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SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND METRONIDAZOLE IN BULK AND TABLET FORMULATION BY UV SPECTROPHOTOMETRY

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Keywords:

UV, Simultaneous equation method, Q-absorbance ratio method, Ciprofloxacin, Metronidazole

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ABSTRACT: Three simple and economical UV-spectrophotometric methods have been developed and validated for simultaneous estimation of ciprofloxacin (CIP) and metronidazole (MET) in a tablet dosage form using distilled water as a green solvent. The proposed methods were; simultaneous equation method (method A), Q-absorbance ratio method (method B), and area under curve method (method C). λ_{max} of CIP & MET in distilled water were found to be 271 nm and 320 nm, respectively. The isoabsorptive point was observed at 290 nm. The linearity was obtained in the concentration range of 1-9 µg/ml, and 2-18 µg/ml for CIP and MET respectively by methods A, B & C. Validation parameters were carried out. All three methods were found to be linear, accurate, precise, and specific. Good results were achieved using distilled water as solvent due to its greater solubility, reproducible readings with maximum absorbance. Among the three methods, method C was found to be the most sensitive. Hence, this method can be recommended for the routine analysis of this drug combination.

INTRODUCTION: Ciprofloxacin (CIP) is chemically 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid **Fig. 1**. It is a fluoroquinolone antibiotic useful for the treatment of various infections caused by Gram-positive, Gram-negative organisms and against *Mycobacterium tuberculosis*. The bactericidal action of CIP results from inhibition of the enzymes topisomerase 2 (DNA gyrase) and topisomerase 4, which are required for bacterial DNA replication, transcription repair, and recombination [1, 2]. Metronidazole (MET) is designated chemically as 2-(2-methyl-5-nitro-1*H*-imidazole-1-yl) ethan-1-ol **Fig. 2**.

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It is a prodrug unionized and the most useful antiprotozoal nitroimidazole derivative. It has been found to possess efficacy against obligate anaerobic bacteria due to their ability to intracellularly reduce MET to its active form, which then covalently binds to DNA, disrupts its helical structure, inhibiting the bacterial nucleic acid synthesis and results in bacterial cell death [3, 4].

A survey of literature has revealed several analytical methods for the determination of CIP in pharmaceutical dosage form and biological fluids, including spectrophotometry [5-9], spectrofluorimetry [10], HPLC [11-13], potentiometry [14], electrical micro-titration [15], and HPTLC [16]. CIP in admixtures with MET [17] and ampicillin has been determined by NMR [18]. HPLC methods either with fluorescence detection or coupled with mass spectrometry (LC/MS) for determination of CIP in human plasma [19, 20], and by SPE-UHPLC-PDA [21] have also been published. MET has been determined by several methods involving spectrophotometry [22]

and HPLC [23-26]. For CIP & MET, recently, more and more work has also been done on the use of HPLC-DAD [27] and ultra-performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) [28, 29]. So, far to the best of our knowledge, no UV spectrophotometric analytical method is available in the literature for simultaneously analyzing these two drugs by the area under curve method using distilled water as a green solvent. It was felt necessary to develop simple, precise, and rapid methods for quantitative determination of CIP and MET. Therefore, this paper describes the development and validation of methods to simultaneously quantify these two drugs by three simple UV spectrophotometric methods, *i.e.*, simultaneous equation method, Q-absorbance ratio method, and area under curve method in bulk and tablet formulation and to compare the results. The proposed methods were optimized and validated as per ICH guidelines [30].

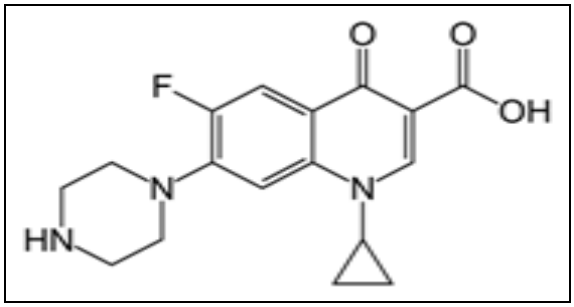

FIG. 1: STRUCTURE OF CIPROFLOXACIN

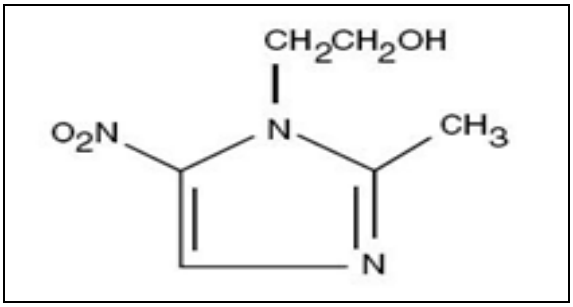

FIG. 2: STRUCTURE OF METRONIDAZOLE

MATERIALS AND METHODS:

Instruments: Absorbance measurements were made on double beam UV-visible spectro-photometer, model 1800, Shimadzu, Japan; with software UV Probe 2.10 and 1 cm matched quartz cells. Standard and sample drugs were weighed by keroy weigh balance (KRT 12HS). Ultrasonicator (1.5 l) was used to sonicate the standard and formulation samples.

