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Simultaneous determination of norfloxacin and metronidazole from combined tablet dosage formulation by spectrophotometry

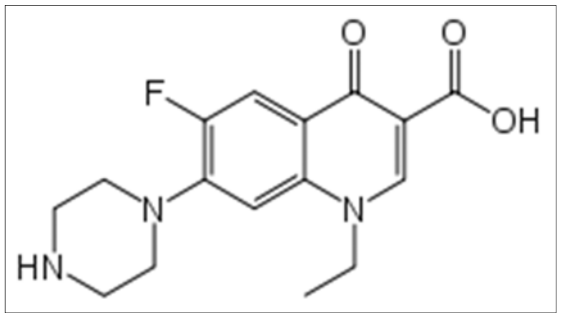
Pralhad Rege, Kyle Meyer and Neha Kapadia

Abstract
In present study, a successful attempt has been made to develop a simple, sensitive, accurate and economical Spectrophotometric method for simultaneous estimation of Norfloxacin and Metronidazole in tablet dosage form. The method is based on the simultaneous equations. Norfloxacin and Metronidazole shows absorbance maxima at 275 nm and 325 nm in 0.1N sodium hydroxide, respectively. The linearity was obtained in the concentration range 1 µg / mL to 10 µg / mL for standard N at 275 nm and 2 µg / mL to 20 µg / mL for standard M at 325 nm.. In given method the quantification of the drugs was done by using simultaneous equation method. The results of the analysis have been validated statistically and by assay, recovery studies. Therefore the proposed validated method can be successfully applied for routine quality control analysis and simultaneous determination of norfloxacin and metronidazole in combined drug formulations.

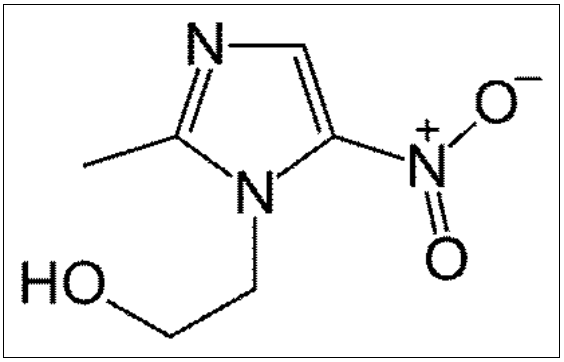
Keywords: Norfloxacin, metronidazole, simultaneous equation, spectrophotometric method and validation

Introduction
In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are most common. Drugs with antifungal and antiprotozoal activity have been used in the treatment of the same.
Norfloxacin is a synthetic chemotherapeutic agent occasionally used to treat common as well as complicated urinary tract infections. It is sold under various brand names.
Since its establishment in 1946, the Japanese Society of Chemotherapy (JSC) has been and currently is involved in the development of synthetic antibacterial agents from nalidixic acid and pipemidic acid, leading to new quinolones. Subsequent work led to the birth of a new era with the introduction of norfloxacin as the first new quinolone in Japan in 1984 and then in many other countries throughout the world. Since the discovery of norfloxacin (1980), around 10,000 new analogues have been described.
Norfloxacin was first patented in 1979. Kyorin granted Merck & Company, Inc., an exclusive license (in certain countries, including the United States), to import and distribute Norfloxacin under the brand name Noroxin. The U.S. Food and Drug Administration (FDA) approved Noroxin for distribution in the United States on October 31, 1986. Since the approval of Noroxin in 1986, there have been numerous upgrades to the warning sections of the package inserts, as well as recent restrictions placed upon the use of Noroxin to treat urinary tract infections (UTIs).
Metronidazole is one of the rare examples of a drug developed against a parasite which has since gained broad use as an antibacterial agent. Metronidazole was shown to be efficacious against Entamoeba histolytica, the cause of amebic dysentery and liver abscess. Giardia lamblia (also known as *G*. duodenalis) was treated with metronidazole after this luminal parasite was recognized as a cause of malabsorption and epigastric pain in the 1970s.
The antibacterial activity of metronidazole was discovered by accident in 1962 when metronidazole cured a patient of both trichomonad vaginitis and bacterial gingivitis However, it was not until the 1970s that metronidazole was popularized for treatment of infections caused by gram-negative anaerobes such as bacteroides or gram-positive anaerobes such as clostridia Presently, metronidazole, which is inexpensive, has good tissue penetration, and

produces relatively mild side effects, is on the formulary at most hospitals for prophylaxis against anaerobic infection after bowel surgery, for treatment of wound abscess, and for treatment of antibiotic-associated colitis caused by Clostridium difficile. Metronidazole is an important part of combination therapy against Helicobacter pylori, a major cause of gastritis and a risk factor for stomach cancer.



