

Research Article



Development and Validation of Spectrophotometric Method for Determination of Gentamicin and Curcumin in Bulk Powder

Rajput Pratima Nemu*, Anjana Adhyapak, V.S. Mannur, V.S.Mastiholimath, Tushar Powalkar

*Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi,
KLE Academy of Higher Education and Research, Nehrunagar, Belagavi, Karnataka, India.

*Corresponding author's E-mail: rajputpratima96@gmail.com

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ABSTRACT

Gentamicin is a class of broad spectrum fluoroquinolone antibiotic used as antibacterial agent and its antibacterial effect found to be enhance in presence of Curcumin. In the present research attempt has been to develop new analytical method for determination of Gentamicin and Curcumin in its bulk powder. UV-Visible Spectrophotometric method was developed by utilizing solvent system composed of water. Gentamicin and Curcumin showed maximum absorbance wavelength at 207 nm and 421 nm respectively. The method was optimized and validated in terms of specificity, selectivity, linear range, precision, robustness, ruggedness, reproducibility and solutions stability as per ICH guidelines. Analytes showed linear response between the concentration ranges of 2-10 μ g/mL for Gentamicin and 4-24 μ g/mL for Curcumin. Method was found to be precise, robust, rugged and reproducible with %RSD values less than 2%. Hence newly developed, optimized and validated UV-Spectrophotometric method was found to be simple, selective, specific, linear, precise, robust, rugged, reproducible and economical and can be used for simultaneous determination of Gentamicin and Curcumin in bulk drug combination.

Keywords: Antibacterial, Gentamicin, Curcumin, Spectrophotometric, Stability.

INTRODUCTION

Gentamicin is a broad spectrum amino glycoside type antibiotic that is isolated from *Micromonospora purpurea*. Gentamicin kills bacteria by damaging the plasma membrane and binding to the 16s ribosomal RNA, leading to the inhibition of microbial protein synthesis. It is effective against wide spectrum of gram positive and gram negative bacteria¹.

Gentamicin is not a single drug but in fact a mixture of number of minor components and three major components, gentamicin C1, C1a and C2. It is hydrophilic in nature and is distributed to body water. Moreover it is excreted as the same drug by kidneys, by glomerular filtration. In old age drug interaction due to increasing prevalence of polypharmacy, decreasing renal efficiency and reduced lean body weight are the major factors that may affect the pharmacokinetic of gentamicin².

Curcumin chemically, (1E, 6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione is major natural polyphenolic pigment present in the turmeric root (*Curcuma Longa*. Family, Zingiberacea) and used as spice, cosmetics and also as Indian traditional medicine. Biologically it has potent activities which include anticancer, anti-inflammatory and anti-amyloid activities³.

Synergistic antibacterial activities of Curcumin with antibiotic Gentamicin against *Staphylococcus aureus* have been reported by Teaw SY and Ali SA in their research work and they have concluded that Curcumin enhances the antibacterial effect of Gentamicin (Teaw SY *et al.*,

2015). This promotes us to carry out the research on development and validation of suitable analytical method for determination of Gentamicin and Curcumin in bulk drug combination. As in future there is need to develop and optimize a formulation containing both Gentamicin and Curcumin for their synergistic antibacterial effect and to analyze the drug contents in the formulation⁴.

Literature survey reviewed that Gentamicin were analyzed by UV-spectrophotometric⁵, HPLC⁶ methods also few UV-spectrophotometric⁷, RP-HPLC⁸ methods, also HPTLC⁹ methods reported for analysis of Curcumin in single and combination with other drugs in various pharmaceuticals and Ayurvedic dosage forms. But no analytical method was reported for simultaneous determination of Gentamicin and Curcumin. Hence in the present research work an attempt has been made to apply suitable validated analytical method for simultaneous determination of both analytes in bulk combination.

MATERIALS AND METHODS

Drug samples

Gentamicin having potency of 99.98% with batch No. 180411043 was obtained as gift sample from Shree Anand Life Sciences Ltd. Belagavi and Curcumin having potency 95% with batch no. RD/18398 was obtained from natural remedies, Bengaluru.