Chemicals: Gift samples of active pharmaceutical ingredients CIP and MET were provided by MSN Labs, Hyderabad, Telangana, India, respectively. The formulation CIPFUN – M (CIP - 500 mg and MET - 250 mg) was purchased from Hanmakonda local market. Reagents like acetonitrile, methanol and distilled water used in analytical methods selection were of HPLC grade from Merck, Mumbai, India. Standard methanol and sodium hydroxide of desired normality were prepared.

Solubility Test: The solubility of CIP and MET were determined by dissolving the drugs in different solvents like methanol, distilled water, aqueous sodium hydroxide solution, dilute hydrochloric acid, *etc.* and solvent with a high degree of solubility is selected for experimental work. From the above solvents, distilled water is selected for analysis because both drugs were completely soluble.

Preparation of Standard Stock Solution: Standard stock solutions of both drugs were prepared separately by dissolving accurately weighed 10 mg of CIP and 10 mg of MET into 100 ml clean dry volumetric flasks.

20 ml of distilled water was added for complete dissolution, and the volume was made up to the mark with distilled water (stock solution). 5 ml of CIP and 5ml of MET were pipetted from the above stock solutions into 10 ml volumetric flasks and diluted up to the mark with distilled water.

Preparation of Working Standard Solutions: From the above stock solutions, desired concen-trations were prepared by transferring specific volume to separate 10 ml volumetric flasks and volume was made up to 10 ml with purified distilled water.

Sample Solution Preparation: Accurately weighed a quantity of tablet powder equivalent to 5 mg of CIP and MET samples into a 100 ml clean dry volumetric flask.

Added 20 ml of distilled water to dissolve it completely and made the volume up to the mark with distilled water (stock solution). It was sonicated for 15 min and filtered with Whatman filter paper. From the above filtrate, a volume of 10 ml of CIP and MET was pipetted into a 100 ml

volumetric flask and diluted up to the mark with distilled water to get 5 µg/ml sample solution.

Method Development:

Selection of Suitable Detection Wavelength: UV scan of CIP and MET was done individually, and both were overlayed upon each other to get the required wavelength. The maximum wavelength of CIP and MET were found to be 271 & 320 nm, respectively. The isoabsorptive point was found to be 290 nm **Fig. 3** and **Fig. 4**. These wavelengths were effective in the determination of both the drugs at a time.

