SENNOS

Journal of Drug Delivery and Biotherapeutics

Journal homepage: https://sennosbiotech.com/JDDB/1

Research Article

Development and Validation of Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Ciprofloxacin and Ornidazole; in Combined Pharmaceutical Dosage Form

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ARTICLEINFO

ABSTRACT

The present research work demonstrates an analytical method development for simultaneous estimation of Ciprofloxacin and Ornidazole in combined dosage form using Q-absorbance ratio concept. While method development, two different wavelengths one representing iso-absorptive point (290 nm) and other representing the λ_{max} of Ornidazole (311 nm) were used. Optimum response was obtained in solvent system that comprises water and methanol in ratio of 80:20 v/v. Proposed UV method was found to be linear over the concentration range of 1-12 µg/ml for Ciprofloxacin and that of 1-20 µg/ml for Ornidazole. Based on recovery studies after standard addition, accuracy of proposed method was found to be in between 99.54 to 100.24 and 99.53 to 100.04% for Ciprofloxacin and Ornidazole respectively. Intra-day precision of the method in terms of % relative standard deviation was found to be in between 0.15 to 0.83 and 0.10 to 1.34 for Ciprofloxacin and Ornidazole respectively. Inter-day precision range of the method for Ciprofloxacin and Ornidazole was found to be in between 0.12 to 0.83 and 0.10 to 1.34 respectively. LOD and LOQ of proposed UV method were 0.01037µg/ml and 0.03142µg/ml for Ciprofloxacin and 0.01929µg/ml and 0.05848µg/ml for Ornidazole. Proposed UV method was robust and rugged in nature. Proposed method was successfully used for the estimation of Ciprofloxacin and Ornidazole contents of marketed formulation consisting of APIs and the common excipients.

Keywords: UV- visible spectrometry; Q absorbance ratio; Analytical Validation; Ciprofloxacin; Ornidazole

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Received date: 01-May-2024 Revised date: 21-May-2024, Accepted date: 01-Jun-2024

Crossref DOI: https://doi.org/10.61920/jddb.v1i01.32

1. Introduction

Ciprofloxacin is a broad spectrum anti-biotic active against gram+ve and gram–ve bacteria. It acts by inhibiting the enzymes DNA gyrase and to po-isomerase which are essential for bacterial replication. It is mainly used in the infections of urinary tract, GI tract and skin tissues by bacteria. Chemically ciprofloxacin is 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid (Figure. 1.)

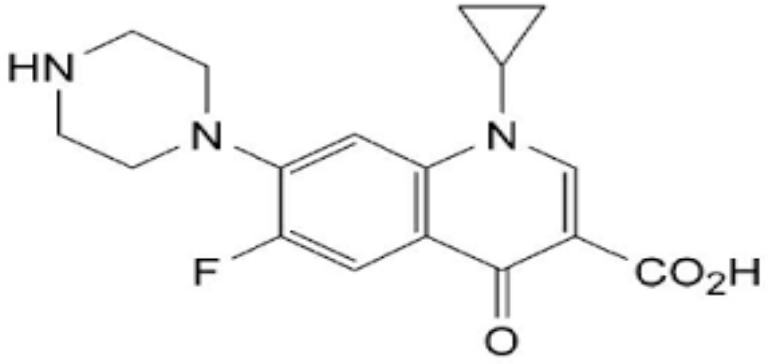

Fig. 1: Chemical structure of Ciprofloxacin

Ornidazole is used in the treatment of amoebiasis and other protozoal infections. Ornidazole is chemically 1-chloro-3-(2-methyl5nitroimidazole-1-yl)-propan-2-ol. Ornidazole (OND), a 5-nitroimidazole (Figure.2) is used in the treatment of protozoal infections and in the treatment and prophylaxis of anaerobic infections. It has been investigated for use in Crohn's disease after bowel resection. Ornidazole is converted into reduction products that interact with DNA to cause the destruction of the helical DNA structure and strand leading to inhibition of protein synthesis causing cell death in susceptible organisms.

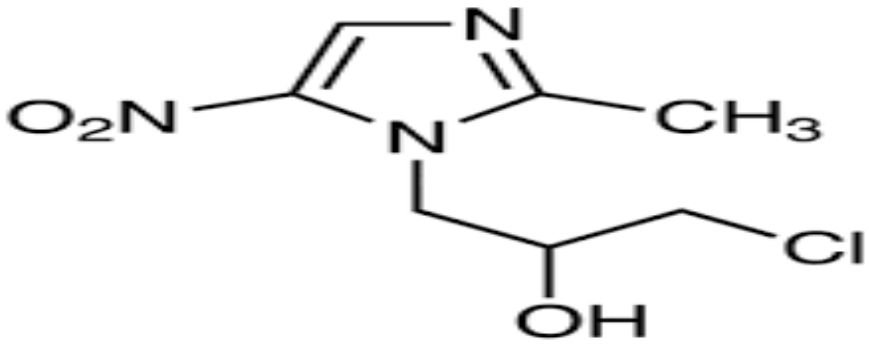

Fig. 2. Chemical structure of Ornidazole

Considering the therapeutic and commercial importance of both drugs it was envisaged that development of simple, economic, accurate, precise yet sensitive UV-visible spectrophotometric method with ability of simultaneous estimation of both Ciprofloxacin and Ornidazole will be worth, it would be useful in routine analysis of Ciprofloxacin and Ornidazole composition in near future [14-20].

