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Simultaneous Spectrophotometric Assay for Estimation of Norfloxacin and Metronidazole in Tablets

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The present work deals with the simple, accurate, precise and economical method for the simultaneous estimation of norfloxacin and metronidazole in tablet dosage form by Vierodt's UV spectrophotometric method. The absorption maxima (λ_{max}) of norfloxacin and metronidazole in 0.1 N HCl were found to be 278.0 and 277.5 nm respectively. Since the λ_{max} values of the chosen drugs were found to be significantly close, 278 nm was undertaken as a measuring wavelength for the present study. The drugs of combination also shows significant absorption in the wavelength 320 nm which was chosen as the second wavelength for the experiment. Both of the drugs followed Beer's law in the concentration range 1-10 and 5-25 µg/mL, respectively. The A1 % values for norfloxacin and metronidazole in the wavelength 278 nm and 320 nm were estimated to be 1273 and 320; 346 and 51, respectively.

Key Words: Norfloxacin, Metronidazole, Spectrophotometry.

INTRODUCTION

Norfloxacin (NRX) (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)-3-quinoline carboxylic acid)[1], is a broad spectrum fluoroquinolone antibacterial agent used in the treatment of various bacterial infections caused by gram-positive and gram negative microorganisms. It is a widely used and broad spectrum antibiotic having broader spectrum of activities than many other antibiotics like aminoglycosides, macrolides and 3rd generation cephalosporins. Due to some better therapeutic activity and lower toxicity, it is also used preferentially than many other fluoroquinolones. Metronidazole (MRN) is a very popular drug mainly used as antiamoebic agent in g.i. tract infections. Chemically metronidazole is 1-(2-hydroxy ethyl)-2-methyl-5-nitroimidazole. Metronidazole in combination with norfloxacin is used in intra-abdominal infection. Norfloxacin is official in IP[2], BP and USP. The IP and USP described HPLC methods and BP[3] describes non-aqueous titration methods[4] for estimation of norfloxacin. Literature survey[5,6] revealed HPLC and antibiotic assay methods

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for its determination in dosage forms and biological fluids[7]. The combination of NRX and MRN is not official in any pharmacopoeia; hence no official method is available for the estimation of NRX and MRN in their combined dosage form. Literature survey also revealed that there is no simple spectrophotometric method available for estimation of these drugs in combined dosage form. The present communication describes one simple, accurate, sensitive, rapid and economical method for simultaneous estimation of NRX and MRN in tablet dosage form. The Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer with spectral width 2 nm, wavelength accuracy 0.5 nm with 10 nm matched quartz cells was used for experiments[8-10].

EXPERIMENTAL

The chemicals used were of analytical grade. An AFCOSAT- ER 200A Digital balance, an ultrasonic cleaner (Frontline FS 4), Dissolution test apparatus (Veego, single station), norfloxacin I.P. 99.55 % and metronidazole USP 99.235 % (Caplet India Pvt. Limited) and double-glass distilled water were used for the study.

The solubility of NRX and MRN were checked in various solvents and 0.1 (N) HCl was found to be the common solvent for both the drugs undertaken for the study. The standard stock solution of NRX and MRN was prepared by dissolving 10 mg of each drug in 100 mL volumetric flask separately using glass-distilled water. Final working standard solutions of 10 µg/mL of NRX and MRN were prepared by diluting 10 mL of the above solutions separately in two 100 mL volumetric flasks.

