
Simultaneous Estimation of Metronidazole and Nalidixic Acid In two Component Pharmaceutical Dosage forms by Ultraviolet Spectrophotometry

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A method for simultaneous estimation of metronidazole and nalidixic acid in two component tablet and suspension formulations was established based on dual wavelength spectrophotometry. No prior separation is required. Sodium hydroxide (0.1 N) was used as solvent. Beer Lambert's law was obeyed in the concentration ranges employed for the analysis. The results of analysis have been validated statistically and by recovery studies. The method was found to be simple, rapid, accurate, reproducible and economic.

METRONIDAZOLE and nalidixic acid are official in IP, BP and USP. The IP¹⁻⁴ suggests a titrimetric method for metronidazole (bulk drug and dosage forms) and nalidixic acid (bulk drug). A spectrophotometric method is described for nalidixic acid tablets. Various other methods for the estimation of metronidazole, in formulations, include spectrophotometric⁵⁻⁷, polarographic⁸ and HPLC⁹ techniques. Non-aqueous titrimetric¹⁰, colorimetric^{11,12} and HPLC¹³ methods have been reported for determination of nalidixic acid in dosage forms. But none of the method described the simultaneous estimation of both drugs in combined dosage forms.

The present work describes a dual wavelength spectrophotometric method for the simultaneous determination of metronidazole and nalidixic acid in two component tablet and suspension dosage forms.

EXPERIMENTAL

A Shimadzu UV / Vis recording spectrophotometer (Model : 160 A) with 10 mm matched quartz cells was used in the present study.

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Standard stock solution of strength 50 mcg/ml each of nalidixic acid (NA), metronidazole (MZ) and metronidazole benzoate (MZB) were made in 0.1 N sodium hydroxide (alkaline solution), separately. The six mixed standards of nalidixic acid - metronidazole, and nalidixic acid - metronidazole benzoate having the composition shown in Table - 1 were made in alkaline solution.

Selection of Sampling Wavelength :

Two-Component Tablet Formulations:

The combination of nalidixic acid and metronidazole is available commercially in tablet formulations. To determine the sampling wavelength of nalidixic acid, it was considered as a component of interest and metronidazole as an interfering component, and vice-versa. The standard solution of nalidixic acid and metronidazole was prepared in 0.1 N sodium hydroxide. Nalidixic acid gave two peaks at 258 nm and 335.4 nm., whereas metronidazole has one peak at 321.8 nm., as evident from the UV spectrum of the two drugs (fig-1). The wavelength at which one of the peaks of nalidixic acid appears i.e., 335.4 nm (where metronidazole has considerable absorbance) was selected as the first wavelength (λ_1) for the estimation of nalidixic acid. The wavelength at which metronidazole exhibits absorbance equal to that at λ_1 was selected as the second wavelength (λ_2), which was 306.6 nm. Similarly, the two

Table - 1
Concentration of Nalidixic Acid and Metronidazole / Metonidazole Benzoate in the six mixed standards

Concentration of Component ($\mu\text{g/ml}$)	Standard Solution Number					
	1	2	3	4	5	6
NA	0.0	2.5	5.0	7.5	10.0	12.5
MZ / MZB	0.0	12.5	10.0	7.5	5.0	2.5

NA - Nalidixic acid
 MZ - Metronidazole
 MZB- Metronidazole benzoate

Table - 2
Results of Analysis of Commercial Tablet and Suspension formulations

Commercial Samples		Label claim (mg/tab or 5 ml)		Found* (mg/tab or 5 ml)		Percentage found	
		NA	MZ/MZB	NA	MZ/MZB	NA	MZ/MZB
TABLET	T1	300	200	296.8	198.5	98.33	99.25
	T2	300	200	297.6	199.0	99.20	99.50
	T3	150	200	149.2	199.1	99.46	99.55
SUSPENSION	S1	150	160	149.3	160.1	99.53	100.06
	S2	150	160	148.9	159.7	99.26	99.81
	S3	150	160	150.1	159.8	100.06	99.87

Commercial Samples		Standard Deviation		Coeff. of Variance		Standard Error	
		NA	MZ/MZB	NA	MZ/MZB	NA	MZ/MZB
Tablet		0.2650	0.1607	0.2671	0.1616	0.5750	0.5760
Suspension		0.4069	0.1305	0.4085	0.1306	0.5770	0.4400

* - Mean of five readings
 NA - Nalidixic acid
 MZ - Metronidazole
 MZB - Metronidazole benzoate

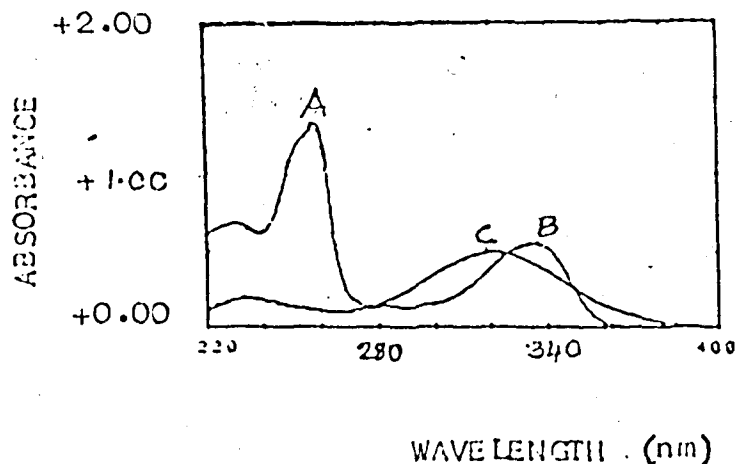
wavelengths selected for the estimation of metronidazole were 321.8 nm and 343.8 nm.