2. Experimental

2.1 Instrumentation

A double beam UV-visible spectrophotometer (V-530, Jasco) with spectra manager software was used for the

method development and validation. Matched quartz cells with 3 cm height and 1 cm path length were used for spectral measurements. Analytical balance (Vibra HT, Essae) was used for the weighing purpose.

2.2 Material and Methods

All chemicals and reagents used for the method development purpose were of analytical or HPLC grade. Pure Ciprofloxacin and Ornidazole standard were purchased from the TCI chemicals (INDIA) Pvt. Ltd.

2.3 Preparation of standard stock solution

Ciprofloxacin and Ornidazole was weighed separately (5 mg each) and transferred to the 5 ml pre-calibrated volumetric flasks and dissolved in 5 mL of methanol and sonicated for 15 min, to achieve a stock solution of 1000 µg/ml (Stock-1). Stock 1 was suitably diluted to achieve solution of 100µg/ml (stock 2).

2.4 Determination of maximum wavelength (λ_{max})

Stock-2 of Ciprofloxacin and Ornidazole was diluted suitably to obtain solutions of 10µg/ml strength. Resultant Ciprofloxacin and Ornidazole solutions were scanned over wavelength range of 800 to 200 nm using medium scanning speed. Obtained spectra were analyzed using Spectra Manager software and the λ_{max} were identified.

2.5 Preparation of calibration curve

Stock 2 of Ciprofloxacin was diluted suitably so as to achieve seven different calibration standards representing CAL STD 1(1µg/ml), CAL STD 2(2µg/ml), CAL STD 3 (4µg/ml),CAL STD 4(6µg/ml), CAL STD 5(8µg/ml), CAL STD 6(10µg/ml) and CAL STD 7(12 µg/ml) strength whereas Stock 2 of Ornidazole was diluted to obtain calibration standards with CAL STD 1(1µg/ml), CAL STD 2(2µg/ml), CAL STD 3(4µg/ml), CAL STD 4(8µg/ml), CAL STD 5(12µg/ml), CAL STD 6(16µg/ml) and CAL STD 7(20 µg/ml) strength. From the full spectrum measurement mode (Figure 3 and 4) of stock-2 of Ciprofloxacin and Ornidazole, two different wavelengths viz. 290 nm and 311 nm were identified as λ_{max}. The calibration curves representing concentration vs. absorbance were plotted (Figure 3 and Figure 4 respectively).

2.6 UV-spectrophotometric method

2.6.1. Q-Absorption ratio analysis method

Q-Absorption ratio method comprises use the ratio of absorption at two selected wavelengths (one representing is o-absorptive point and other representing λ_{max} of one of the two components). Proposed method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths is a constant value, independent of concentration and path length. The solutions of CAL STD 12µg/ml ofCiprofloxacin and Ornidazole were scanned in the wavelength range of 400 to 200nm to obtain overlain spectra (fig 5). Two wavelengths, 290nm as iso-absorptive point and 311nm (λmax of Ornidazole) were selected for the formation of Q-absorbance ratio equation.

The concentration of the individual components was calculated by using the following equations;

Cx = Qm

-Qy/Qx-Qy) ×A1/ax 1

Cy = Qm-Qy/Qy-Qx) ×A1 /ax1

Where, Qm = A2 /A1, A 1 is absorbance of sample at iso-absorptive point,

A2 is absorbance of sample at λ_{max} of one of the two components,

Qx = ax2 /ax1,Qy = ay2 /ay1,

ax 1and ax 2 represent absorptivities of Ciprofloxacin at λ1 and λ2,

ay 1 and ay 2 denote absorptivity is of Ornidazole at λ1 and λ2 respectively;

Cx and Cy be the concentration of Ciprofloxacin and Ornidazole respectively.

3 Validation of UV- visible spectrophotometric methods

The developed method for simultaneous estimation of Ciprofloxacin and Ornidazole was validated as per ICH guidelines. Different parameters like linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated [21-25].

3.1 Linearity and Range

Linearity of the proposed UV method was established using seven different CAL STDs of Ciprofloxacin and Ornidazole. CAL STDs of Ciprofloxacin and Ornidazole were analyzed at respective wavelengths of maximum absorbance. Calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R^2value was important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

3.2 Accuracy

Accuracy may often be expressed as % recovery by the assay of known added amount of analyte. To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%) of its predefined concentration. To the predefined concentrations, different amounts of Ciprofloxacin and Ornidazole were added (standard addition method) and the accuracy was calculated based on percent recovery. For calculating the percent recovery following formula was used.

% RC= (SPS-S/SP) × 100

Were,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

3.3 Precision (Inter-day and Intra-day precision)

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of predefined samples. The study was performed at three concentration levels (Ciprofloxacin: LQC-1.5, MQC-20and HQC-39 µg/ml and Ornidazole: LQC-1.5, MQC-6 and HQC-11.5 µg/ml). Samples (n=5) were analyzed at three different time intervals of a day. Study was repeated on three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

3.4 Robustness

Robustness of the method was assessed by analyzing MQC STDs of Ciprofloxacin and Ornidazole 6µg/mL and 10µg/ml Respectively at ±1nm of pre-identified wavelength of maximum absorbance for both Ciprofloxacin and Ornidazole. The results were calculated in terms of % RSD.

