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Development and Validation of Norfloxacin and Metronidazole in Combined Dosage form by vierordts's Method

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ABSTRACT

A simple, rapid, precise and highly selective spectrophotometric method was developed for simultaneous estimation of Norfloxacin and metronidazole in tablet dosage form. This method involves the measurement of absorbances of Norfloxacin and Metronidazole at the wavelength of 272nm (λmax of Norfloxacin) and 334nm (λmax Metronidazole) .0.1 N NaOH was used as solvent. Linearity was observed in the concentration range of 1-10 µg/ml for Norfloxacin and 1-19µg/ml for Metronidazole. The accuracy of the method was confirmed by recovery studies of tablet dosage forms and was found to be 101 and 102% for Norfloxacin and metronidazole respectively. The method showed good reproducibility and recovery with % RSD less than 2. The LOD of Norfloxacin and metronidazole and LOQ of Norfloxacin and metronidazole was found to be 0.3µg/ml and 1.16µg/ml and 0.79µg/ml and 2.4µg/ml respectively. The developed method was free from interferences due to excipients present in tablets .Thus the proposed method was found to be rapid, specific, precise, accurate and cost effective quality control tool for the routine analysis of Norfloxacin and metronidazole in bulk and combined dosage form.

Keywords: Norfloxacin (Nor); Metronidazole(MTZ), UV spectrophotometric method; simultaneous equation method

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INTRODUCTION

Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. chemically 1-ethyl-6-fluro-4-oxo-7-(piperazin-1yl)-1,4-di hydroquinoline-3-carboxylicacid.its molecular formula is $C_{16}H_{18}FN_3O_3$ having molecular weight 319.330 g/mol. Norfloxacin is a fluoroquinolone antibacterial agent, structurally related to nalidixic acid. Due to it's relatively poor oral bioavailability, its clinical use is limited to the treatment of urinary tract infections and uncomplicated gonorrhea. Norfloxacin is bactericidal via its effects on DNA gyrase, an enzyme responsible for counteracting the excessive supercoiling of DNA during replication or transcription. Although human cells do not contain DNA gyrase, they do contain a topoisomerase enzyme that functions in the same manner.

Metronidazole is chemically 2-(2-methyl-5-nitrol Himidazolyl Ethan-1ol.its molecular formula is C6H9N3O3 having molecular formula $C_6H_9N_3O_3$. Metronidazole is a synthetic antibacterial and antiprotozoal agent that belongs to the nitroimidazole class. Metronidazole is effective therapy against protozoa such as Trichomonas vaginalis, amebiasis, and giardiasis. Metronidazole is also useful in treating Crohn's disease, antibiotic-associated diarrhea, and rosacea. It acts selectively aganist anaerobic bacteria's as only these bacteria are capabale to reduce it to its active form intracellularly. Reduced it then disrupts DNA's helical structure, thereby inhibiting bacterial nucleic acid synthesis. This eventually results in bacterial cell death.

Combination of Norfloxacin & Metronidazole is used in the treatment of diarrhoea, amoebiasis, giardiasis ,orodental infection

There are very few analytical methods reported for simultaneous estimation of Norfloxacin and Metronidazole. Hence, investigation of new analytical methods is in need for the quantitative estimation of Norfloxacin and Metronidazole, in combination in pharmaceutical dosage form

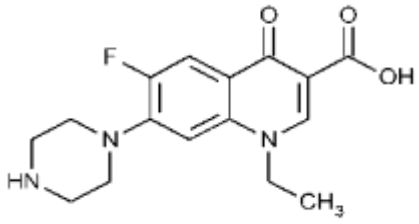

Figure 1: Norfloxacin Structure

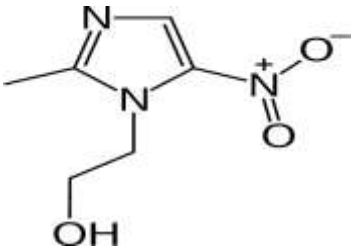

Figure 2:Metronidazole structure

MATERIALS AND METHODS

Instruments:

Shimadzu UV-Visible Spectrophotometer (Model UV-1800),Shimadzu digital electronic balance (BL 220H), fast clean ultra sonic cleaning system (Life care equipments Pvt Ltd).

