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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CIPROFLOXACIN AND CURCUMIN IN BULK POWDER

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ABSTRACT: Ciprofloxacin is a class of broad-spectrum fluoroquinolone antibacterial agent and produces a synergistic effect in combination with Curcumin. In the present research work, we have developed a new analytical method for the determination of Ciprofloxacin and Curcumin in its bulk powder. The UV-spectrophotometric method was developed by utilizing a solvent system composed of methanol: water (50:50 v/v). Ciprofloxacin and Curcumin showed maximum absorbance wavelength at 275 nm and 430 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 a linear response between the concentration ranges of 1-6 µg/mL. This method was found to be precise, robust, rugged and reproducible with % RSD values less than 2%. Hence, a 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 Ciprofloxacin and Curcumin in bulk drug combination.

INTRODUCTION: Ciprofloxacin hydrochloride, chemically 1-cyclopropyl -6-fluoro-1, 4-dihydro-4-oxo-7 (1- piperazinyl)-3-Quinoline carboxylic acid hydrochloride is a class of fluoroquinolone antibiotic effective against both gram-positive and gram-negative bacteria [1]. It acts by inhibiting enzymes such as DNA gyrase, topoisomerase-II, topoisomerase-IV and interferes with the growth of bacteria [2]. It is used both in human and veterinary medicine to treat various infectious diseases such as infection of bones, joints, endocarditis, gastroenteritis, respiratory tract infections, cellulitis and urinary tract infections [3].

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Curcumin chemically, (1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-dioneis major natural polyphenolic pigment present in the turmeric root (*Curcuma longa*.). Biologically it has potent activities which include anti-septic, anti-bacterial, anti-cancer, anti-inflammatory and anti-amyloidal activities [4-5].

Synergistic antibacterial activities of Curcumin with antibiotic Ciprofloxacin 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 Ciprofloxacin [6]. This promotes us to carry out research on the development and validation of a suitable new analytical method for the determination of ciprofloxacin and curcumin in bulk drug combinations. Based on the above fact, there is a necessity to develop and optimize a formulation containing both ciprofloxacin and curcumin for their synergistic antibacterial effect

and to analyze the drug contents in the formulation. Literature survey reviewed that ciprofloxacin can be analyzed by UV-spectrophotometric [7], RP-HPLC and HPTLC [8] methods also few UV-spectrophotometric [9], RP-HPLC [10] and HPTLC [11] 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 ciprofloxacin and curcumin. Hence, in the present research an attempt has been made to develop and validate a new UV-Spectrometric analytical method for simultaneous determination of both analytes in bulk combination.

MATERIALS AND METHODS:

Drug Samples: Ciprofloxacin having potency of 99.98% was procured from Micro Labs Pvt. Ltd. Bengaluru. Curcumin having potency 95% was procured 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 storehouse of KLE College of Pharmacy, Belagavi.

Instruments and Apparatus: UV-Spectro-photometer of Shimadzu make and 1800 model having UV probe software were used for analysis.

Method Development: The development of UV spectrophotometric method was started with the selection of the solvent system and determination of the maximum wavelength of absorption of UV light. The solubility of ciprofloxacin and curcumin was checked in various solvents by performing practically and also through the literature survey.

A literature survey revealed that ciprofloxacin is soluble in water and curcumin is soluble in methanol. In order to select the solvent, few trials were carried out in different solvents. Finally, a mixture of solvents containing Methanol: Water (50:50% v/v) was used.

Solutions containing both analytes were scanned between the range of 800-200 nm and the UV spectrum was obtained in single and combination. Ciprofloxacin and curcumin showed maximum absorbance wavelength of 275 nm and 430 nm respectively.

Method Validation: In order to prove the suitability of method, optimized method parameters were validated as per ICH guidelines Q2A; Q2B [12-13].

Specificity and Selectivity: Solutions containing ciprofloxacin and curcumin were scanned between the range of 800-200 nm and the spectrum was obtained. A spectrum of blank solvent was obtained and compared for any interference at maximum wavelength of absorbance of analytes.

Linear Range Response: 10 mg of ciprofloxacin and curcumin was weighed separately and transferred into 10 mL of volumetric flask. Few mL of dimethylformamide was used to dissolve both drugs and volume was made up to the mark using a solvent to obtained 1000 µg/mL of analytes. From this stock serial dilutions were made to obtained 1, 2, 3, 4, 6 µg/mL solutions of ciprofloxacin and curcumin. The resulted solutions were prepared in triplicates and absorbance was measured at 275 nm and 430 nm respectively for ciprofloxacin and curcumin analytes.