Reagents and chemicals

All the chemicals and reagents used for the experiment were pure and analytical grade and obtained from the store house of KLE College of Pharmacy, Belagavi.

Instruments and apparatus

UV-Spectrophotometer of Shimadzu make and 1800 model having UV probe software were used for analysis.

Method Development

Development of UV Spectrophotometric method was started with the selection of solvent system and determination of maximum wavelength of absorption of UV light. Solubility of Gentamicin and Curcumin was checked in various solvents by performing practically and also through the literature survey. Literature survey revealed that Gentamicin is soluble in water and Curcumin is soluble in methanol. In order to select single solvent system few trials were carried out in different solvents. Finally mixture of solvents containing Water was used and solutions containing both analytes were scanned between range of 800-200 nm and UV spectrum was obtained in single and combination. Gentamicin and Curcumin showed maximum absorbance wavelength of 207 nm and 421 nm respectively.

Method Validation

In order to prove the suitability of method, optimized method parameters were validated as per ICH guidelines (ICH guidance Q2A; Q2B) ¹⁰⁻¹¹:

Specificity and selectivity

Solutions containing Gentamicin and Curcumin were scanned between the range of 800-200 nm and spectrum was obtained and also spectrum of blank solvent was obtained and compared for any interference at maximum wavelength of absorbance of analytes by solvent.

Linear range response

10 mg of Gentamicin and Curcumin was weighed separately and transferred into 100 mL of volumetric flask. Curcumin was dissolved in methanol and Gentamicin was dissolved in water as first stock. Second serial dilutions were made with water for both Curcumin and Gentamicin as for Curcumin (4-24 µg/ml) and Gentamicin (2-12 µg/ml). The resulted solutions were prepared in triplicates and absorbance's was measured at 207 nm and 421 nm respectively for Gentamicin and Curcumin analytes.

Detection and Quantification limit of analytes

Limit of detection and quantification was calculated by using statistical calculations using following formulas.

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

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

Precision

Six replicates of solution containing Gentamicin and Curcumin were prepared and absorbance of each solution was measured at 207 nm and 421 nm respectively. On same day at different time intervals and on different days to obtained system precision, intraday precision and interday precision data and absorbance were measured and % RSD was calculated.

Precision at LOQ level

The precision at LOQ concentration of each analyte were performed. Six replicates of each analyte were prepared from standard stock solution at their LOQ concentration and absorbance of each was measured and %RSD calculated.

Ruggedness and Reproducibility

In order to prove ruggedness and reproducibility of method six replicates of solutions containing both analytes were prepared and absorbance's of each replicate solutions were measured by different analyst and also by using different instruments and %RSD was calculated for absorbance's obtained.

Solution and standard stock solution stability

In order to obtained the stability of solvent and stock solutions, fresh stock was prepared and dilutions were made using fresh solvent and absorbance's of each dilutions containing both analytes were compared with that of old stock dilutions and % RSD for absorbance's was calculated.

RESULTS AND DISCUSSION

Development

For the simultaneous estimation of Gentamicin and Curcumin the basic criterion is that, both analytes should be soluble in only single solvent system as we are analyzing both analytes simultaneously. Hence it was the most critical step in the method. Many trials were made and finally we have concluded that both analytes are soluble in water and hence it was chosen as the solvent. Detection wavelength for both analytes was obtained by scanning analytes solution in spectrophotometer. Spectrum showed the maximum absorbance's at 207 nm and 421 nm respectively for Gentamicin and Curcumin. Developed method parameters are presented in Table 1.