Norfloxacin
(IUPAC) name:1-ethyl- 6- fluoro- 4-oxo- 7-piperazin- 1-yl- 1 *H*- quinoline-3-carboxylic acid
Formula: $C_{16}H_{18}FN_3O_3$
Molecular Weight: 319.33 g



Metronidazole
(IUPAC) name 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol
Formula: $C_6H_9N_3O_3$
Molecular Weight: 171.15 g

Materials and Methods (Experimental)

Instrument

Systronics UV – VIS spectrophotometer type 118, single beam spectrophotometer with spectral width of 2.0 nm. Wavelength accuracy is + 1.0 nm and a pair of 10 mm matched quartz cells was used to measure the absorbance. The wavelength ranges from 200 – 999.9 nm obtained by a 1200 grooves/mm grating with Czerny – Turner Mount. Fused Silica deuterium lamp and tungsten iodide sources are used to cover the wavelength range. High sensitivity, wide range, reliable silicon photocell is used as the detector. The photometric readings of %T (Percent Transmittance), ABS (Absorbance) and CONC (Concentration) are displayed on an electronic display.

Materials

Pure standard of norfloxacin and metronidazole was obtained from Cipla pharmaceutical Pvt. Ltd. The tablet formulations of the said combination were purchased from a local pharmacy (The label claim contained 400mg of norfloxacin and 500mg of metronidazole.) All the solutions were prepared in double distilled water. All the reagents use were of AR grade.

Preparation of standard and sample solutions

Standard drug solution

A Primary Stock solution of Standard NF was prepared by dissolving 10 mg in 50 mL of 0.1N NaOH to obtain a 200 ppm solution and Standard MNZ was prepared by dissolving 20 mg in 50 mL of 0.1N NaOH to obtain a 400 ppm solution. 10 mL of the primary stock solution was diluted to 100 mL with 0.1N NaOH. 20 µg / mL and 40 µg / mL of standard N and Standard M, respectively, was used for the preparation of the calibration curve.

Sample Solution

Commercial sample (Nor-metrogyl (Lekar Pharma Limited) containing of norfloxacin and metronidazole in combination was procured. Sample contained a label claim of 400mg of norfloxacin and 500mg of metronidazole per tablet. Ten tablets of given sample was weighed and powdered for the analysis. The powder (41 mg) equivalent to 10 mg of norfloxacin and 20 mg of metronidazole was accurately weighed and transferred to a 50 mL standard flask and the sample was dissolved in 0.1N NaOH. The mixture was then mixed and allowed to settle to room temperature. The sample mixture was then filtered with Whatman filter paper no. 41 to removed additives which did not dissolve in the solvent (0.1N NaOH) used. 10mL of this solution was then diluted with 0.1N NaOH to 100mL. Absorbances of these solutions were measured at appropriate wavelengths, i.e. at 275nm and 325nm.

Procedure

Working standard solutions were scanned in the entire range of 200-400 nm to determine λmax of both the drugs. The λmax of norfloxacin and metronidazole were found at 275 nm and 325 nm respectively. By appropriate dilution of two standard drug solutions with 0.1N NaOH, the solution containing 5 µg / mL of norfloxacin and 10 µg / mL of metronidazole i.e. working concentration of NF and MNZ, respectively, was scanned separately in the range of 200 – 400 nm to determine the wavelength of maximum absorption for both the drugs. NF and MNZ showed absorbance maxima at 275nm (λ_1) and 325nm (λ_2) respectively The linearity was obtained in the concentration ranges of 1-10 µg/ml for norfloxacin and 2-20 µg/ml for metronidazole. The absorptivity coefficients of these two drugs were determined by using calibration curve equation. Two simultaneous equations were formed using these absorptivity coefficients values as follows

$A_1 = 136.4\ C_N + 15.6\ C_M$ - (1)
$A_2 = 53.7\ C_N + 54.6\ C_M$ - (2)

Where, A_1 and A_2 are the absorbances of sample at 275 nm and 325 nm respectively. C_N and C_M are the concentration (µg/ml) in of Norfloxacin and Metronidazole in sample respectively. From the resulting concentration obtained after solving above equations, then amount of NF and MNZ present in the given sample was found out.

Analytical Method Validation

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The System suitability was determined with a solution containing 5 µg / mL of NF and 10 µg / mL of MNZ i.e. at the working concentrations of NF and MNZ. For this 2.5 mL of Standard solution was diluted to 10mL with 0.1N

NaOH. These solutions were analyzed with five replicates and the mean was used for the whole calculation.

Specificity

The specificity of method was confirmed by recording the spectra of both the standard solution and the drug sample solutions. The spectra obtained from the drugs sample solution were found to be identical to those obtained for standard solution.