3.5 Ruggedness

Ruggedness of the method was established by analyzing triplicate samples of Ciprofloxacin and Ornidazole CAL STD 6µg/ml and CAL STD 10µg/ml respectively on two different UV-Visible spectrophotometers viz. V-530, Jasco and BA-UV-2600, Bio age. Results were expressed in terms of % RSD.

3.6 Limit of Detection and Quantification

To determine the limit of detection and quantification (LOD and LOQ), the standard deviations (σ) of response and slope of calibration curve (S) were used. Detection of limit was calculated by (3.3×σ/S) and quantification limit was calculated by (10×σ/S).

4 Application of Method

4.1 Estimation of Ciprofloxacin and Ornidazole content in pharmaceutical formulation

The marketed pharmaceutical formulation of Ciprofloxacin and Ornidazole (Brand Name CIPLOX-OZ) was analyzed to estimate the contents of above-mentioned pharmaceutical formulation, 5 mg of formulation was accurately weighed and transferred to calibrated volumetric flask. The contents were dissolved in 5 ml of methanol and obtained solution was filtered through 0.45 µm syringe filter. Filtered solution was suitably diluted and analyzed for Ciprofloxacin and Ornidazole content by using proposed UV-Visible spectrophotometric method.

4.2 *In vitro* drug release studies

In vitro drug release testing of Ciplox-oz marketed tablets 1000mg was performed in phosphate buffer PH 6.8 as dissolution media (900ml) using USP apparatus

II (paddle) at 100 RPM for time interval 5,10,15,20,30,40 min.The temperature was maintained at 37°C±0.5°C. An aliquot (5ml) withdraw at specific time intervals and drug content was determined by UV-spectrometer at 290nm and 311nm. The selected dissolution parameters for the study where based on official dissolution method of Ciplox-oz tablet USP and the method reported in the literature.

5. Results and Discussion

5.1 Determination of wavelength of maximum absorbance (λ_{max})

Identification of wavelength having maximum absorbance is prerequisite for quantitative UV analysis. Solution with absorbance value less than 1 were appropriate for the determination of wavelength having maximum absorbance. Considering the above-mentioned point determination of λmax of Ciprofloxacin and Ornidazole solution of 10 µg/ml concentration each were carried out by full scan mode of UV-Visible spectrophotometer. The full scan mode was processed by Jasco UV software and λmax were determined. The λmax was found to be 290 nm and 311nm for Ciprofloxacin and Ornidazole (Fig. 3 and Fig. 4) respectively. The overlain spectra of both drugs shown in Fig. 5. The two wavelengths were used for the analysis of the drugs were 290 nm (Iso-absorptive point) and 311nm (λmax of Ornidazole) at which the calibration curves were prepared for both the drugs.

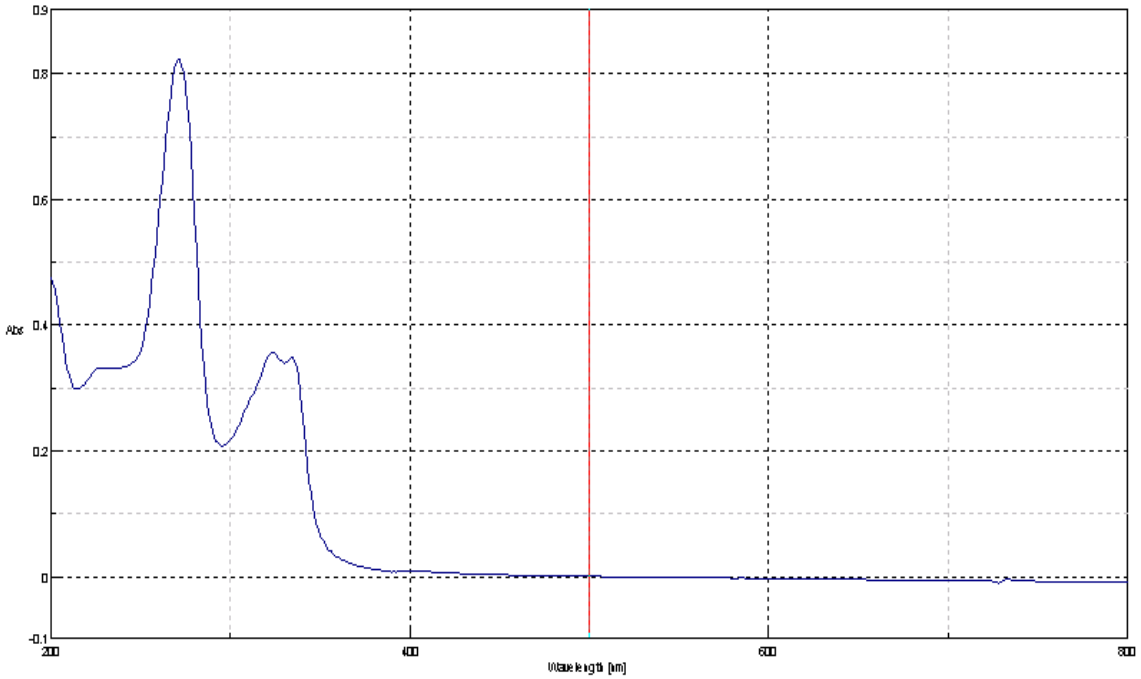

Fig.3: UV-visible spectra of Ciprofloxacin (290 nm)