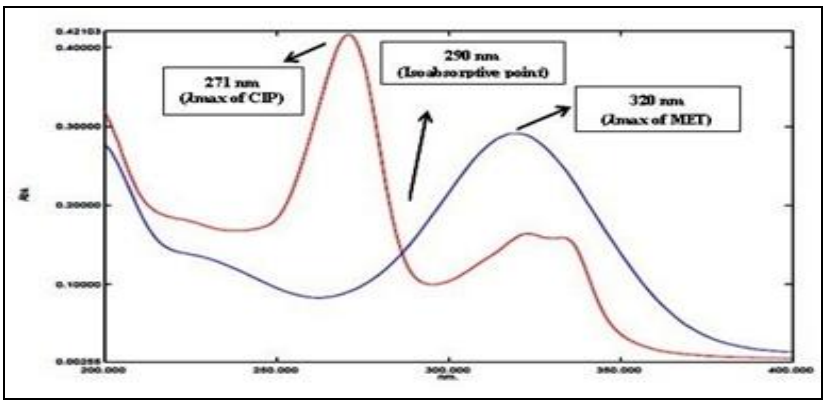

FIG. 3: OVERLAY SPECTRUM OF CIP AND MET

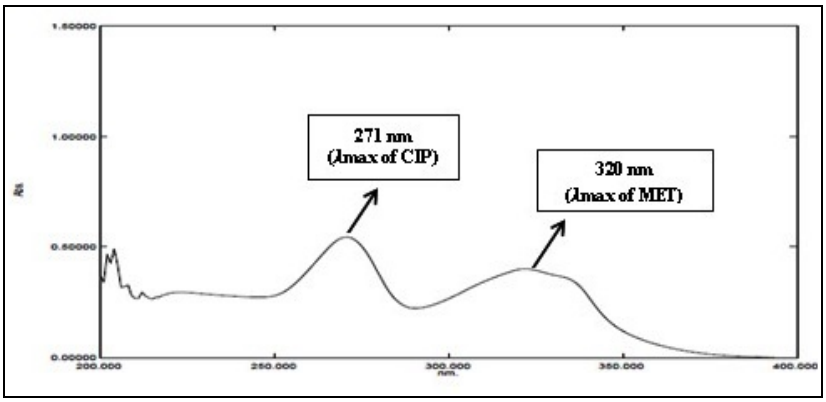

FIG. 4: OVERLAY SPECTRUM OF TABLET FORMULATION (CIP AND MET)

Simultaneous Equation Method (Method A): 1 µg/ml solutions of CIP and MET were prepared separately in distilled water, and the solutions were scanned against blank in the entire UV range to determine the λ_{max} values. Clear peaks were observed at 271 nm for CIP and 320 nm for MET. Hence these wavelengths were chosen as λ_{max} values for each drug, respectively. The absorbances were measured at both wavelengths. Standard solutions of CIP and MET in the concentration range 1- 5 µg/ml were prepared, and the absorbance of these solutions was measured at 271 and 320 nm. Calibration curves were plotted to verify Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. If a sample contains two absorbing drugs (X= CIP and Y=MET) each of which absorbs at the λ - max of the other (λ1 and λ2), it may be possible to determine both the drugs by the simultaneous equation method. Criteria for obtaining maximum precision is that below-mentioned ratio should lie outside the range 0.1-2.0

(A2/A1) / (ax2/aX1) and (aY2/aY1) / (A2/A1)

The information required is (I) the absorptivities of X at λ1 and λ2 are aX1and aX2 (II), the absorptivities of Y at λ1 and λ2 are aY1and aY2 (III), and the absorbance of the diluted sample at λ1 and λ2 are A1 and A2. Let Cx and Cy be the concentration of X and Y respectively in the sample. The absorbance of the mixture is the sum of the individual absorbances of X and Y. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm)

At λ1, A1= ax1bCx+ay1bCy …. (1)

At λ2, A2= ax2bCx+ay2 bCy……. (2)

For measurements in 1 cm cells b=1 Rearrange eq. (2)

$$Cy = A2 - ax2\ Cx\ / ay2$$

Substituting for Cy in eq (1) and rearranging

$$CX = (A1\ ay2 - A2\ ay1) / (ax1\ ay2 - ax2\ ay1)$$

$$Cy = (A2\ ax1 – A1\ ax2) / (ax1\ ay2 - ax2\ ay1)$$

Where, Cx and Cy are the concentrations of CIP and MET measured in gm/100 ml in sample solutions, A1 and A2 are absorbances of mixture at selected wavelengths 271 and 320 nm, respectively.

Q-absorbance Ratio Method / Q-Analysis (Method B): The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance, which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length.

For example, two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as the Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbencies are measured at two wavelengths, one being the λ_{max} of one of the components (λ2) and the other being wavelength of equal absorptivity of two components (λ1) *i.e.*, an isoabsorptive point.

A series of standard solutions of CIP and MET in the concentration range of 1-5 µg/ml were prepared, and the absorbance of these solutions was measured at 290 nm (isoabsorptive point).

Calibration curves were plotted to verify Beer's law and the absorptivity values calculated at the respective wavelength for both the drugs. The absorptivity values were reported. The concentration of two drugs in a mixture was calculated by using the following equations:

$$Cx = Qm – Qy / Qx – Qy \times A1 / ax1……5$$

$$Cy = Qm – Qx / Qy – Qx \times A1 / ay1….6$$

Where, Qm = A2/A1 Qx = ax2/ax1 Qy = ay2/ay1 A1 is absorbance of mixture at isobestic point *i.e.*, 290 nm. A2 is absorbance of mixture at 320 nm, λ_{max} of MET. Ax1 and ax2 represent absorptivities of CIP at 271nm & 320 nm. ay1and ay2 denotes absorptivities of MET at 271nm &320 nm. Cx and Cy are the concentration of CIP and MET.

Area Under Curve Method (Method C): The method C is used in case of the presence of broad spectra and absence of sharp peaks. This method is based on the calculation of the integrated value of absorbance between the selected two wavelengths.