The addition of the standard solution to the drug sample solution for recovery analysis did not change the characteristics of spectra. This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

Linearity and Range

A good linearity was achieved for NF and MNZ in the concentration ranges of 1-10 µg/ml and
2-20 µg/ml respectively. The calibration curves were constructed with concentration (C) against absorbance of both drugs.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. For LOD and LOQ analysis twenty readings for blank recorded then their standard deviation calculated i.e. for LOD= (SD×3+ Mean absorbance of Blank) and for LOQ= (SD×10 + Mean absorbance of Blank.).

Intra-day and Inter-day precision

The intra-day and inter-day precision was used to study the variability of the method. According to USP, ruggedness is the degree of reproducibility of the results obtained under variety of test conditions. It is expressed as percent RSD. It is also called as reproducibility or intermediate precision. It is the analysis of same sample under variety of normal test condition such as different laboratories, different analysts, different instruments, different lots of reagents, different days etc.

It was checked by recording the absorbance as well as spectra of standard solutions of NF and MNZ i.e. working concentrations for NF (4 µg/ml, 5 µg/ml, 6 µg/ml) and for MNZ (8 µg/ml,
10 µg/ml, and 12 µg/ml) with five replicates (both at intra-day (five times within 24 hour) and inter-day (two times each. during 3 days intervals) to check the precision.

Sensitivity

Sensitivity refers to the smallest quantity that can be accurately measured. It also indicates the capacity of the method to record or measure small variations in concentrations. In the case of spectrophotometric methods, a parameter known as "Sandell's Sensitivity" is used to evaluate, the sensitivity of the method. It is the amount required to give an absorbance of 0.001 unit in one square centimeter path.

Assay

For estimation of drugs from commercial formulations, twenty tablets were weighed accurately and finely powdered. 41 mg of powder which is equivalent to (10mg of norfloxacin and 20mg of metronidazole) was accurately weighed and transferred to 50ml volumetric flask and dissolved in 25ml of 0.1N NaOH and sonicated for 10 mins. The solution was filtered through whatman filter paper No.41 and residue was washed thoroughly with given solvent. The filtrate and washings were combined in 50ml volumetric flask and diluted with 0.1N NaOH. The spectra were obtained and absorbance was measured at 275 nm and 325nm and finally concentration of both the drugs was calculated using equations 1 and 2. The amount of NF and MNZ present in the given sample found out. Assay studies were carried out at three different levels i.e. 80%, 100%, 120% level.

The validated spectrophotometric method was used for the simultaneous quantitative determination of NF and MNZ from the formulation. Quantification has been done by simultaneous equation method (SEM). Two samples of different brands were used for the determination. The absorptivity coefficients of these two drugs were determined by using calibration curve equations 1 and 2 mentioned earlier.

Further two more equations were derived with the equations (1) and (2) which are given below:

$$C_N = \frac{(\lambda_2{}^{\varepsilon 2} \text{ X } A\lambda_1) - (\lambda_1{}^{\varepsilon 2} \text{ X } A\lambda_2)}{(\lambda_1{}^{\varepsilon 1} \text{ X } \lambda_2{}^{\varepsilon 2}) - (\lambda_1{}^{\varepsilon 2} \text{ X } \lambda_2{}^{\varepsilon 1})} \quad \text{----- (3)}$$

$$C_M = \frac{(\lambda_1{}^{\varepsilon 1} \text{ X } A\lambda_2) - (\lambda_2{}^{\varepsilon 1} \text{ X } A\lambda_1)}{(\lambda_1{}^{\varepsilon 1} \text{ X } \lambda_2{}^{\varepsilon 2}) - \text{x X} \lambda_2{}^{\varepsilon 1})} \quad \text{----- (4)}$$

C_N and C_M were calculated where C_N = Concentration of standard NF and C_M = Concentration of standard MNZ. From their concentration the amount of NF and MNZ in the tablet formulation is calculated, where,

$\lambda_1{}^{\varepsilon 1}$ = 136.4 X 10^{-3} (N at 275 nm) $\lambda_2{}^{\varepsilon 1}$ = 53.7 X 10^{-3} (N at 325 nm)

$\lambda_1{}^{\varepsilon 2}$ = 15.6 X 10^{-3} (M at 275 nm) $\lambda_2{}^{\varepsilon 2}$ = 54.6 X 10^{-3} (M at 325 nm)

$A\lambda_1$ = Absorbance of Standard N at 275 nm $A\lambda_2$ = Absorbance of Standard M at 325 nm

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard NF and MNZ solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e. from 80% to 120% level.

