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Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Ciprofloxacin and Metronidazole in their Combined Dosage Form

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ABSTRACT:

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Ciprofloxacin and Metronidazole in combined dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Ciprofloxacin and Metronidazole show an isoabsorptive point at 295 nm in methanol. The second wavelength used is 279 nm, which is the λ-max of Ciprofloxacin in methanol. The linearity was obtained in the concentration range of 2-10 µg/ml for Ciprofloxacin and Metronidazole. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of Ciprofloxacin. The method was successfully applied to pharmaceutical dosage form because no interference. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS: Ciprofloxacin, Metronidazole, absorbance ratio method, isoabsorptive point, validation, simultaneous.

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INTRODUCTION:

Ciprofloxacin (CIPRO) is chemically 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid [1]. It is official in IP[2], BP[3], USP[4] and EP[5]. IP, BP and EP describe Potentiometric Titration and Liquid Chromatography method for its estimation. Literature survey reveals Solid-phase UV spectrophotometric method [6] and HPLC [7] methods for determination of CIPRO in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals RP-HPLC [8] and UV-spectrometry [9] methods for determination of CIPRO with other drugs in combination. Metronidazole (METRO) is chemically 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethan-1-ol is a Antibiotics. Metronidazole is official in IP, BP, EP, USP[3] and EP[4] describes Potentiometric titration and liquid chromatography method for its estimation. Literature survey reveals HPLC [10], UV Spectrophotometry [11] method for the determination of METRO. Literature survey also reveals HPLC [12], UV Spectrophotometry [13] and HPTLC [14] method for determination of METRO with other drugs in combination. The combined dosage forms of CIPRO and METRO used as anti-crohn's drug [15]. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CIPRO and METRO in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric method for simultaneous estimation of CIPRO and METRO in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective Spectrophotometric method based on First derivative Spectrophotometric method for simultaneous estimation of both drugs in their combined dosage form.

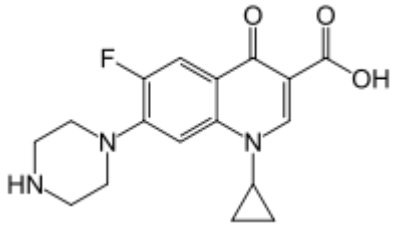

Figure 1: Structure of Ciprofloxacin

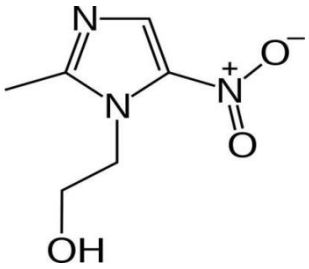

Figure 2: Structure of Metronidazole

MATERIALS & METHODS

MATERIALS

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study. CIPRO and METRO bulk powder was kindly gifted by Torrent Research Center, Ghandhinagar, Gujarat, India.. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) were used in the study.

METHODS

Preparation of Standard Solutions

A 10 mg of standard CIPRO and METRO were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100µg/ml each of CIPRO and METRO.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that CIPRO and METRO show an isoabsorptive point at 295 nm. The second wavelength used is 279 nm, which is the λ-max of CIPRO. Working standard solutions having concentration 2, 4, 6, 8, and 10.0 µg/ml for CIPRO and METRO were prepared in methanol and the absorbances at 295 nm (isoabsorptive point) and 279 nm (λ-max of CIPRO) were measured and

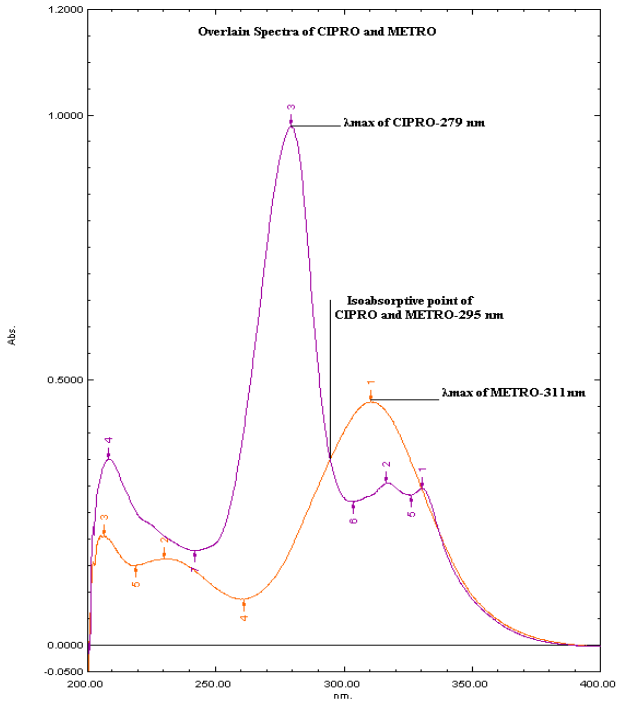

Figure 3 Overlain absorption spectra of Ciprofloxacin (279 nm) and Metronidazole (311 nm) Showing isoabsorptive point (295 nm) in methanol

absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM - QY) / (QX - QY)] \times A_1/ax_1 \ldots\ldots\ldots (1)$$

$$CY = [(QM - QX) / (QY - QX)] \times A_1/ay_1 \ldots\ldots\ldots (2)$$

Where, A_1 and A_2 are absorbances of mixture at 279 nm and 295 nm; ax1 and ay1 are absorptivities of CIPRO and METRO at 279 nm; ax2 and ay2 are absorptivities of CIPRO and METRO respectively at 295 nm; $QM = A_2 / A_1$, $QX = ax_2 / ax_1$ and $QY = ay_2 / ay_1$.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guideline.[16]