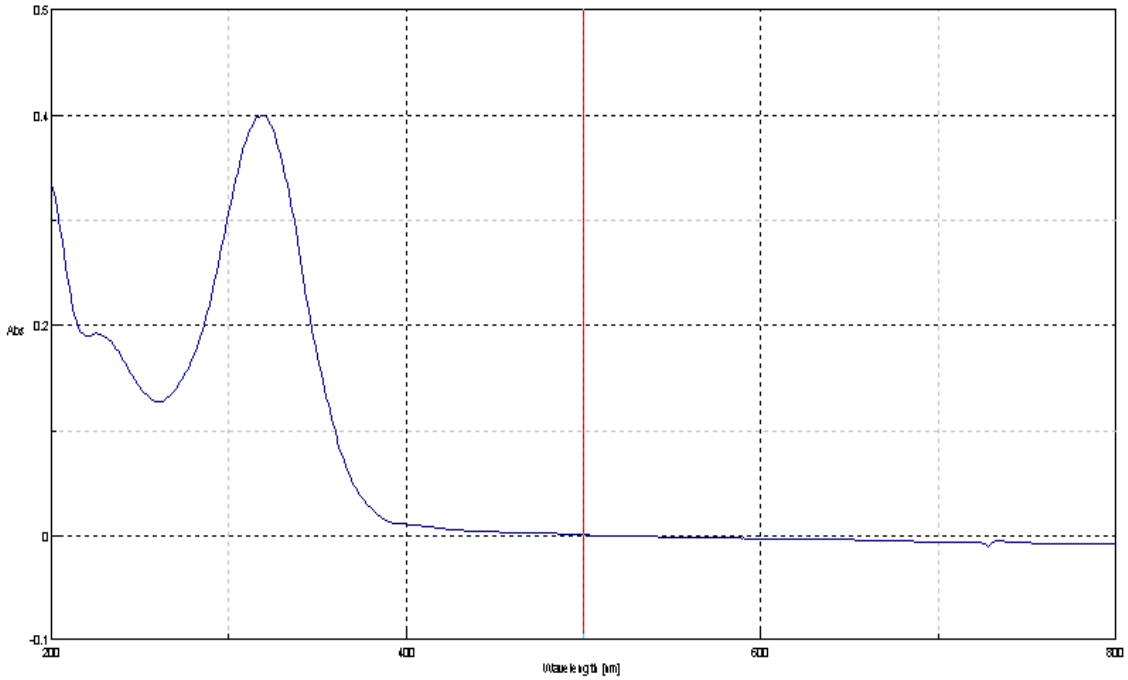

Fig.4: UV-visible spectra of Ornidazole (311 nm)