Table 1: Method validation parameters for the determination of Norfloxacin and Metronidazole

Parameters	SEM	
	Norfloxacin	**Metronidazole**
System suitability (n=5) %RSD	0.68%	0.45%
Linearity range (µg/mL)	1to 10 µg/ml	2 to 20 µg/ml
Correlation coefficient (R^2)	0.9991	0.9993
LOD (µg mL^{-1})	0.0625 µg/ml	0.0625 µg/ml
LOQ (µg mL^{-1})	0.125 µg/ml	0.125 µg/ml
Intraday precision (n=5) %RSD	0.75%	0.65%
Interday precision (n=5) %RSD	0.60%	0.45%
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%
Sandell's Sensitivity (µg / cm^2)	0.007	0.015

SEM: - Simultaneous Equation Method

Table 2: Result of Assay studies of Norfloxacin and Metronidazole

	Method (SEM)	
	Norfloxacin	**Metronidazole**
Labeled claim (mg)	400mg	500mg
Drug found in mg	398.8 mg	492.1 mg
% Assay	99.7%	98.42 %
% RSD (n=5)	0.31	0.47

Sample Used
Nor-metrogyl (Lekar Pharma Limited)
Batch No- 3ANK2037
Label claim- Metronidazole- 500mg & Norfloxacin - 400mg

Table 3: Results of Recovery Experiment

Method Name	Level of % Recovery	% Recovery Found		Standard Deviation (SD)		% (RSD) (N=5)	
		NF	**MNZ**	**NF**	**MNZ**	**NF**	**MNZ**
SEM	0	100.6%	100.2%	0.037	0.032	0.57	0.73
	80 %	99.8%	99.4%	0.041	0.04	0.35	0.51
	100 %	101.01%	101%	0.051	0.045	00.39	0.50
	120%	100.2%	99.7%	0.01	0.07	0.69	0.79

Results and Discussion

1. The proposed method was found to be simple, accurate, sensitive, precise and economical.
2. The given method has been validated as per as ICH guidelines. Method validation parameters for the determination of Norfloxacin and Metronidazole is given in (Table.1) i.e. system suitability, the mean % RSD was found to be less than 1.for both NF and MNZ, which was found to be well within the acceptable limit. Regression analysis (Linearity and Range) for both the drugs was > 0.999 indicate the precision of the validated method. Lower value of LOD and LOQ confirm the sensitivity of the specified method.
3. Repeatability and also inter and intra-day precision was studied where % RSD was found to be less than 1) for both drugs.(Table.1)
4. Accuracy was determined for the given method by calculating the percent recovery at different levels i.e. from 80% to 120% level. The percentage recovery at three different was found to be from 98.00 % to 102.00 % for both the drugs. (Table 3).
5. Quantification of drugs from formulation was done out by assay analysis for the given method. Assay studies were carried out at three different levels i.e. 80%, 100%, 120% level. The percentage assay at three different levels for NF and MNZ was found to be from 98.00 % to 102.00 %. (Table 2).

Conclusion

Application to Analysis of pharmaceutical formulation

All these factors lead to the conclusion that, above validated method mentioned in this paper for simultaneous estimation of norfloxacin and metronidazole by spectrophotometric technique can be recommended for routine quality control analysis in their combined pharmaceutical dosage forms.

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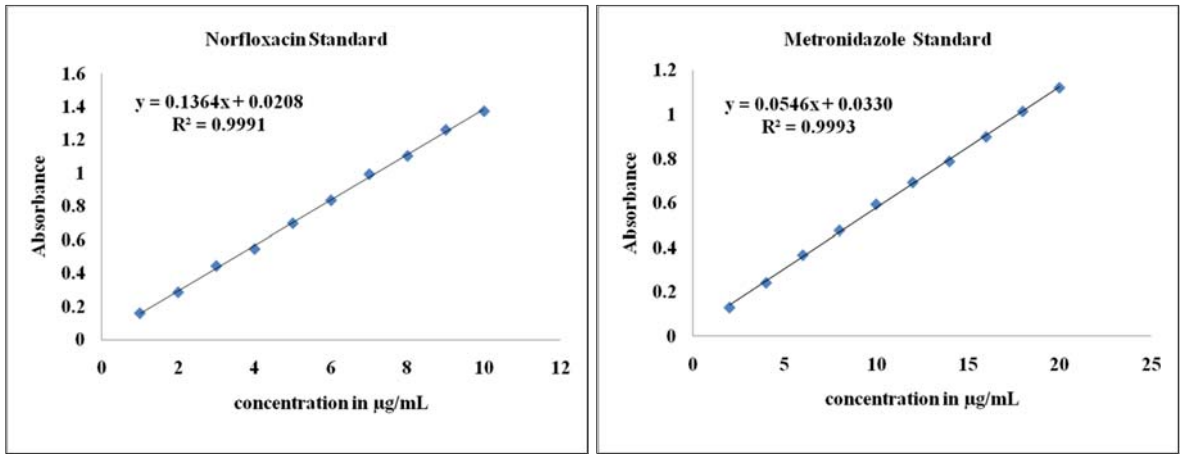

Fig 1: Linearity Graph

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