This wavelength range is chosen depending on several trials performed to obtain a good linear relationship between area under curve and concentration. This method utilizes two wavelength ranges.

From the overlain spectra of both the drugs, the area under the curve is determined at both selected wavelength ranges 250-285 nm for CIP & 306-336 nm for MET within the above-selected wavelength ranges, the area under curve was determined for both drugs (CIP & MET-10 µg/ml & 16 µg/ml) and sample solutions were analyzed and concentration of CIP & MET in sample solution were calculated using "Cramel's Rule" and "Matrix method".

$$CM = X^{N}_{\lambda1\text{-}\lambda2}\ AUC_{\lambda3\text{-}\lambda4} - X^{N}_{\lambda3\text{-}\lambda4}\ AUC_{\lambda1\text{-}\lambda2} / X^{N}_{\lambda1\text{-}\lambda2}\ X^{M}_{\lambda3\text{-}\lambda4} - X^{N}_{\lambda3\text{-}\lambda4}\ X^{M}_{\lambda1\text{-}\lambda2}$$

$$CN = X^{M}_{\lambda1\text{-}\lambda2}\ AUC_{\lambda3\text{-}\lambda4} - X^{M}_{\lambda1\text{-}\lambda2} AUC_{\lambda1\text{-}\lambda2} / X^{N}_{\lambda1\text{-}\lambda2}\ X^{M}_{\lambda3\text{-}\lambda4} - X^{N}_{\lambda3\text{-}\lambda4}\ X^{M}_{\lambda1\text{-}\lambda2}$$

Where, C^M&C^N: concentration of components M and N in a sample. $AUC^{M}_{\lambda1\text{-}\lambda2}$: area under the curve for component M at wavelength ranges λ1-λ2 $AUC^{M}_{\lambda3\text{-}\lambda4}$: area under the curve for component M at wavelength ranges λ3-λ4 $AUC^{N}_{\lambda1\text{-}\lambda2}$: area under the curve for component N at wavelength ranges λ1-λ2 $AUC^{N}_{\lambda3\text{-}\lambda4}$: area under the curve for component N at wavelength ranges λ3-λ4.

$$X_{\lambda1\text{-}\lambda2} = AUC_{\lambda1\text{-}\lambda2} / \text{Conc. in g/l}$$

$$X_{\lambda3\text{-}\lambda4} = AUC_{\lambda3\text{-}\lambda4} / \text{Conc. in g/l}$$

$$AUC_{\lambda1\text{-}\lambda2} = AUC^{M}_{\lambda1\text{-}\lambda2} + AUCN_{\lambda1\text{-}\lambda2}$$

$$AUC_{\lambda3\text{-}\lambda4} = AUC^{M}_{\lambda3\text{-}\lambda4} + AUCN_{\lambda3\text{-}\lambda4}$$

The overlay spectrum of CIP and MET by the area under the curve method was presented in **Fig. 5** and **Fig. 6**.