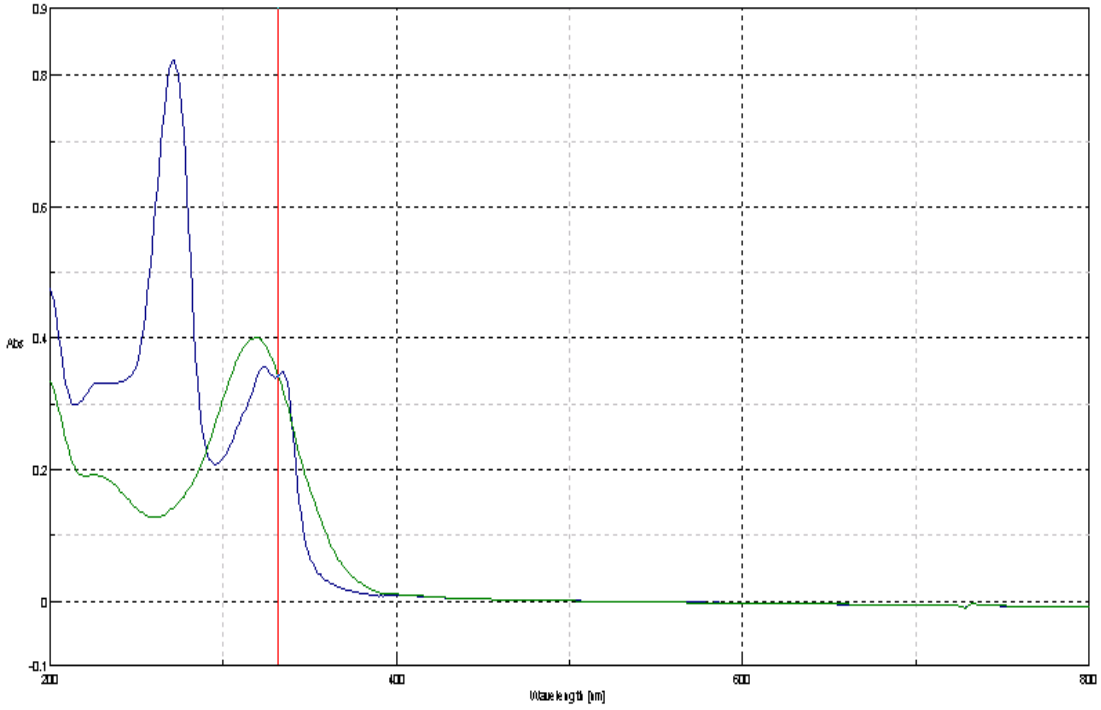

Fig. 5: Overlain spectra of Ciprofloxacin and Ornidazole

5.2 Preparation of Calibration Curve

(A) Calibration Curve for Ciprofloxacin

Calibration curve for Ciprofloxacin consists of different concentrations of standard solution ranging from 1 - 10µg/ml. The solutions were prepared by pipetting out CAL STD 1 (1µg/ml), CAL STD 2(2µg/ml), CAL STD 3(4µg/ml), CAL STD 4(6µg/ml), CAL STD 5(8µg/ml), CAL STD 6(10µg/ml) and CAL STD 7(12µg/ml) of the working standard solution of Ciprofloxacin (100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent. The absorbance of the solutions was measured at 290nmand 311nm against solvent ratio of methanol: water (20:80) as a blank. Calibration curve was plotted at both wavelengths and

two equations were formed using the absorptivity (Figure 6).

(B) Calibration Curve for Ornidazole

Calibration curve for Ornidazole consists of different concentrations of standard solution ranging from 1-20µg/ml. The solutions were prepared by pipetting out CAL STD 1(1µg/ml), CAL STD 2 (2µg/ml), CAL STD 3(4µg/ml), CAL STD 4(8µg/ml), CAL STD 5(12µg/ml), CAL STD 6 (16µg/ml) and CAL STD 7(20 µg/ml) of the working standard solution of Ornidazole(100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent ratio. The absorbance of the solutions was measured at 290 nm and 311nm against solvent ratio of methanol: water (20:80) as a blank. Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity (Figure 7).

6. Method validation

6.1 Linearity and Range

Linearity and range are the key parameters of analytical method which demonstrates the limit within the intended method to be used for its optimum performance. Considering the importance of linearity and the range, seven points' calibration curves of Ciprofloxacin between the range 1-10 µg/ml and Ornidazole between the range 1-20 µg/ml were plotted. The concentrations and the respective mean absorbance values of Ciprofloxacin and Ornidazole are mentioned in (Table 1 & Table 2). Calibration curve was subjected to least square regression analysis yielded an equation; y = 0.016X+0.002 and y = 0.017X+0.002 with correlation coefficient for Ciprofloxacin and Ornidazole in 290nm respectively (Fig. 6) and other too least square regression analysis yielded an equation; y = 0.066X + 0.023 and y = 0.029X +0.008 with correlation coefficient for Ciprofloxacin and Ornidazole in 311nm respectively (Fig. 7). The linearity study revealed that the developed UV method was found to be linear adherence to the system of Beers Law over the concentration range of 1 to 12µg/ml for Ciprofloxacin and 1 to 20µg/ml for Ornidazole.