Chemicals:

Analytical pure samples of Norfloxacin and metronidazole were provided by Dr reddys laboratory as a gift sample. Formulation,Nor-metrogyl manufactured by j.b. chemicals and pharmaceuticals ltd was procured from a local pharmacy in Hyderabad. 0.1NaoH was used as solvent.

Methods:

The absorbance of both drugs was found to be maximum in 0.1N NaOH. So, 0.1N NaOH was used as solvent and λmax of NOR and MTZ was fixed as 272 (Figure 3) and 334 (Figure 3) respectively.

Preparation of NOR standard stock solution:

Standard stock solution of NOR was prepared by dissolving 10mg of drug in 100ml of 0.1N NaOH to get a concentration of 100µg/ml.

Preparation of MTZ standard stock solution:

Standard stock solution of NOR was prepared by dissolving 10mg of drug in 100ml of 0.1N NaOH to get a concentration of 100µg/ml.

Selection of wavelength:

From the spectra of drugs in 0.1N NaOH, the wavelengths were selected as272 AND 334 nm(figure:3) for Norfloxacin and Metronidazole respectively. Different concentrations of Norfloxacin (1 to 10 µg/ml.) Metronidazole (1 to 19 µg/ml.), scanned and absorbance's were noted at the wavelengths. From the data, it was noted that, at wavelengths 272 and 334nm good linearity was observed and hence these wavelengths were fixed for the study

VALIDATION PARAMETERS:

Linearity

The linearity of the response for Metronidazole and Norfloxacin was determined by preparing standard solutions of Metronidazole 1-19 µg/ml and Norfloxacin 1-10 µg/ml. The Correlation coefficient (r) was found 0.998 for MTZ and 0.998 for NOR as shown in Figure 6 and 7 respectively. It indicate that the response was linear over the concentration range. overlain spectra of Nor(1-10 µg/ml) and MTZ(1-19 µg/ml) shown in figure 4,5.

Accuracy:

Accuracy was performed at 50%, 100% levels by Standard addition method. Each concentration was analyzed 3 times and average recoveries were measured as shown in Table 2 To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1hr and

on three different days for intra and inter day study, respectively. The Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated. The results are given in the table 2.

Precision:

The Precision of the method was established by carrying out the analysis of the analyte using the proposed developed method. The low value of %RSD showed that the methods were precise. The precision values are shown in Table 3.

Limit of detection and quantification: It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection limit(LOD) and quantification limit (LOQ) may be expressed as

$$LOD = 3.3 \times N/S$$

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve. The LOD values of Norfloxacin and Metronidazole were found to be 0.3µg/ml and 1.16µg/ml respectively and the LOQ values were found to be 0.79µg/ml and 2.4µg/ml respectively as shown in table 4.

Analysis of marketed formulation

Twenty tablets are powdered and the average weight was calculated. A quantity equivalent to 10 mg of drug was dissolved in water and to it 2mg of standard Norfloxacin , 5 mg of Metronidazole and Norfloxacin was added (standard addition method), such that the sample contains 10mg each of Norfloxacin and Metronidazole . Finally the volume was made up to get a working concentration of 100µg/ml each of Norfloxacin and Metronidazole. Absorbances were noted at 272 nm and 334 nm, The concentration of each analyte was determined using the simultaneous equation (Figure. 8) (Table 5).

RESULTS AND DISCUSSION:

This method is considered simple, reliable, selective providing satisfactory accuracy, precision with lower limits of detect

From the optical characteristics obtained with the proposed method it was found that the drugs obey linearity with in concentration range of 1-10µg/ml for NOR and 1-19µg/ml for MTZ.(Figure 4) &(figure 5) From the precision studies it was found that the % RSD is less than 2% which indicates that the method has good reproducibility (Table 3)

The LOD values of Norfloxacin and Metronidazole were found to be 0.3µg/ml and 1.16µg/ml respectively and the LOQ values were found to be 0.79µg/ml and 2.4µg/ml respectively.

From the results of recovery studies, it was found that the % recovery values of the pure drugs from the reanalyzed solutions of formulations were in between 99.8-101%, which indicates that the method is accurate and reveals that commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method.