Detection and Quantification Limit of Analytes: Limit of detection and quantification was calculated by using statistical calculations using the formula:

LOD = 3.3 × standard deviations of y-intercept / Slope of the calibration curve

LOQ = 10 × standard deviations of y-intercept / Slope of the calibration curve

Precision: Six replicates of a solution containing ciprofloxacin and curcumin were prepared and absorbance of each solution was measured at 275 nm and 430 nm respectively.

On the same day at different time intervals and on different days to obtained system precision, intraday precision and inter-day precision data and absorbance were measured and % RSD was calculated.

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

Robustness: Solvent system composed of Methanol: Water (50:50% v/v) was used for the dilutions. For the robustness analysis solvent system containing Methanol: Water (49:51% v/v) and Methanol: Water (51:49% v/v) was prepared and six replicates of solutions containing both analytes were prepared by using each solvent system and absorbance's was measured and % RSD was 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 study 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 was calculated.

RESULTS AND DISCUSSION:

Development: For the simultaneous estimation of ciprofloxacin and curcumin the basic criterion is that both analytes should be soluble in the same solvent 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 a mixture of methanol and water and hence it was chosen as the solvent. Detection wavelength for both analytes was obtained by scanning analytes solution in a spectrophotometer. The spectrum showed the maximum absorbance's at 275 nm and 430 nm respectively for ciprofloxacin and curcumin. The developed method parameters are presented in **Table 1**.

TABLE 1: DEVELOPED METHOD PARAMETERS

S. no.	Parameters	Specifications
1	Analytes	Ciprofloxacin and Curcumin
2	Solvent	Methanol: Water (50:50% v/v)
3	Maximum wavelength of Ciprofloxacin	275 nm
4	Maximum wavelength of Curcumin	430 nm

Validation

Specificity and Selectivity: Spectrum of solvent showed no interference of absorbance at maximum wavelengths of ciprofloxacin and curcumin and both analytes selectively showed maximum wavelength at 275 nm and 430 nm respectively. Hence method was found to specific and selective. UV-spectrum of ciprofloxacin and curcumin was showed in **Fig. 1** and **Fig. 2** respectively. UV-Spectrum of both analytes in combination were shown in **Fig. 3**.

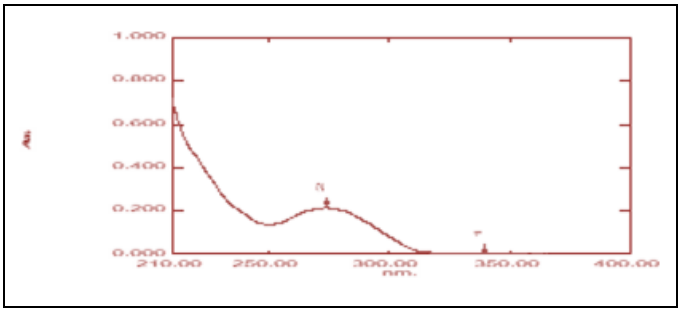

FIG. 1: UV-SPECTRUM OF CIPROFLOXACIN

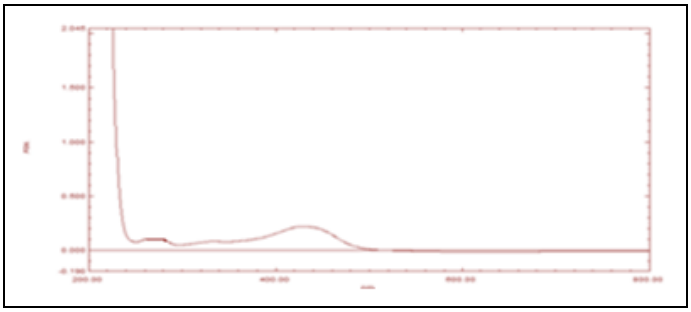

FIG. 2: UV-SPECTRUM OF CURCUMIN

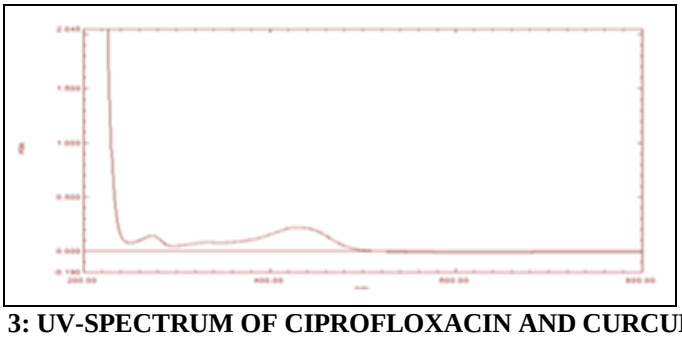

FIG. 3: UV-SPECTRUM OF CIPROFLOXACIN AND CURCUMIN

Linear Range Response: The standard calibration curve was plotted using concentration *vs.* absorbances obtained by each linear dilution of both analytes. Each concentration showed linear absorbance's range between the concentration range of 1, 2, 3, 4, 6 µg/mL with a regression equation of 0.997 and 0.998 for Ciprofloxacin and Curcumin respectively. Linearity range data was presented in **Table 2**, the overlay spectrum of linearity of ciprofloxacin and curcumin was showed in **Fig. 4** and a standard calibration curve was presented in **Fig. 5** and **Fig. 6**.

