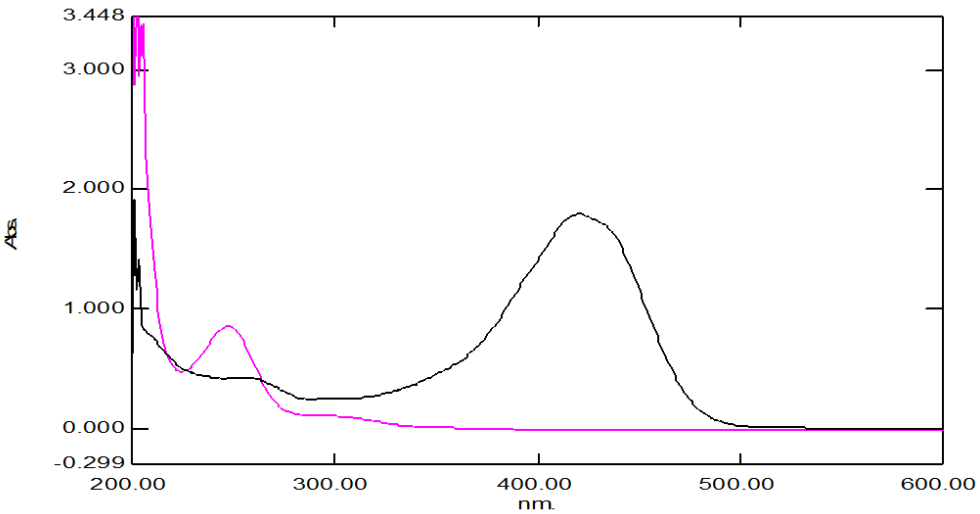
Table 1. Absorptivity Values for Simultaneous Estimation of Gentamicin sulphate and Curcumin

No	Drug	Parameters		Absorptivity	
1	Gentamicin sulphate	ax_1	ax_2	0	0.012
2	Curcumin	ay_1	ay_2	0.2485	0.061
3	Mixture	A1	A2	0.497	0.146

Each value is an average of three determinants.C1 and C2 were found to be 2 ìg/ml in mixture

Table 2. Accuracy Study

Accuracy Parameters		Curcumin			Gentamicin sulphate	
Initial amount(µg/ml)	3 µg/ml	3 µg/ml	3 µg/ml	2µg/ml	2µg/ml	2 µg/ml
Added amount (µg/ml)	2.4 µg/ml	3 µg/ml	3.6 µg/ml	1.6 µg/ml	2 µg/ml	2.4 µg/ml
%Recovery	98.1%	96%	98.4%	96.1%	98.3%	95.4%
%RSD	0.15%	0.14%	0.19%	0.23%	0.19%	0.21%



Graph 3. Overlay spectra of Gentamicin Sulphate and Curcumin

curve that can be computed with reasonable accuracy and precision is referred to as the LOQ. For LOQ22, the noise to signal ratio should be 1:10. Equation 1 has been used to calculate the values of LOD and LOQ.

$$LOD=3.3\sigma/S;\ LOQ=10\sigma/S$$

σ is standard deviation S is slope

The LOD and LOQ for Gentamicin sulphate in phosphate buffer were found to be 0.024µg/ml and 0.045µg/ml, respectively and values for Curcumin were 0.024µg/ml and 0.037 µg/ml, respectively which indicates adequate sensitivity of method

Table 3. Curcumin and Gentamicin Sulphate Precision Study

Curcumin Precision Study		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.18±0.017	0.53%
6 µg/ml	6.13±0.029	0.47 %
9 µg/ml	9.04±0.012	0.132 %
Intermediate Precision Evening (Day1)		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.06±0.026	0.84%
6 µg/ml	6.08± 0. 015	0.24%
9 µg/ml	9.19±0.019	0.20%
Intermediate Precision Morning Inter day (Day 11)		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.13± 0.027	0.86%
6 µg/ml	6.26±0.012	0.19%
9 µg/ml	9.28±0.016	0.17%
Gentamicin Sulfate Precision Study		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.07± 0.014	0.45%
6 µg/ml	6.29± 0.019	0.30 %
9 µg/ml	9.17± 0.011	0.11%
Intermediate Precision Evening (Day1)		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.24± 0.015	0.46%
6 µg/ml	6.19± 0.012	0.19
9 µg/ml	9.37±0.017	0.18
Intermediate Precision Morning Inter day (Day 11)		
Conc. taken	Conc. Observed± SD	RSD
3 µg/ml	3.30± 0.028	0.84%
6 µg/ml	6.24±0.019	0.30
9 µg/ml	9.24±0.014	0.15%

CONCLUSION

In first-order derivative spectroscopy, Curcumin and Gentamicin Sulphate have zero crossing points of 420 nm and 244 nm, respectively. The spectrophotometric technique used methanol and distilled water as the solvent. The linearity of Curcumin and Gentamicin sulphate was established throughout a concentration range of 2–12 g/ml, with correlation values of 0.995 and 0.996, respectively. Curcumin and Gentamicin Sulphate were reported to have mean percent recoveries of 98.88 percent and 98.54 percent, respectively. In both inter day and intraday testing, the method was proven to be repeatable. The method has been demonstrated to be accurate and dependable. The approach was successfully applied to pharmaceutical formulation with no influence from excipients, according to the recovery investigation. Recovery trials were used to examine the outcomes of the analysis, which were statistically evaluated. Gentamicin Sulphate LOD and LOQ in phosphate buffer were determined to be 0.024g/ml and 0.045g/ml, respectively, and Curcumin LOD and LOQ were 0.024g/ml and 0.037 g/ml, respectively, indicating appropriate sensitivity of the method.

Conflict of Interest

There are no conflict of interest.

Funding Sources

There is no funding sources.

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