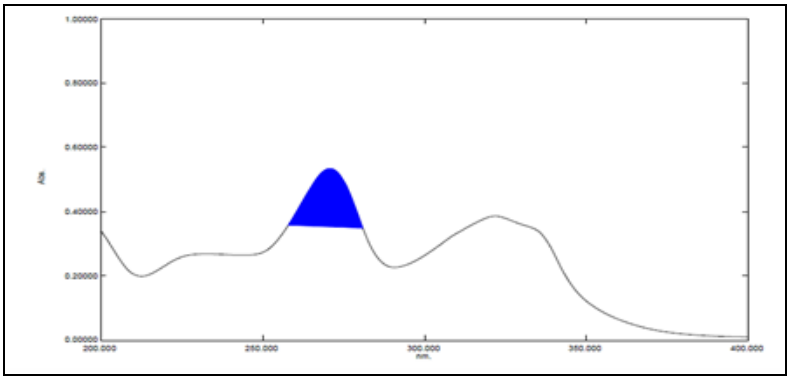

FIG. 5: OVERLAY SPECTRUM OF AREA UNDER CURVE SAMPLE FORCIP

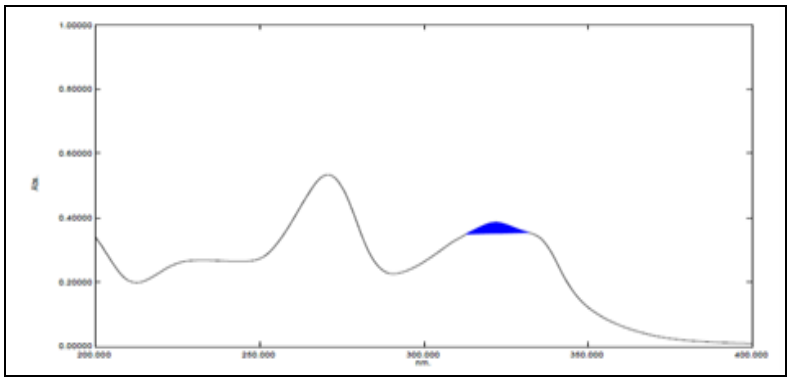

FIG. 6: OVERLAY SPECTRUM OF AREA UNDER CURVE SAMPLE FOR MET

Validation of Proposed Methods (Methods A, B and C): The method was validated according to ICH guidelines.

Linearity: Accurately weigh and transfer 10 mg of CIP and 10 mg of MET working standard into a 100 ml clean dry volumetric flask. Add about 20 ml of distilled water to dissolve it completely and make the volume upto the mark with distilled water to get 100 µg/ml of stock solution. The method was validated for linearity by taking standard in level–I, level–II, level–III, level–IV, level–V (Linearity results for CIP and MET). Linearity was plotted among absorbance and concentration, and the response was found to be linear over the range of 1-10 µg/ml for CIP and 2- 20 µg/ml for MET of targeted concentration.

Preparation of level–I (1 ppm of CIP & 2 ppm of MET), level–II (2 ppm of CIP & 4 ppm of MET), level–III (3 ppm of CIP & 6 ppm of MET), level–IV (4 ppm of CIP & 8 ppm of MET) level–V (5 ppm of CIP & 10 ppm of MET) was carried out. Then absorbance was measured at three wavelengths. A graph of absorbance versus concentration in µg/ml (on X-axis concentration and Y-axis Peak absorbance) was plotted and calculated the correlation coefficient.

Accuracy (Recovery Studies): It is the closeness of agreement between the true value and found value is expressed as % recovery. It is determined by calculating % recovery. To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of CIP and MET at 80 %, 100 %, and 120 % of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the % recovery. Recovery samples are prepared in triplicate at each level, the samples at different levels and the percentage mean recovery for CIP and MET were calculated by using the formula

$$\% \text{ Recovery} = \text{Amount found/amount taken} \times 100$$

The absorbance for standard solution, Accuracy-80 %, Accuracy-100 %, Accuracy-120 % solutions was measured. Calculated the amount found added for CIP & MET and calculated the individual recovery and mean recovery values**.**

Precision: Precision is the degree of repeatability of the analytical method under normal operational conditions. The repeatability, inter-day precision, and intraday precision were studied by comparing

the assays by performing replicates of six on the same day (morning, afternoon, evening) and three different days, respectively. The results documented as standard deviation and % RSD.

$$\% \text{ RSD} = \text{SD/Mean} \times 100$$

Where, SD is the standard deviation.

Precision studies were performed in triplicate at three different concentration levels covering the entire linearity range for CIP (1, 5, 9 µg/ml) and for MET (2, 10, 18 µg/ml).

Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample, which can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using the calibration curve.

The formulae for LOD & LOQ are:

$$\text{LOD} = 3.3\ \sigma / S$$

$$\text{LOQ} = 10\ \sigma / S$$

Where, σ is mean standard deviation of y-intercepts of regression lines, S is slope of the standard curve.

RESULTS AND DISCUSSION: In the present work, we developed three UV spectroscopic methods and validated them as per ICH guidelines. UV-spectrophotometric methods have superiority over HPLC methods for being simple, rapid, cost-effective, and time-saving. Regression analysis of the Beer plots showed a good correlation in the concentration ranges of 1.0–9.0 µg/ml (CIP) & 2–18 µg/ml (MET) for methods A, B, and C using distilled water as a universal green medium **Tables 1, 2** and **3 Fig. 7** to **Fig. 12**. Results were found to be linear in the concentration range of level I, II, III, IV, and V of CIP and MET with r^2 values for methods A, B, and C of 0.998, 0.997,0.997 (CIP), and 0.998,0.996,0.998 (MET), respectively.