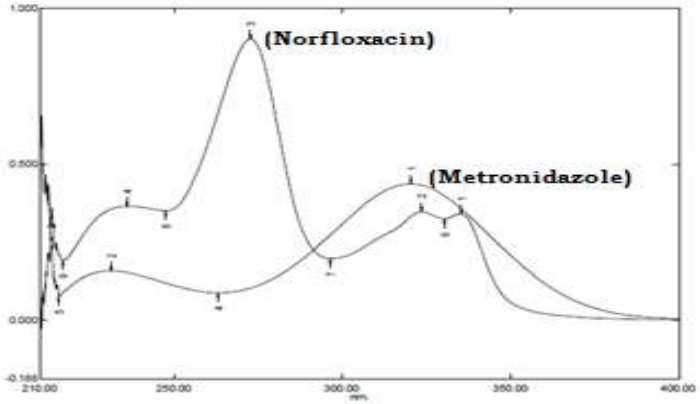

Figure :3 Overlain UV Spectra of standard solution of Norfloxacin(7µg/ml)and Metronidazole(7µg/ml)

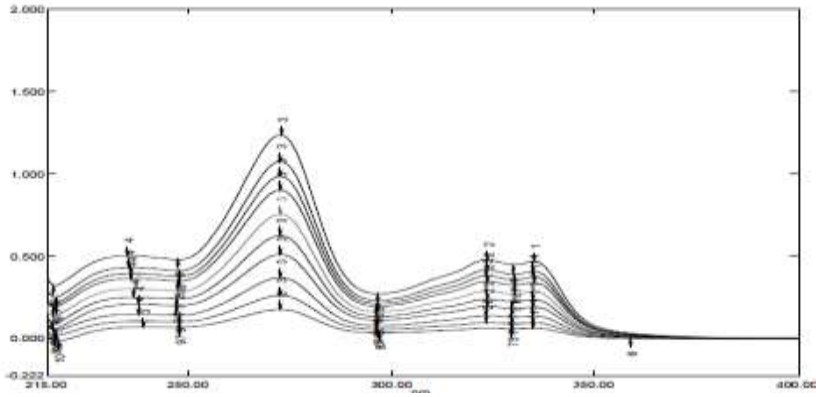

Figure 4:overlain UV spectra of Norfloxacin (1-10µg/ml)

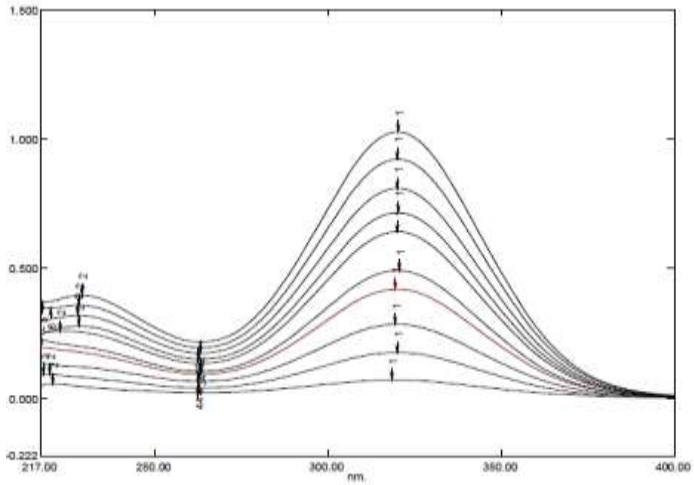

Figure:5 overlain UV spectra of Metronidazole (1-19µg/ml)