RESULTS AND DISCUSSION

In the first method, working standard solutions were scanned in the entire UV-range 200-400 nm to record the λ_{max} of both the drugs. The λ_{max} of NRX and MRN were found to be 277 and 278 nm. But these two wavelengths were found to be very close to each other to provide notably different absorbance values for the two drugs. For that purpose, the scanned spectra of the two drugs were overlain and a second wavelength 320 nm was chosen from the point of intersection of the two spectra where both of the drugs have significant absorbance values (Fig. 1). Two set of dilutions of the two drugs were prepared separately for working in the two wavelengths from the standard stock solutions. For estimation in the wavelength 278 nm, six dilutions were prepared having dilutions 1, 2, 4, 6, 8 and 10 µg/mL of both NRX and MRN. Similarly for wavelength 320 nm, five dilutions were prepared of those two drugs in concentrations 5, 10, 15, 20 and 25 µg/mL using 0.1 N HCl. The absorbances of the resulting solutions were measured at the specified wavelengths and calibration curves were plotted in these wavelengths. The absorptivity coefficients (A 1 %, 1 cm) of these two drugs were calculated from their calibration curve equations (Table-1). According to Vierodt's method[11], two simultaneous equations were formed using the absorptivity coefficients of NRX and MRN in the specified wavelengths.

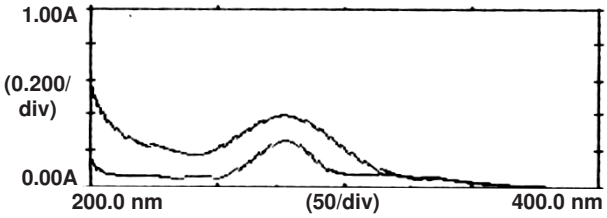

Fig. 1. Overlain spectra of norfloxacin and metronidazole

TABLE-1
VALIDATION AND SENSITIVITY OF PROPOSED METHOD

Parameters	Wavelength 1		Wavelength 2	
	NRX	MRN	NRX	MRN
Wavelength for measurement (nm)	278	278	320	320
Sandell's sensitivity (μg/mL/cm^2/0.001 abs.unit)	-0.07227		0.07514	
Absorptivity (or Extinction) coefficient (0.01 abs. g cm^{-3})	0.55625		1.6078	
Regression values:	1273.0172	319.9832	345.9984	50.9928
I. Slope (Y*):	0.1273	0.032	0.346	0.0051
II. Intercept on Y-axis	0.0102	-0.0168	-0.0016	-0.0072
Correlation coefficient (r^2)**	0.9983	0.9997	0.9985	0.9927
Standard error	0.01907	0.00506	0.00507	0.00158

* Y = mx + C, where 'C' is the concentration in μg/mL and 'Y' is the absorbance unit.
**Six replicate samples for norfloxacin and five replicate samples for metronidazole.

From the absorptivity coefficient values, two equations were constructed such as:

$$A_{278} = 1273\ C_x + 320\ C_Y \quad (1)$$

$$A_{320} = 346\ C_x + 51\ C_Y \quad (2)$$

where A_{278} and A_{320} are absorbance values of the mixed sample systems at 278 and 320 nm respectively and C_x and C_Y are concentrations in (μg/mL) of norfloxacin and metronidazole, respectively.

Twenty tablets were purchased from the market (each containing NRX 400 mg and MRN 500 mg) and subjected to dissolution in the dissolution test apparatus under standard conditions. After 1 h, the aliquot mixture from the medium was withdrawn and filtered so that a tablet stock solution of 18 mg/mL concentration was prepared. From this, suitable dilution (5:100) was made so that a final working standard solution of concentration 900 μg/mL was made. From this stock solution, suitable volumes were withdrawn and diluted so that a series of final dilutions were made in the range of 9-30 μg/mL for the combined formulation of norfloxacin and metronidazole. All the concentrations were measured in both the specified wavelengths at 278 and 320 nm and absorbance values were noted. Putting these absorbance values into eqns. 1 and 2 concentration values were calculated for norfloxacin and metronidazole. From all these replicate values, tablet content of both norfloxacin and metronidazole were calculated and the average was estimated to get a final batch content of norfloxacin and metronidazole (Table-2).