Regarding method A, the highest % recoveries for CIP and MET were found to be 99.75 % and 94.5 %, respectively. For method B, the highest % recoveries for CIP and MET were found to be 99.6 % and 100 %, respectively. In method C, the highest % recoveries for CIP and MET were 103.5 % and 104.8 %,, respectively **Table 4**. The interday and intraday precision for CIP and MET were found to be <2% in **Table 5, 6, 7,** and **8.**

The LOD and LOQ for CIP and MET results are shown in **Table 9**. Amongst the three methods, the lowest LOD and LOQ values for CIP and MET were observed in method C. The LOD and LOQ values obtained by method B for CIP (0.09 µg/ml and 0.28 µg/ml) and MET (0.07µg/ml and 0.22µg/ml) were found to be less than the earlier reports on their simultaneous estimation method by Q-absorbance ratio method in methanol. The validation parameters of CIP and MET are summarized in **Tables 10** and **11**, respectively. The developed methods have several advantages over the earlier reported spectrophotometric methods for simultaneous determination of CIP and MET, like the area under curve method (method C) is simpler, sensitive, accurate, and precise. The novelty of the method lies in making use of an economical green solvent like distilled water instead of using methanol, as reported in the previous method.

TABLE 1: LINEARITY RESULTS FOR CIP AND MET BY METHOD A

S. no.	CIP		MET	
	Concentration (µg/ml)	Absorbance*	Concentration (µg/ml)	Absorbance*
01	1	0.108±0.001	2	0.127±0.001
02	2	0.213±0001	4	0.228±0.001
03	3	0.292±0.003	6	0.345±0.001
04	4	0.394±0.002	8	0.448±0.003
05	5	0.472±0.001	10	0.564±0.001
06	6	0.581±0.002	12	0.676±0.003
07	7	0.675±0.001	14	0.811±0.004
08	8	0.764±0.002	16	0.921±0.001
09	9	0.897±0.002	18	0.997±0.003

*Data represents mean ± SD (n=3).

TABLE 2: LINEARITY RESULTS FOR CIP AND MET BY METHOD B

S. no.	CIP		MET	
	Concentration (µg/ml)	Absorbance*	Concentration (µg/ml)	Absorbance*
01	1	0.030±0.002	2	0.101±0.003
02	2	0.053±0.001	4	0.156±0.001
03	3	0.074±0.002	6	0.224±0.002
04	4	0.094±0.001	8	0.287±0.004
05	5	0.118±0.003	10	0.335±0.003
06	6	0.138±0.001	12	0.401±0.001
07	7	0.154±0.002	14	0.457±0.002
08	8	0.182±0.002	16	0.536±0.002
09	9	0.201±0.001	18	0.586±0.002

*Data represents mean ± SD (n=3).

TABLE 3: LINEARITY RESULTS FOR CIP AND MET BY METHOD C

S. no.	CIP		MET	
	Concentration (µg/ml)	Absorbance*	Concentration (µg/ml)	Absorbance*
01	1	1.120±0.002	2	0.321±0.001
02	2	1.733±0.001	4	0.545±0.002
03	3	2.652±0.002	6	0.863±0.003
04	4	3.374±0.001	8	1.143±0.001
05	5	4.321±0.001	10	1.457±0.001
06	6	4.987±0.003	12	1.748±0.002
07	7	5.732±0.001	14	2.129±0.001
08	8	6.565±0.002	16	2.326±0.002
09	9	7.719±0.001	18	2.569±0.001

*Data represents mean ± SD (n=3).

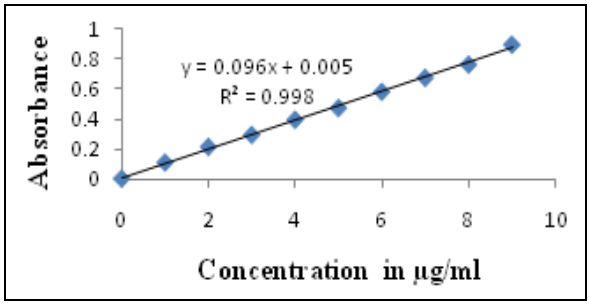

FIG. 7: LINEARITY GRAPH FOR CIP (A)

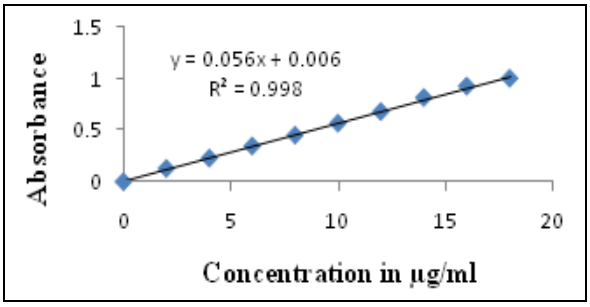

FIG. 8: LINEARITY GRAPH FOR MET (A)