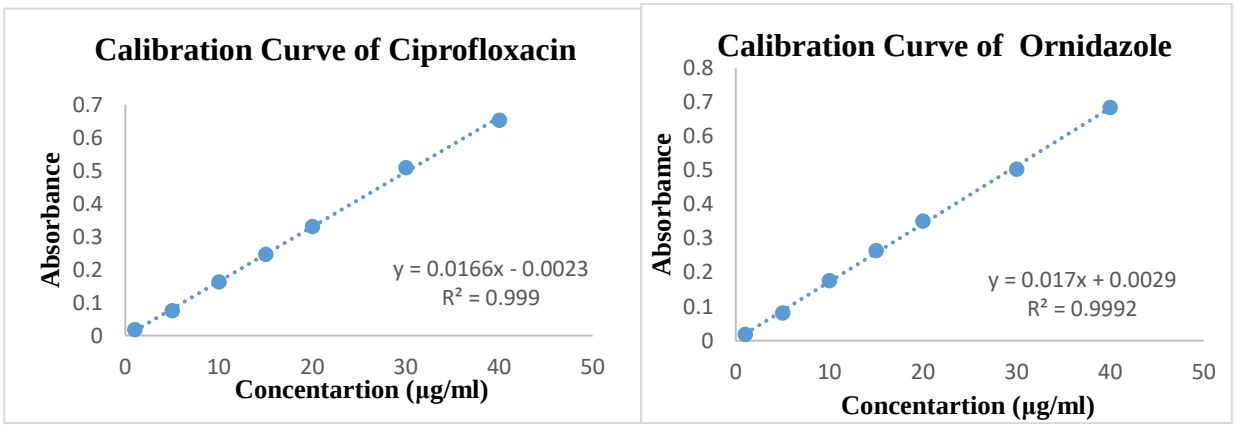

Fig. 6: **Calibration curve of Ciprofloxacin and Ornidazole at 290 nm**

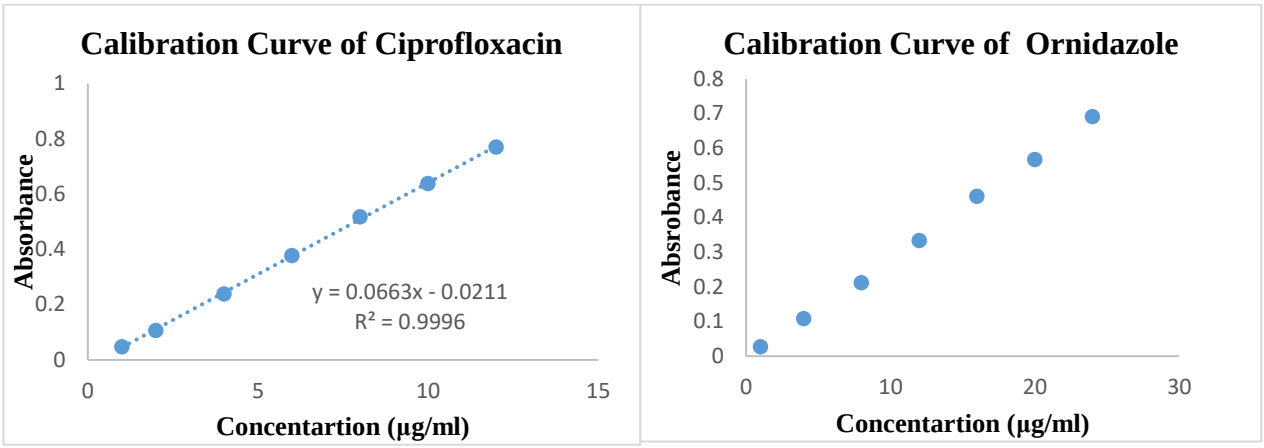

Fig.7: Calibration curve of Ciprofloxacin and Ornidazole at 311 nm

Table 1: Calibration data at Iso absorptive Point (290nm)

	CIPROFLOXACIN		ORNIDAZOLE	
Sr No.	**Conc. (µg/ml)**	**Absorbance**	**Conc.(µg/ml)**	**Absorbance**
1	1	0.0176± 0.0016	1	0.0184 ± 0.0014
2	5	0.0745 ±0.0027	5	0.081 ± 0.0025
3	10	0.1624 ± 0.0047	10	0.1752± 0.0037
4	15	0.2457 ±0.0025	15	0.2636 ± 0.0029
5	20	0.3305 ± 0.0034	20	0.3504± 0.0041
6	30	0.5088 ± 0.0019	30	0.5021 ± 0.0048
7	40	0.6525 ± 0.0056	40	0.6829 ± 0.0051

Table 2: Calibration data at λmax (311nm)

	CIPROFLOXACIN		ORNIDAZOLE	
Sr No.	**Conc. (µg/ml)**	**Absorbance**	**Conc.(µg/ml)**	**Absorbance**
1	1	0.0482 ± 0.0021	1	0.0273 ± 0.0011
2	2	0.1075 ± 0.0037	2	0.054 ± 0.0049
3	4	0.2389 ± 0.0018	4	0.109 ± 0.0026
4	6	0.3785 ± 0.0027	8	0.2121 ± 0.0017
5	8	0.5188 ± 0.0063	12	0.3342 ± 0.0033
6	10	0.6387 ± 0.0044	16	0.4619 ± 0.0041
7	12	0.7709 ± 0.0042	20	0.5683 ± 0.0054

6.2 Accuracy

Accuracy is the measure of how close the experimental value is to the true value. The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an

accepted reference value. Sometimes it termed as trueness. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. Accuracy of UV method for Ciprofloxacin and Ornidazole was established by recovery studies. The results of accuracy studies, determined that the developed UV method is highly accurate as the percent recovery was found to be between 99.53 to 100.24% (table 3).

Table 3: Recovery studies for Ciprofloxacin and Ornidazole

Origin level (µg/ml)	CIPROFLOXACIN				Origin level (µg/ml)	ORNIDAZOLE			
	Conc. (%)	Amount Added	% Recovery	% RSD		Conc. (%)	Amount Added	% Recovery	% RSD
1.5	80	1.2	99.58 ± 0.29	0.1138	1.5	80	1.2	100.04± 0.35	0.1786
20	100	20	100.24 ± 0.18	1.6110	6	100	10	99.92 ± 0.14	0.2749
39	120	46.8	99.65 ± 0.32	0.3658	11.5	120	23.4	99.53 ± 0.25	0.7310

6.3 Precision

Precision is the variability among replicate measurements, i.e., how close the values in a series of results are to each other. Precision of the assay was determined by repeatability and intermediate precision, which was studied by comparing the assays on 3 different days. It is expected that an analytical method should generate reproducible outcomes. Precise analytical method leads to accurate results. Considering the importance of reproducible and accurate results, Inter-day, intra-day variations were studied to determine repeatability and intermediate precision of the proposed analytical method. Intermediate precision was determined by analyzing three different levels of Ciprofloxacin and Ornidazole.concentrations at CAL STD 1.5µg/ml, CAL STD 20µg/ml,CAL STD 39µg/ml and CAL STD 1.5µg/ml, CAL STD 6µg/ml, CAL STD 11.5µg/ml respectively. The results were expressed in terms of mean absorbance values, percent assay and % RSD for the intra-day and inter-day precision study, demonstrated in Table 4-7, respectively for Ciprofloxacin and Ornidazole. Percentage RSD values of intra-day precision study were found to be between 0.15 and 0.83 for Ciprofloxacin and between 0.10 and 1.34 for Ornidazole. Whereas those of inter-day precision study were between 0.12 and 0.83 for Ciprofloxacin and between 0.10 and 1.34 for % RSD values Ornidazole. Use was less than 2, demonstrated the precision of developed UV method.