LINEARITY (CALIBRATION CURVE)

The calibration curves were plotted over a concentration range of 2-10 µg/ml for CIPRO and METRO. Appropriate aliquots from the standard stock solutions of CIPRO and METRO were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of CIPRO (2-10 µg/ml). Aliquot from the stock solution of CIPRO (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2-10 µg/ml (0.2, 0.4, 0.6, 0.8, and

1.0ml). Series B consisted of varying concentrations of METRO (2-10 µg/ml). Appropriate volume of the stock solution of METRO (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol to get final concentration in range of 2-10 µg/ml (0.2, 0.4, 0.6, 0.8, and 1.0 ml). The absorbances of solution were then measured at 279 nm and 295 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

METHOD PRECISION (REPEATABILITY)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (*n* = 6) for CIPRO and METRO (6 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

INTERMEDIATE PRECISION (REPRODUCIBILITY)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CIPRO and METRO (4, 6 and 8 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

ACCURACY (RECOVERY STUDY)

The accuracy of the method was determined by calculating the recoveries of CIPRO and METRO by the standard addition method. Known amounts of standard solutions of CIPRO and METRO were at added at 50, 100 and 150 % level to prequantified sample solutions of CIPRO and METRO (5µg/ml for CIPRO and 4µg/ml for METRO). The amounts of CIPRO and METRO were estimated by applying obtained values to the respective regression line equations.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by

Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S \quad \text{..... (3)}$$

$$LOQ = 10 \times \sigma/S \quad \text{..... (4)}$$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve.

ANALYSIS OF DRUGS IN SAMPLE:-

The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 279 nm (λ-max of CIPRO) and 295nm (isoabsorptive point) respectively, and ratios of absorbance

Table 1: Regression Analysis Data and Summary of Validation Parameters for CIPRO and METRO by Q-Absorbance Spectrophotometric Method

Parameters	CIPRO	METRO	CIPRO & METRO
Wavelength (nm)	279	279	295
Beer's law limit (µg /ml)	2-10	2-10	2-10
Regressionequation (y = a + bc)	Y=0.123X-0.017	Y=0.021X+0.001	Y= 0.043X-0.004
Slope (b)	0.123	0.021	0.043
Intercept (a)	0.017	0.001	0.004
Correlation coefficient (r^2)	0.9983	0.9993	0.9974
LOD[a] (µg/ml)	0.146	0.246	0.156
LOQ[b] (µg /ml)	0.445	0.745	0.474
Repeatability (% RSD[c], n =6)	0.1584	0.6313	0.3148
Precision (%RSD, n = 3)			
Interday	0.41-0.53	0.56-1.92	0.89-1.58
Intraday	0.15-0.32	0.85-1.30	0.44-0.88
Accuracy ± S.D[d]. (%Recovery, n= 5)	98.84 ± 0.54	98.52 ± 0.42	99.24 ± 0.46

Table 2: Recovery Data of CIPRO and METRO by Spectrophotometric Method

Drug	Amount taken (µg/ml)	Amount added (%)	%Recovery ± S. D. (n=5)	
			At 279 nm	At 295 nm
CIPRO	5	50	98.36 ± 0.19	*99.53 ± 0.77*
	5	100	98.93 ± 0.56	*99.29 ± 0.41*
	5	150	99.23 ± 0.87	*98.91 ± 0.22*
METRO	4	50	98.01 ± 0.50	99.53 ± 0.77
	4	100	98.90 ± 0.55	99.29 ± 0.41
	4	150	98.67 ± 0.21	98.1 ± 0.22

Table 3: Analysis of CIPRO and METRO by Spectrophotometric Method

Mixture	Label Claim (mg)		Amount Found (mg)		% Label Claim ± S.D. (n=6)	
	CIPRO	METRO	CIPRO	METRO	CIPRO	METRO
I	125	100	124.9	100.14	99.92 ± 0.44	101.40 ± 0.52

were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample was calculated using above equation (1) and (2). The analysis procedure was repeated three times with combined dosage form.

RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength (Beckett et al., 1997), which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 279 nm (λ-max of CIPRO) and 295nm (isoabsorptive point) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CIPRO (279 nm) and METRO (311 nm) showing isoabsorptive point (295 nm) in methanol is shown in Figure 3. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table 2). The method was successfully used to determine the amounts of CIPRO and METRO present in the combined dosage form. The results obtained were in good agreement with the corresponding labeled amount (Table 3). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 1. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of CIPRO and METRO in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of CIPRO and METRO hence the method was also economic for estimation of CIPRO and METRO from synthetic mixture. The common excipients and other additives are usually present in the synthetic mixture do not interfere in the analysis of CIPRO and METRO in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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