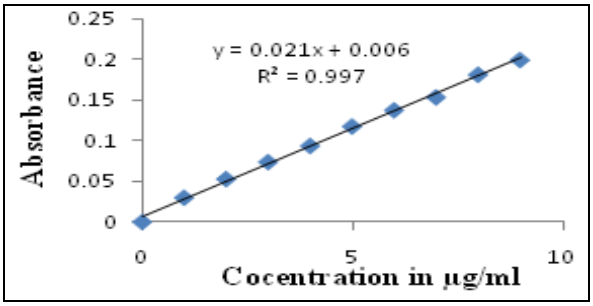

FIG. 9: LINEARITY GRAPH FOR CIP (B)

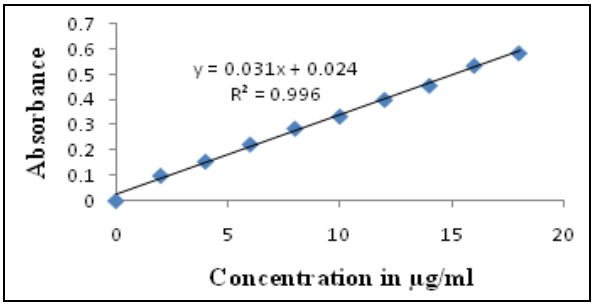

FIG. 10: LINEARITY GRAPH FOR MET (B)

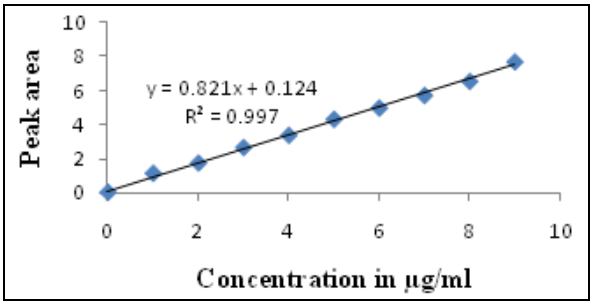

FIG. 11: LINEARITY GRAPH FOR CIP (C)

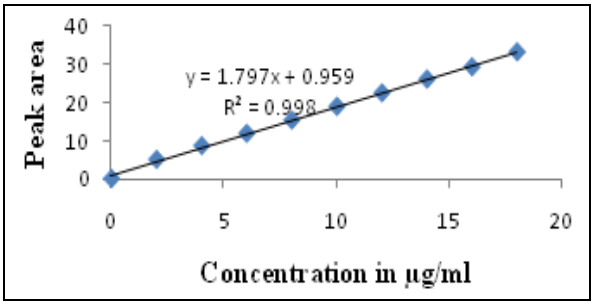

FIG. 12: LINEARITY GRAPH FOR MET (C)

TABLE 4: % RECOVERIES OF CIP AND MET FOR METHOD A, B AND C.

Method	CIP			MET		
	Amount Added (µg/ml)	Amount Found* (µg/ml)	% Recovery*	Amount Added (µg/ml)	Amount Found* (µg/ml)	% Recovery*
Method A	4	3.99±0.01	99.75±0.212	4	3.11±0.03	77.75±0.510
	5	4.93±0.02	98.6±0.238	5	4.01±0.02	80.2±0.420
	6	4.92±0.02	82.9±0.241	6	5.67±0.01	94.5±0.411
Method B	4	3.01±0.03	75.25±0.422	4	3.07±0.02	76.75±0.530
	5	4.98±0.02	99.6±0.002	5	4.88±0.02	97.6±0.328
	6	5.67±0.03	94.5±0.246	6	6.00±0.01	100±0.120
Method C	4	3.22±0.02	80.5±0.502	4	4.04±0.03	101±0.110
	5	4.29±0.02	85.8±0.438	5	5.24±0.04	104.8±0.320
	6	6.21±0.01	103.5±0.322	6	5.90±0.03	98.33±0.239

*Values given in the table are the mean ± SD of three replicate experiments.

TABLE 5: INTRADAY PRECISION FOR CIP

Standard (CIP)	Method A* (271 nm)	Method B* (290 nm)	Method C* (250-285 nm)
Sample–1	0.4134±0.0008	0.1623±0.0006	6.1109±0.0006
Sample–2	0.4143±0.0009	0.1638±0.0008	6.0103±0.0007
Sample–3	0.4123±0.0006	0.1632±0.0006	6.1064±0.0006
%RSD	0.19	0.38	0.76

*Values given in the table are the mean ± SD of six observations.