Two Component Suspension Formulations :

The commercial suspension formulations contain nalidixic acid and metronidazole benzoate. The standard

solution of nalidixic acid and metronidazole benzoate was prepared in 0.1 N sodium hydroxide. Similar procedure was used for selection of λ_1 and λ_2 , as described above. The two wavelengths selected for estimation of nalidixic acid and metronidazole benzoate were 334.6 nm and 312.2 nm, and 322.2 nm and 343.6 nm respectively.

Fig. 1: Overlain Spectra of Nalidixic Acid (NA) and Metronidazole (MZ)



Absorbance maxima are obtained for nalidixic acid at 258 nm (A) and 335.4 nm (B) and for metronidazole at 321.8 nm (C)

All the mixed standards were scanned at the respective λ_1 and λ_2 and the absorbance (A1 and A2) were obtained. The A1A2 values versus corresponding concentration of each drug (component of interest) was plotted to get the calibration curves.

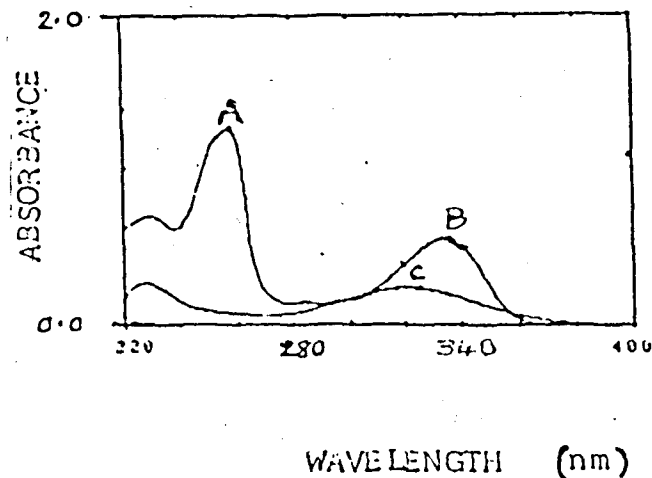
Preparation and Analysis of Two Component Tablet Sample Solutions :

Twenty tablets were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 30 mg of nalidixic acid was weighed accurately from three different brands of tablets, treated with alkaline solution (75 ml) and filtered through Whatman No. 40 filter paper, separately. The residue was washed thoroughly with alkaline solution. The filtrate and washings were combined in 100 ml volumetric flasks. Aliquot of solutions were diluted to obtain final concentration of 6 mcg/ml of nalidixic acid and 4 mcg/ml of metronidazole. The concentration of nalidixic acid and metronidazole, was determined by analyzing these solutions. The results of analysis and the statistical parameters are given in Table-2.

Preparation and Analysis of Two Component Suspension Sample Solution:

One milliliter each of suspension was taken from three different brands, after thorough shaking, and transferred to 100 ml volumetric flasks, separately. The pipette was

Fig. 2: Overlain Spectra of Nalidixic Acid (NA) and Metronidazole Benzoate (MZB)



Absorbance maxima are obtained for nalidixic acid at 258 nm (A) and 334.6 nm (B) and for metronidazole benzoate at 322.2 nm (C)

rinsed with little amount of alkaline solution into the respective volumetric flasks. The mixture was treated with alkaline solution (75 ml) and filtered through Whatman No. 40 filter paper. The residue was washed thoroughly with alkaline solution. The filtrate and washings were combined. Aliquot of solutions were diluted to obtain final concentration of 6 mcg/ml of nalidixic acid and 6.4 mcg/ml of metronidazole benzoate. The concentration of nalidixic acid and metronidazole benzoate was determined by analyzing these solutions. The results of analysis and the statistical parameters are given in Table-2.

Recovery Studies:

The recovery studies conducted by addition of different amount of pure drugs to a preanalysed tablet / suspension sample solution gave satisfactory recovery data which are tabulated in Table - 3.

RESULTS AND DISCUSSION

"The absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest, independent of the interfering component" is the basic principle underlying dual wavelength method of analysis. The proposed method was found to be simple, rapid and accurate for routine

Table - 3 : Recovery Studies

Sample	Concentration of added amount of drug in final dilutions ($\mu\text{g/ml}$)		Recovery ($\mu\text{g/ml}$)		Percent Recovery ($\mu\text{g/ml}$)	
	NA	MZ / MZB	NA	MZ / MZB	NA	MZ / MZB
T1	5.5	5.0	5.46	4.90	99.28	98.00
T2	5.5	5.0	5.47	5.00	99.27	100.00
T3	4.5	4.5	4.50	4.47	100.00	99.30
S1	6.0	6.0	6.01	5.91	100.10	98.50
S2	5.0	5.0	4.90	4.99	98.00	99.80
S3	6.0	6.0	5.94	5.95	99.00	99.10

NA - Nalidixic acid

MA - Metronidazole

MZB- Metronidazole benzoate

simultaneous estimation of metronidazole and nalidixic acid in oral dosage forms, requiring no prior separation. When metronidazole and metronidazole benzoate was estimated, it was considered as component of interest and nalidixic acid as interfering component and vice-versa. The interference of interfering component was neglected by selecting the proper λ_1 and λ_2 for the component of interest.

The values of standard deviation and coefficient of variance were satisfactorily low, and the recoveries were close to 100% indicating the reproducibility and accuracy of the method.

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