Table 4: Intra-day precision data of UV method for Ciprofloxacin

Sr. No			Morning			Afternoon			Evening		
	Wavelength (nm)	Conc. (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	290	1.5	1.515	101.01	1.258	1.521	101.4	1.581	1.505	100.1	0.541
	311	1.5	1.529	100.18	1.276	1.54	102.7	0.653	1.559	101.7	0.189

2	290	20	20.16	100.8	0.754	20.01	100.1	0.169	20.03	100.1	0.062
	311	6	6.06	101	0.268	6.04	100.7	0.448	6.06	101.1	0.104
3	290	39	39.4	101.1	0.415	39.29	100.8	0.317	39.07	100.2	0.043
	311	11.5	11.54	101.3	0.278	11.51	100.1	0.024	11.51	100.1	0.024

Table 5: Inter-day precision data of UV method for Ciprofloxacin

Sr. NO	Wavelength (nm)	Conc. (µg/ml)	Day 1			Day 2			Day 3		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	290	1.5	1.50	100.92	1.12	1.50	100.58	0.7848	1.50	100.17	0.95
	311	1.5	1.53	102	1.03	1.50	100.47	0.2626	1.50	100.17	0.25
2	290	20	20.06	100.34	0.32	20.03	100.16	0.1284	20.0	100.19	0.24
	311	6	6.05	100.93	0.27	6.02	100.47	0.0561	6.03	100.6	0.05
3	290	39	39.26	100.67	0.25	39.02	100.06	0.0482	39.03	100.1	0.08
	311	11.5	11.5	100.17	0.10	11.51	100.11	0.0217	11.5	100.12	0.02

Table 6: Intra-day precision data of UV method for Ornidazole

Sr. NO	Wavelength (nm)	Conc. (µg/ml)	Morning			Afternoon			Evening		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	290	1.5	1.501	100.1	1.176	1.494	99.84	1.30	1.531	101.5	0.993
	311	1.5	1.508	100.5	0.748	1.508	100.5	0.748	1.508	100.5	0.748
2	290	20	20.03	100.0	0.094	20.03	100.2	0.061	20.04	100.2	0.085
	311	6	6.02	100.3	0.128	6.017	100.3	0.128	6.02	100.3	0.164
3	290	39	39.01	100.1	0.066	39.03	100.1	0.092	39.03	100.1	0.069
	311	11.5	11.54	100.3	0.086	11.54	100.3	0.124	11.53	100.3	0.131

Table 7: Inter-day precision data of UV method for Ornidazole

Sr. NO	Wavelength (nm)	Conc. (µg/ml)	Day 1			Day 2			Day 3		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	290	1.5	1.53	102	1.15	1.51	100.9	1.54	1.52	101.7	1.5
	311		1.50	100.5	0.74	1.52	101.8	0.33	1.52	101.8	0.33
2	290	12	20.0	100.1	0.07	20.02	100.12	0.05	20.02	100.1	0.05
	311		6.01	100.3	0.14	6.01	100.17	0.01	6.01	100.1	0.09
3	290	19.5	39.02	100.0	0.07	39.0	100.2	0.04	39.07	100.2	0.04
	311		11.5	100.3	0.11	11.5	100.31	0.08	11.5	100.3	0.08

6.4 Robustness

Robustness examines the effect that operational parameters such as temperature, mobile phase composition, detection wavelength etc., have on the analysis results. If the influence of parameter is said to be within a previously specified tolerance, the parameter is said to be within the methods robustness range. Robustness study of proposed UV method was evaluated by using three different solvents. The method was found to be robust as indicated by the % RSD values which are less than 2%. The% RSD values were found to be between 0.09 and 0.64 for Ciprofloxacin and between 0.06 and 0.20 for Ornidazole., shown in Table 8 for Ciprofloxacin and Ornidazole respectively. Percentage RSD values were below 2 depict that the proposed UV method was robust in nature.

Table 8: Robustness study for Ciprofloxacin and Ornidazole

Molecule	Conc. (µg/ml)	Solvent Ratio (Water: Methanol)	λ_{max}	Absorbance	%RSD
Ciprofloxacin	6	79:21	290	0.0644	0.6461
			311	0.3788	0.1402
		80:20	290	0.0643	0.4988
			311	0.3787	0.0929
		81:19	290	0.0643	0.7009
			311	0.3787	0.1403
Ornidazole	10	79:21	290	0.1755	0.1740
			311	0.3119	0.1785
		80:20	290	0.1754	0.1441
			311	0.3126	0.1153
		81:19	290	0.1755	0.2
			311	0.3127	0.0665

Ruggedness

Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different instruments, different elapsed assay times, different assay temperatures, different days etc. Ruggedness analytical methods are free from environmental/external factors impact. The ruggedness of proposed UV method, for Ciprofloxacin and Ornidazole solutions were analyzed by using two different UV-Visible spectrophotometers. Sample analysis resulted into % RSD values between 0.09 and 1.10 for Ciprofloxacin and between 0.99 and 1.18 for Ornidazole. Results showed that the proposed UV method was rugged as % RSD values were less than 2, shown in Table 7.