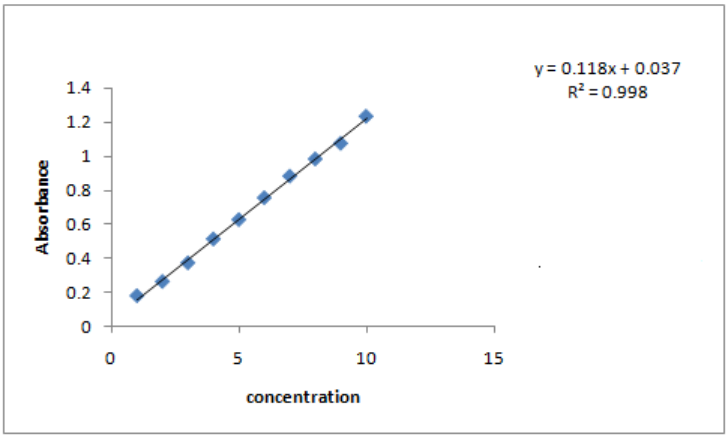

Figure 6: Calibration Graph of Norfloxacin at 272nm

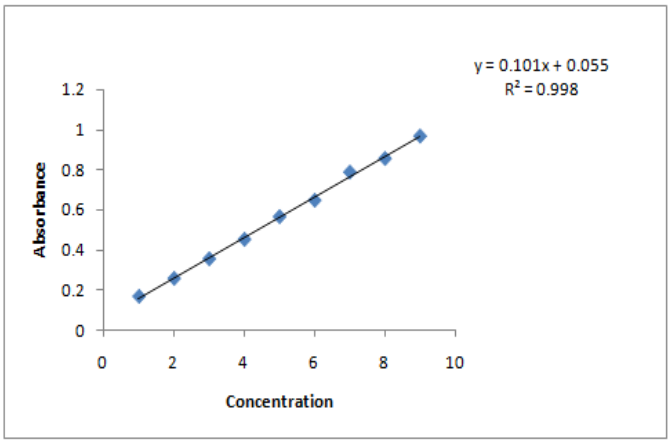

Figure 7:Calibration Graph of Metronidazole at 334nm

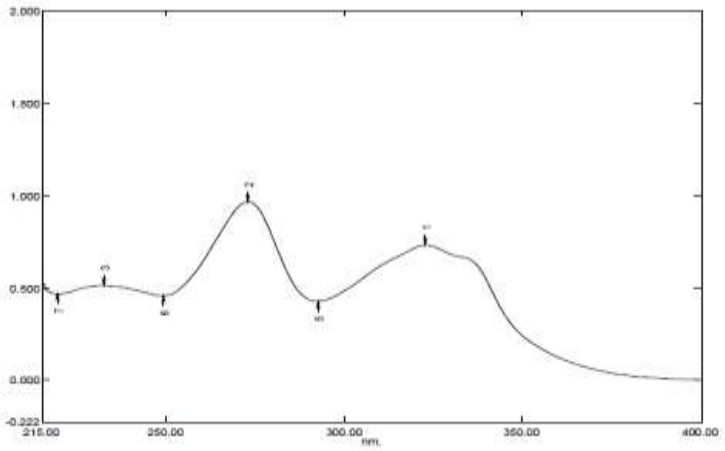

Figure: 8 UV Spectrum of Formulation

Table 1: Linearity and correlation coefficient

Parameters	Norfloxacin	Metronidazole
Regression equation	Y=0.118X+0.037	Y=0.101X+0.055
Linearity	1-10µg/ml	1-19µg/ml
Correlation coefficient	R^2= 0.998	R^2= 0.998

Table 2: Accuracy

Drug	% Amount Added	Amount taken(mg)	Amount recovered(mg)	% Recovery	% *RSD
Norfloxacin	50%	5	4.88	97.7,98.2	0.3
	100%	10	10.1	101,100.1	0.6
Metronidazole	50%	5	5.05	101,99.5	1.0
	100%	10	10.2	102,101.5	0.3

%RSD of two observations

Table 3: precision studies

Drug	Concentration µg/ml	Intraday Precision % RSD	Inter day Precision %RSD
Norfloxacin	7	0.5	0.3
Metronidazole	7	0.2	0.4

Table 4: LOD and LOQ studies

Validation Parameters	Norfloxacin (µg/ml)	Metronidazole (µg/ml)
(LOD) µg/ml	0.3	1.16
(LOQ) µg/ml	0.79	2.4

Table 5: Analysis of Formulation

Drug	Labelled amount	Amount found	% Label claim	% *RSD
Norfloxacin	400	404	101	0.8
		399.2	99.8	
Metronidazole	500	499	99.8	0.8
		504	101.1	

%RSD of two observations

CONCLUSION

The proposed UV spectrophotometric method for the simultaneous estimation of Norfloxacin and Metronidazole in pharmaceutical dosage form are simple and reliable providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved are good. Hence, this method can be recommended for routine and quality control analysis of the drugs in pure and pharmaceutical dosage form.

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