Table 1: Developed method parameters

Sr. No.	Parameters	Specifications
1	Analytes	Gentamicin and Curcumin
2	Solvent	Water
3	Max of Gentamicin	207 nm
4	Max of Curcumin	421 nm



Validation

Specificity and selectivity

Solvent spectrum obtained showed no interference of absorbance at maximum wavelengths of Gentamicin and Curcumin and both analytes selectively showed maximum wavelength at 207 nm and 421 nm respectively. Hence method was found to be specific and selective. UV spectrum of Gentamicin and Curcumin was showed in Fig. 1. and Fig. 2 respectively. UV-Spectrum of both analytes in combination was showed in Fig. 3. stability of 5 days (Table 7,8).

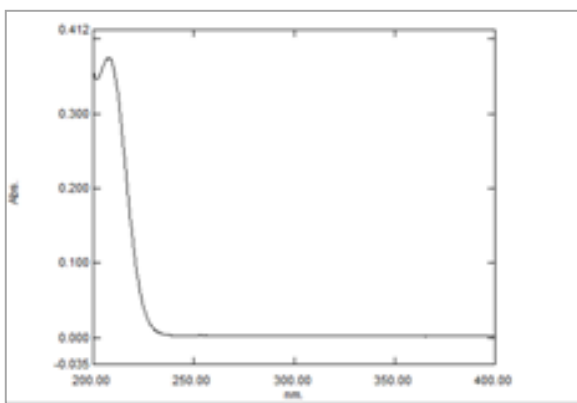


Figure 1: UV-Spectrum of Gentamicin

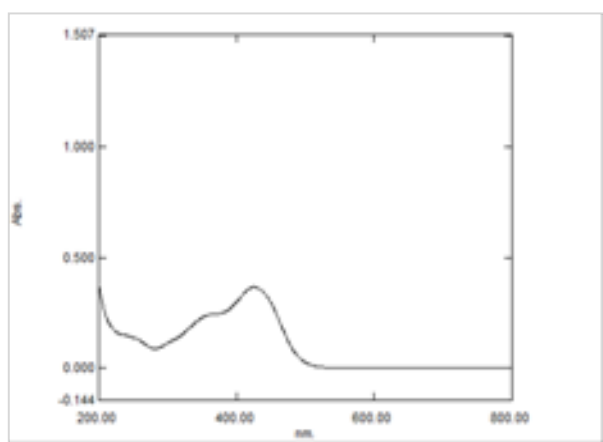


Figure 2: UV-Visible Spectrum of curcumin

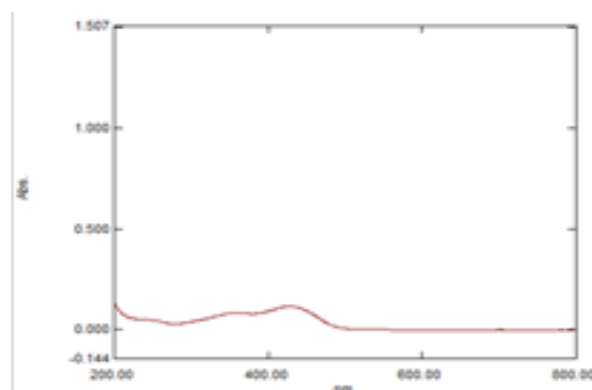


Figure 3: UV-Visible Spectrum of Gentamicin and Curcumin

Linearity and Range

Standard calibration curve was plotted using concentration vs absorbance's obtained by each linear dilution of both analytes. Each concentration showed linear absorbance's range between the concentration range of 4-24µg/mL for cucumin and 2-12 µg/mL for gentamicin with regression equation of 0.9998 and 0.9998 for Gentamicin and Curcumin respectively. Linearity range data was presented in Table 2, overlay spectrum of linearity of Gentamicin and Curcumin was showed in Fig. 4and standard calibration curve was presented in Fig.5 and Fig.6.

Detection and Quantification limit of analytes

LOD values of Gentamicin and Curcumin was found to be 0.39 µg/mL and 0.38 µg/mL. LOQ of values of Gentamicin and Curcumin was found to be 1.18 µg/mL and 1.16 µg/mL.

Precision

Method was found to be precise as the %RSD calculated for six replicates solution of both analytes at each precision level was found to be less than 2% and data were presented in Table 3, 4 and 5.