TABLE 6: INTRADAY PRECISION FOR MET

Standard (CIP)	Method A* (320 nm)	Method B* (290 nm)	Method C* (306-336 nm)
Sample–1	0.6013±0.0049	0.1508±0.1516	2.1214±0.0095
Sample–2	0.6129±0.0080	0.1527±0.1606	2.1415±0.099
Sample–3	0.6042±0.0090	0.1514±0.0577	2.1215±0.0069
%RSD	0.81	0.50	0.44

*Values given in the table are mean ± SD of six observations.

TABLE 7: INTERDAY PRECISION FOR CIP

Standard (CIP)	Method A* (271nm)	Method B* (290 nm)	Method C* (250-285 nm)
Sample–1	0.3247±0.0019	0.1341±0.0007	4.3075±0.0038
Sample–2	0.3256±0.0055	0.1342±0.0008	4.3035±0.0040
Sample–3	0.3212±0.0045	0.1356±0.0006	4.3129±0.0047
%RSD	0.58	0.52	0.08

*Values given in the table are mean ± SD of six observations.

TABLE 8: INTERDAY PRECISION FOR MET

Standard (CIP)	Method A* (320 nm)	Method B* (290 nm)	Method C* (306-336 nm)
Sample–1	0.5243±0.0013	0.7023±0.0001	1.6028±0.0045
Sample–2	0.5226±0.0030	0.7095±0.0003	1.6111±0.0056
Sample–3	0.5210±0.0004	0.7119±0.0016	1.6023±0.0077
%RSD	0.25	0.57	0.24

*Values given in the table are mean ± SD of six observations.

TABLE 9: LOD & LOQ FOR CIP AND MET

Method	CIP		MET	
	LOD (µg/ml)	LOQ (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
Method A	0.15	0.16	0.07	0.23
Method B	0.09	0.28	0.07	0.22
Method C	0.01	0.04	0.02	0.09

TABLE 10: VALIDATION RESULTS FOR CIP

S. no.	Parameter	Method A	Method B	Method C
01	Wavelength (nm)	271	290	250-285
02	Linearity (µg/ml)	1-10	1-10	1-10
03	Regression equation	y= 0.96x+0.005	y=0.021x+0.006	y=0.821x+0.124
04	(y = mx + c)*			
	Correlation coefficient (r2)	0.998	0.997	0.997
05	Intraday precision (% RSD)	0.19	0.38	0.76
06	Interday precision (% RSD)	0.58	0.52	0.08
07	% Recovery (Accuracy)	99.75	99.6	103.5
08	LOD (µg/ml)	0.15	0.09	0.01
09	LOQ (µg/ml)	0.16	0.28	0.04

*Where y = absorbance and x = concentration, y = mx + c

TABLE 11: VALIDATION RESULTS FOR MET

S. no.	Parameter	Method A	Method B	Method C
01	Wavelength (nm)	320	290	306-336
02	Linearity (µg/ml)	2-20	2-20	2-20
03	Regression equation	y=0.056x+0.006	y=0.031x+0.024	y=1.727x+0.959
04	(y = mx + c)*			
	Correlation coefficient (r2)	0.998	0.996	0.998
05	Intraday precision (% RSD)	0.81	0.50	0.44
06	Interday precision (% RSD)	0.25	0.57	0.24
07	% Recovery (Accuracy)	94.5	100	104.8
08	LOD (µg/ml)	0.07	0.07	0.02
09	LOQ (µg/ml)	0.23	0.22	0.09

*where y = absorbance and x = concentration, y = mx + c

CONCLUSION: The two-drug combination: CIP/MET which is used for the treatment of systematic infections, can be simply analyzed by the adopted rapid, accurate, precise, and specific spectrophotometric methods through simultaneous equation, Q-absorbance ratio, an area under a curve and validated as per ICH guidelines. The developed spectrophotometric methods are considered to be suitable for application in quality control units where economy and time are very important.

The proposed spectrophotometric methods can estimate each drug without interference from the other without prior separation in comparison to HPLC methods which may require some effort to reach to the best chromatographic conditions for their separation. As compared to HPLC methods which require preliminary preparations for the chromate-graphic system before a run, the suggested spectrophotometric methods are considered to be time-saving techniques. Also, they are considered to be more economical where there is no need for expensive solvents in comparison to other reported chromatographic methods, distilled water being the only solvent used throughout the whole experiment. Further, sophisticated equipment is not needed, but instead, simple software programs are used. All the proposed methods were found to be linear, accurate, cost-effective, precise, specific, simple, and practical. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Amongst the three methods, the area under the curve (method C) was found to be most sensitive. Hence this method can be recommended for the routine quality control analysis of this drug combination.

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