TABLE-2
COMPILATION OF RESULTS OF STATISTICAL ANALYSIS OF COMMERCIAL FORMULATIONS

Tablet brand	Tablet component	Label* claim (mg/tabl)	Amount* found (mg/tabl)	Amount* found	SD*	RSD* (%)	SE*	't' calc.
A	Norfloxacin	400	397.24	99.31	18.2718	4.5996	7.4597	19.1778
	Metronidazole	500	546.19	109.23	45.8023	8.3858	18.6993	48.0759
B	Norfloxacin	200	204.67	102.335	5.2551	2.5676	2.1454	5.5158
	Metronidazole	300	345.60	115.20	36.6517	10.6052	14.9635	94.2316

*Average of six determinations, Theoretical 't' values are at 95 % confidence level for (n-1) degrees of freedom. 't' (0.05,5) = 2.2571, SD is the standard deviation, % RSD is per cent relative standard deviation, SE is standard error.

To study the precision, accuracy and reproducibility of the proposed method, recovery studies were done by adding known amount of drugs to the pre analyzed tablet solution and analyzing the drugs by the present method. The procedure was repeated for five times and it was observed that the excipient present in the tablets did not interfere in the estimation of norfloxacin and metronidazole. The occurrence of non-interference of excipients in the analysis of tablet formulations was also validated by scanning of the filtered tablet solutions in the specific range, where no second significant peak came in the scanned spectrum of the two market tablet formulations. The recovery study data are provided in Table-3.

TABLE-3
RESULTS OF RECOVERY STUDIES

Tablet brand	Tablet component NRX (µg/mL)	NRX added (µg/mL)	Recovery (%) ± SD*	Tablet component MRN (µg/mL)	MRN added (µg/mL)	Recovery (%) ± SD*
A	6.0	2	101.82±2.37	7.5	25	102.17±3.62
B	3.6	2	102.56±4.26	5.4	25	100.40±2.35

*Average of six determinations, SD is the standard deviation
Recovery (%) = 100 x (recovered amount/added amount).

The validation parameters were studied at all the specific wavelengths for the method undertaken. Accuracy was determined by calculating the recovery and the average was determined. Precision was calculated as repeatability (standard deviation and relative standard deviation[12]) and inter and intra day variation (% CV) calculated for both the drugs. The method was successfully used to determine the amount of NRX and MRN present in the tablets. The results obtained were in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be simple, accurate, specific and precise. Hence, this method can be successfully applied for the routine analysis of these two drugs in combinations.

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REFERENCES

1. K. Parfitt, In Martindale, The Complete Drug Reference, The Pharmaceutical Press, London, edn. 33, p. 659 (2002).
2. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, New Delhi, India, edn. 4, Vol. 1, p. 489, 523 (1996).
3. British Pharmacopoeia, Her Majesty's Stationary Office, London, Vol. 2, p. 1306, 1398 (2004).
4. J.M. Jalal, A. Hussen and H.S. Khalil, *Anal. Lett.*, **20**, 635 (1987).
5. E.M. Abdel-Moety and O.A. Al-deeb, *Eur. J. Pharm. Sci.*, **5**, 1 (1997).
6. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Current Step 4 Version (2005).
7. S.M. Desai, J.M. Khatri and J.K. Lala, *Indian Drugs*, **27**, 389 (1990).
8. G. Garg, S. Saraf and S. Saraf, *Indian J. Pharm. Sci.*, **69**, 279 (2007).
9. A.V. Kasture and M. Ramteke, *Indian J. Pharm. Sci.*, **67**, 752 (2005).
10. V. Nagulwar, Y.R. Dhurvet, S. Deshpandey, K. Upadhye, S. Bakhle and R. Wadetwar, *Indian J. Pharm. Sci.*, **68**, 639 (2006).
11. A.H. Beckett and J.B. Stenlake, In Practical Pharmaceutical Chemistry, CBS Publishers and Distributors, New Delhi, India, edn. 4, Vol. 2, p. 285 (1998).
12. C.C. Chatterjee, Medical Statistics, In Medical Physiology, Medical Allied Agency, Kolkata, Vol. 2, pp. 101-126 (2004).

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