Table 2: Linearity and range data of Gentamicin and Curcumin

Sr. No.	Concentration Gentamicin	Absorbance's of Gentamicin (207 nm)	Concentration Curcumin	Absorbance's of Curcumin (421 nm)
1	2 µg/mL	0.151	4 µg/mL	0.134
2	4 µg/mL	0.273	8 µg/mL	0.253
3	6 µg/mL	0.399	12 µg/mL	0.392
4	8µg/mL	0.534	16 µg/mL	0.547
5	10 µg/mL	0.662	20 µg/mL	0.81
	r ²	0.9998	r ²	0.9998
	% Curve fitting	99.98%	% Curve fitting	99.98%
	LOD	0.39 µg/mL	LOD	0.38µg/mL
	LOQ	1.18 µg/mL	LOQ	1.16 µg/mL

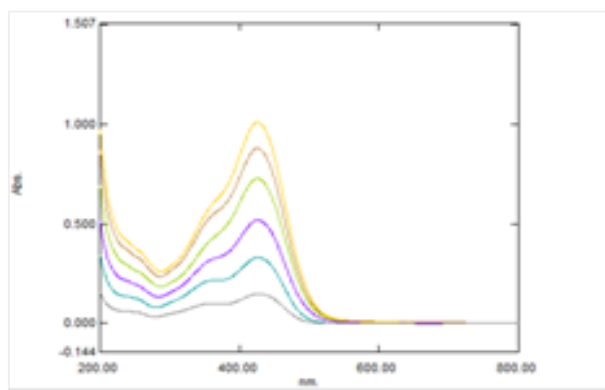


Figure 4: Linearity overlay spectrum of Gentamicin and Curcumin

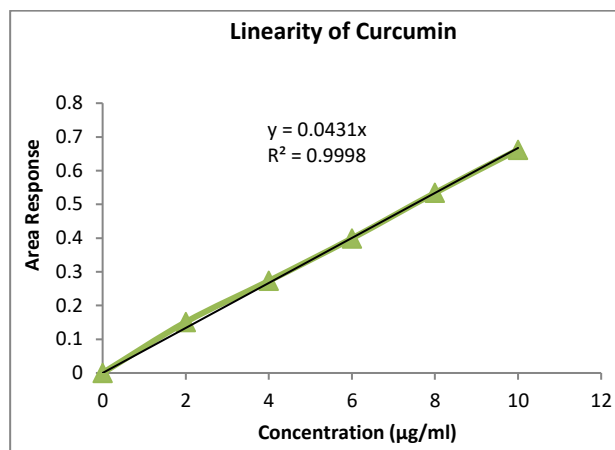


Figure 6: Standard calibration plot of curcuma

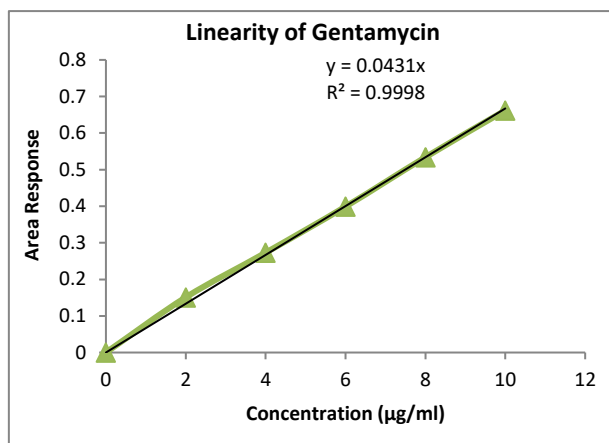


Figure 5: Standard calibration plot of Gentamicin

Table 3: System precision data of Gentamicin and Curcumin

Gentamicin (Conc)	Curcumin (Conc)	Gentamicin (Abs)	Curcumin (Abs)
12µg/mL	6µg/mL	0.350	0.468
12µg/mL	6µg/mL	0.341	0.460
12µg/mL	6µg/mL	0.357	0.480
12µg/mL	6µg/mL	0.348	0.468
12µg/mL	6µg/mL	0.340	0.465
12µg/mL	6µg/mL	0.349	0.462
%RSD		1.81%	1.51%