Table 7: Ruggedness study for Ciprofloxacin and Ornidazole for different instruments

Conc. (µg/ml)	Ciprofloxacin			Conc. (µg/ml)	Ornidazole		
	Instrument/ Analyst	Absorbance	% RSD		Instrument/ Analyst	Absorbance	%RSD
6	Jasco	0.0645	0.50	10	Jasco	0.1741	1.008
6	Bioage	0.3776	0.09	10	Bioage	0.3126	0.122
6	Analyst-I	0.06448	0.53	10	Analyst-I	0.1789	0.99

6	Analyst-II	0.38146	1.10	10	Analyst-II	0.3155	1.18

6.6 Limit of Quantitation (LOQ) and Limit of Detection (LOD)

Generally, LOQ is the first calibration standard. LOQ represents the lowermost concentration that can be analysed. LOD represents the lowest quantity of substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). **LOD** and **LOQ** of proposed UV method were found to be **0.0192** and **0.0584**µg/ml for Ciprofloxacin whereas **0.0192** and **0.0584** µg/ml for Ornidazole, as shown in Table 10 for Ciprofloxacin and Ornidazole, Lower LOQ values indicated that the proposed method would be sensitive enough to quantify the Ciprofloxacin and Ornidazole, content of samples at its lower level.

Table 10: LOD and LOQ for Ciprofloxacin and Ornidazole

Sr. No.	Parameter	Ciprofloxacin	Ornidazole
1	LOD	0.01037	0.01929
2	LOQ	0.03142	0.05848

6.7 Estimation of Ciprofloxacin and Ornidazole content in pharmaceutical formulation:

The developed UV method was successfully applied for estimation of Ciprofloxacin and Ornidazole content in pharmaceutical formulation. The Ciprofloxacin and Ornidazole content in the pharmaceutical formulation was found to be 100.86 % and 100.79% respectively (Table no 11) by Q-Absorbance method**.**

Table 11: Analysis of content in pharmaceutical formulation

Sr no.	Sample(n=5)	Amount present(µg/ml)	Amount found(µg/ml)	Assay%
1	Ciprofloxacin	10	4.1	100.86
2	Ornidazole	10	12.09	100.79

6.8 In vitro drug release studies

The Marketed formulation Ciplox-OZ Tablets of Ciprofloxacin and Ornidazole were evaluated for in vitro drug release studies, which were performed using USP Type-I (Basket) dissolution test apparatus. The volume of the dissolution medium was 900mL with a stirring speed of 100 rpm and the temperature was maintained at 37°C±0.5°C. These conditions were kept constant for all dissolution studies. The study was carried out in pH 6.8 phosphate buffer at 5, 10, 15, 20, 30 and 40min respectively. 5 mL of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium. The collected samples were filtered through 0.45µ filter by discarding initial 4mL of solution. Further diluted 2mL of filtrate to 100mL with dissolution medium and analyzed to assess the percent drug dissolved. The percent drug release was obtained is 99.87% & 99.95% for Ciprofloxacin and Ornidazole respectively.

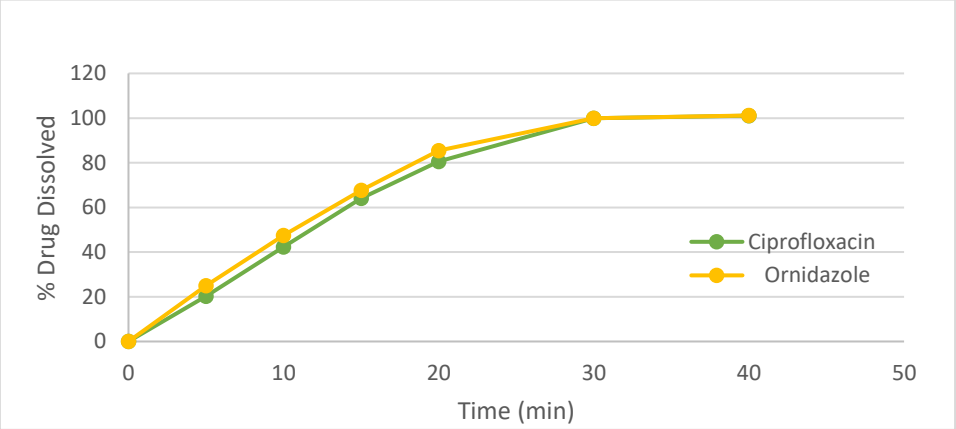

Fig. 7: **Comparative dissolution profile Ciprofloxacin and Ornidazole tablets**

7. Conclusions

The simple, precise, accurate, economic and sensitive UV- visible spectrophotometric method for the Q-absorbance of Ciprofloxacin and Ornidazole in a bulk drug and pharmaceutical formulation was developed and validated. By validating proposed method and relating that obtained values compares with standard values, the satisfactory observation was obtained. The recovery result confirms the drug content accuracy of method. The common additives and some excipient are used in formulation of comined dosage form so the method is easily applied for daily quality control analysis of ciprofloxacin and ornidazole in bulk dosage form. Thus, it can be effectively applied for the estimation of Ciprofloxacin and Ornidazole pharmaceutical formulation.

Acknowledgement

The extra-mural grant support of DST-DPRP, Govt. of India (Ref.:- VI-D&P/626/2018-19/TDT) Sanctioned to P.I. Dr. Sachin S. Bhusari for the proposed research work is highly acknowledged.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

Sachin Bhusari Supervision, Validation, Methodology, Investigation, Writing – original draft, **Madhuri Deshmukh:** Conceptualization, **Pravin wakte**: Administration, Funding, Data Curation

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