TABLE 2: LINEARITY AND RANGE DATA OF CIPROFLOXACIN AND CURCUMIN

S. no.	Concentration	Absorbance's of Ciprofloxacin (275 nm)	Absorbance's of Curcumin (430 nm)
1	1 µg/mL	0.126	0.134
2	2 µg/mL	0.256	0.253
3	3 µg/mL	0.366	0.392
4	4 µg/mL	0.482	0.547
5	6 µg/mL	0.777	0.81
	r^2	0.997	0.998
	% Curve fitting	99.80%	99.80%
	LOD	0.41 µg/mL	0.24 µg/mL
	LOQ	1.24 µg/mL	0.73 µg/mL

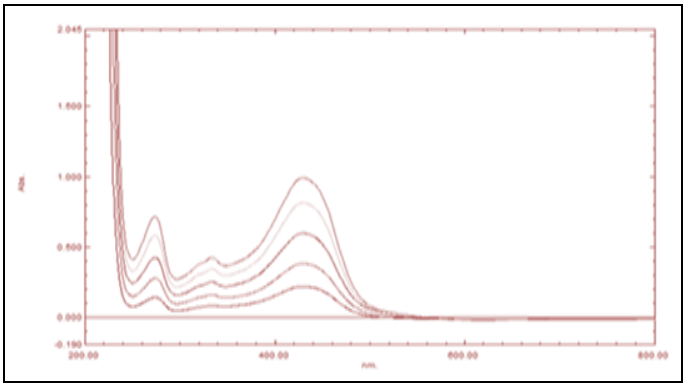

FIG. 4: LINEARITY OVERLAY SPECTRUM OF CIPROFLOXACIN AND CURCUMIN

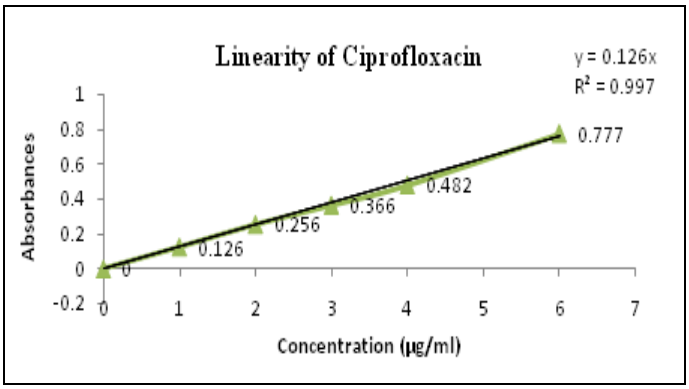

FIG. 5: STANDARD CALIBRATION PLOT OF CIPROFLOXACIN

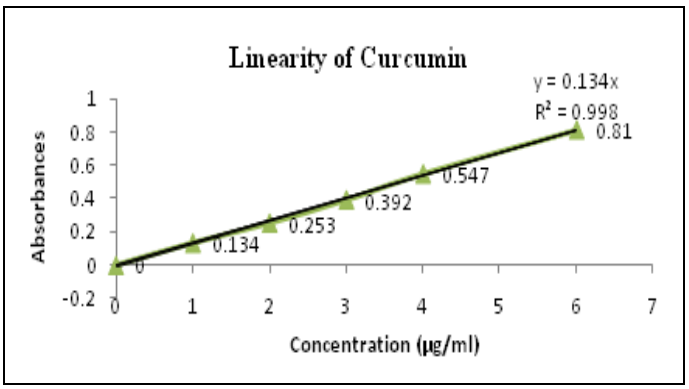

FIG. 6: STANDARD CALIBRATION PLOT OF CURCUMIN

Detection Quantification Limit of Analytes: LOD values of ciprofloxacin and curcumin were found to be 0.41 µg/mL and 0.24 µg/mL. LOQ of values of Ciprofloxacin and Curcumin was found to be 1.24 µg/mL and 0.73 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% **Table 3** and **4**. The data of Precision at LOQ level were shown in **Table 5**.