Table 4: Intraday Precision data of Gentamicin and Curcumin

Conc (Gent)	Conc (Cur)	Cucumin Morning	Gentamicin morning	Curcumin afternoon	Gentamicin afternoon	Curcumin Evening	Gentamicin Evening
16µg	8µg	0.659	0.508	0.620	0.487	0.619	0.443
16µg	8µg	0.664	0.517	0.626	0.475	0.605	0.445
16µg	8µg	0.677	0.507	0.636	0.490	0.630	0.429
16µg	8µg	0.645	0.509	0.635	0.498	0.633	0.447
16µg	8µg	0.658	0.525	0.621	0.492	0.626	0.453
16µg	8µg	0.679	0.531	0.607	0.495	0.634	0.441
% RSD		1.92	1.94	1.73	1.65	1.76	1.81

Table 5: Interday precision data of Gentamicin and curcumin

Precision	Interday-1		Interday-2		Interday-3		
Conc.(Gen)	Conc(Cur)	Gentamicin	Curcumin	Gentamicin	Curcumin	Gentamicin	Curcumin
12 µg/mL	6 µg/mL	0.394	0.494	0.360	0.464	0.336	0.449
12 µg/mL	6 µg/mL	0.397	0.497	0.367	0.478	0.329	0.425
12 µg/mL	6 µg/mL	0.399	0.497	0.358	0.482	0.330	0.444
12 µg/mL	6 µg/mL	0.390	0.480	0.351	0.492	0.339	0.445
12 µg/mL	6 µg/mL	0.389	0.486	0.362	0.477	0.342	0.446
12 µg/mL	6 µg/mL	0.398	0.490	0.368	0.480	0.332	0.444
%RSD		1.07%	1.37%	1.73%	1.89%	1.55%	1.95%

Ruggedness and Reproducibility

% RSD values obtained for each analytes was found to be less than 2% which indicates method was found to be rugged and reproducible as %RSD obtained for

absorbance’s of each replicate of solutions was within the acceptance by change in the analyst and instrument (Table 6).

Table 6: Ruggedness data of Gentamicin and curcumin

Ruggedness		By change in analyst		Change in the instrument	
Conc.(Gen)	Conc.(Cur)	Gentamicin	Curcumin	Gentamicin	Curcumin
12 µg/mL	6 µg/mL	0.355	0.467	0.278	0.453
12 µg/mL	6 µg/mL	0.353	0.477	0.276	0.467
12 µg/mL	6 µg/mL	0.350	0.479	0.274	0.449
12 µg/mL	6 µg/mL	0.354	0.473	0.286	0.452
12 µg/mL	6 µg/mL	0.342	0.463	0.280	0.472
12 µg/mL	6 µg/mL	0.345	0.482	0.273	0.458
% RSD		1.51%	1.54%	1.71%	1.99%

Solution and standard stock solution stability

%RSD for absorbance's obtained by fresh and old dilutions containing Gentamicin and Curcumin was found to be within the acceptance and data obtained showed

the standard stock solution and solvent system showed stability of three days. Data of solution stability were presented in Table 7.

Table 7: solution stability data of Gentamicin and curcumin

Gentamicin			Curcumin		
Concentration	Fresh Solution	Old solution	Concentration	Fresh solution	Old solution
6 µg/mL	0.350	0.340	12 µg/ml	0.446	0.446
6 µg/mL	0.357	0.348	12 µg/ml	0.450	0.450
6 µg/mL	0.345	0.345	12 µg/ml	0.459	0.459
% RSD	1.24%		% RSD	1.24%	

CONCLUSION

The present research concluded that, newly developed Spectrophotometric technique was found to be simple, specific, selective, linear, precise, robust, rugged and reproducible for the simultaneous determination of Gentamicin and Curcumin in its bulk powder combination.

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