TABLE 3: SYSTEM AND INTRADAY PRECISION DATA OF CIPROFLOXACIN AND CURCUMIN

Precision	System precision		Intraday 1st h		Intraday 5th h	
Conc.	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin
4 µg/mL	0.454	0.571	0.459	0.553	0.453	0.547
4 µg/mL	0.453	0.569	0.46	0.554	0.456	0.547
4 µg/mL	0.456	0.568	0.459	0.552	0.459	0.55
4 µg/mL	0.45	0.569	0.455	0.551	0.452	0.547
4 µg/mL	0.451	0.57	0.456	0.548	0.451	0.546
4 µg/mL	0.452	0.57	0.452	0.548	0.452	0.545
%RSD	0.477%	0.184%	0.670%	0.459%	0.674%	0.306%

TABLE 4: INTERDAY PRECISION DATA OF CIPROFLOXACIN AND CURCUMIN

Precision	Interday-1		Interday-2		Interday-3	
Conc.	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin
3 µg/mL	0.371	0.373	0.395	0.388	0.372	0.369
3 µg/mL	0.381	0.379	0.392	0.386	0.37	0.358
3 µg/mL	0.38	0.379	0.382	0.381	0.369	0.36
3 µg/mL	0.385	0.384	0.374	0.378	0.369	0.359
3 µg/mL	0.385	0.384	0.385	0.383	0.368	0.358
3 µg/mL	0.391	0.383	0.387	0.37	0.369	0.358
%RSD	1.755%	1.124%	1.935%	1.693%	0.373%	1.199%

TABLE 5: PRECISION DATA OF CIPROFLOXACIN AND CURCUMIN AT LOQ CONCENTRATION

Ciprofloxacin		Curcumin	
Conc.	Abs	Conc.	Abs
1.24 µg/mL	0.101	0.73 µg/mL	0.097
1.24 µg/mL	0.098	0.73 µg/mL	0.099
1.24 µg/mL	0.103	0.73 µg/mL	1.101
1.24 µg/mL	0.101	0.73 µg/mL	0.102
1.24 µg/mL	0.101	0.73 µg/mL	0.099
1.24 µg/mL	0.102	0.73 µg/mL	0.100
%RSD	1.657%	%RSD	1.757%

Robustness, Ruggedness and Reproducibility: % RSD values obtained for each analytes was found to be less than 2% which indicates method was robust with slight change in the % composition of solvent system and also found to be rugged and reproducible as %RSD obtained for absorbance of each replicate of solution was within the acceptance by change in the analyst and instrument **Table 6** and **7**.

TABLE 6: ROBUSTNESS DATA OF CIPROFLOXACIN AND CURCUMIN

Robustness		Solvent composition 1		Solvent composition 1	
Replicates	Conc.	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin
1	5 µg/mL	0.629	0.691	0.626	0.683
2	5 µg/mL	0.628	0.692	0.618	0.682
3	5 µg/mL	0.628	0.691	0.611	0.674
4	5 µg/mL	0.627	0.691	0.615	0.679
5	5 µg/mL	0.627	0.691	0.616	0.68
6	5 µg/mL	0.625	0.693	0.615	0.682
%RSD		0.218%	0.121%	0.817%	0.483%

TABLE 7: RUGGEDNESS DATA OF CIPROFLOXACIN AND CURCUMIN

Ruggedness		By change in analyst		change in the instrument	
Replicates	Conc.	Ciprofloxacin	Curcumin	Ciprofloxacin	Curcumin
1	4 µg/mL	0.120	0.131	0.130	0.139
2	4 µg/mL	0.125	0.132	0.131	0.132
3	4 µg/mL	0.126	0.132	0.135	0.135
4	4 µg/mL	0.125	0.131	0.129	0.138
5	4 µg/mL	0.123	0.135	0.134	0.134
6	4 µg/mL	0.125	0.134	0.131	0.136
% RSD		1.767%	1.240%	1.776%	1.903%

TABLE 8: SOLUTION STABILITY DATA OF CIPROFLOXACIN AND CURCUMIN

Stability solutions		Ciprofloxacin		Curcumin	
Replicates	Conc.	Fresh	Old	Fresh	Old
1	4 µg/mL	0.129	0.126	0.139	0.137
2	4 µg/mL	0.132	0.128	0.141	0.135
3	4 µg/mL	0.128	0.127	0.142	0.138
%RSD		1.610%		1.862%	

Solution and Standard Stock Solution Stability: % RSD for absorbance obtained by fresh and old dilutions containing ciprofloxacin and curcumin were found to be within the acceptance and data obtained showed the standard stock solution and solvent showed stability for 5 days **Table 8**.

CONCLUSION: The present research concludes that a newly developed spectrophotometric method was found to be simple, specific, selective, linear, precise, robust, rugged and reproducible for the simultaneous determination of Ciprofloxacin and Curcumin in its bulk powder combination.

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CONFLICTS OF INTEREST: There are no conflicts of interest in the work presented in the manuscript.

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