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Simultaneous Estimation of Metronidazole and Ofloxacin in Suspension Dosage Form Using UV-Visible Spectrophotometer

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Four accurate, sensitive and economical procedures for simultaneous estimation of metronidazole and Ofloxacin in suspension dosage form have been developed. The methods employed are Simultaneous equation method, first order derivative method, two wavelengths method and graphics absorbance ratio method for the simultaneous estimation of metronidazole and ofloxacin. The first method employs the formation and solving of simultaneous equations using 324.8 nm and 301.4 nm as the two wavelengths for forming equations. The second method employs first order derivative spectroscopy to eliminate spectral interferenĉe. The third method employs 324.8 nm and 282.8 nm as λ_1 and λ_2 and 301.0 nm and 344.8 nm as λ_1 and λ_2 for metronidazole and ofloxacin respectively. The fourth method employed 266.0 nm as A_1 (isobestic point) and 324.5 nm as A_2 that is the λ_{max} of metronidazole. Both drugs obey Beer's law in the concentration ranges employed for the analysis. The results of analysis have been validated statistically and by recovery studies.

Key Words: Estimation, Metronidazole, Ofloxacin, UV-Visible spectrophotometer.

INTRODUCTION

Metronidazole (MT), 2-methyl-5-nitro-1-H-imidazole-1-ethanol is used as anti-amoebic, anti-trichromonal and anti-giardial. It is official in IP, BP and USP, which suggest a titrimetric method for its estimation[1–3]. Several spectrophotometric and HPLC methods have been reported for the estimation of metronidazole in tablets and biological samples[4–9]. Ofloxacin (OF), (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1H -benzoxazine-6-carboxylic is used as antibacterial and ocular antiinfectives. Its method of analysis is given in USP, as it is an official drug only in USP[10]. Further literature survey revealed some more methods for its estimation from pharmaceutical preparation and includes spectrophotometric, HPLC, RP-HPLC and HPTLC methods[11–24]. There is no single method developed for simultaneous determination of metronidazole and ofloxacin from pharmaceutical preparation in combined dosage form. Hence in the present investigation simple, rapid and reproducible methods were developed for the simultaneous estimation of metronidazole and ofloxacin from their combined dosage form.

EXPERIMENTAL

Shimadzu UV-160A: Spectrophotometer using 1 cm quartz cells was used for the experimental purpose. Shimadzu electronic balance was used for weighing the samples.

N,N-dimethyl formamide (DMF) of analytical grade (S.D. Fine-Chem Limited, Mumbai) was used as solvent. Gift samples of MT and OF were obtained from Ranbaxy Laboratories Ltd., Dewas (M.P.). A combination of both drugs, ofloxacin USP 50 mg and metronidazole 100 mg in each 5 mL in suspension dosage form is marketed by MacLeods Pharmaceutical Ltd. using the trade name Ofloxacin MN.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 50 mg of each drug in 100 mL of DMF to get a concentration 500 µg/mL for both the drugs separately. The absorbance was measured at 324.8 nm for MT and 301.4 nm for OF against DMF. Both the drugs obey Beer's law individually and in mixture within the concentration range of 5–30 µg/mL. Fig. 1 represents the overlain spectra of both the drugs.

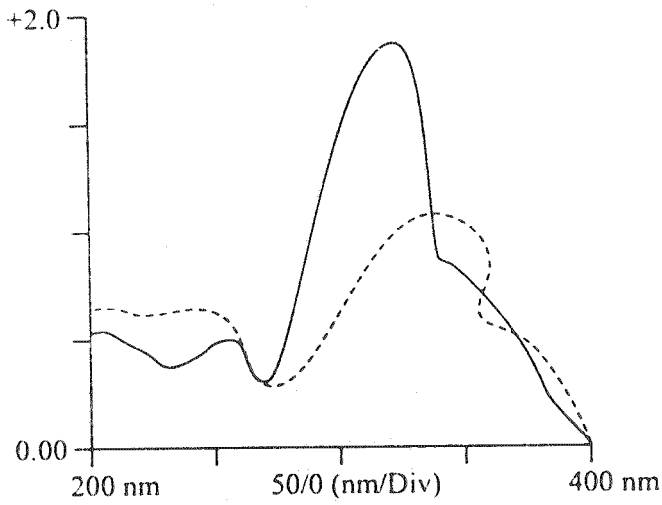

Fig. 1. Overlain spectra of metronidazole and ofloxacin. UV absorption spectra of metronidazole (·····) (20 mcg/mL) and ofloxacin (——) (20 mcg/mL) in DMF

Preparation of sample stock solution

Sample stock solution was prepared by pipetting out 5 mL of the suspension preparation containing 50 mg of ofloxacin USP and 100 mg of metronidazole. The above was diluted up to 100 mL with DMF in 100 mL volumetric flask. Further dilutions were made from this sample stock solution to get the required concentration.

Simultaneous equation method (Method I)

The overlain zero order spectra of metronidazole and ofloxacin are shown in Fig. 1. The figure indicates that absorption maxima of MT is at wavelength 324.8

nm while ofloxacin has absorption maxima at 301.4 nm. Spectrum of standard solution of metronidazole having concentration of 20 μg/mL was recorded in the range of 200–400 nm against DMF. Absorption was determined at wavelength 324.8 nm and 301.4 nm. In the first method, the molar absorptivity coefficients at these wavelengths were calculated. Similarly, spectruna of standard solution of ofloxacin having concentration 20 μg/mL was recorded in the range of 200–400 nm against DMF. Absorption was determined at wavelengths 324.8 nm and 301.4 nm. The molar absorptivity coefficient at these wavelengths was calculated. Molar absorptivity (ε_1) of MT is 8.703×10^3 L/mole cm at 324.8 nm (λ_1) and 5.511×10^3 1/mole cm at 301.4 nm (λ_2), while molar absorptivity (ε_2) of OF is 13.045×10^3 L/mole cm at 324.8 nm (λ_1) and 32.253×10^3 L/mole cm at 301.4 nm (λ_2).

Estimation from marketed preparation

An aliquot of sample stock solution (2 mL) was transferred to 100 mL standard volumetric flask and volume was made up to the mark with DMF. This solution was scanned in the range 200–400 nm against DMF as blank. Absorbances ($A\lambda_1$ and $A\lambda_2$) were recorded at wavelengths 324.8 nm and 301.4 nm. The concentration of each drug was then calculated by using equations given below for both metronidazole and ofloxacin respectively:

$$\text{Concentiation of metronidazole} = (\lambda_2\varepsilon_2 A\lambda_1 - \lambda_1\varepsilon_2 A\lambda_2)/(\lambda_1\varepsilon_1\lambda_2\varepsilon_2 - \lambda_1\varepsilon_2\lambda_2\varepsilon_1) \quad (1)$$

$$\text{Concentiation of ofloxacin} = (\lambda_1\varepsilon_1 A\lambda_2 - \lambda_2\varepsilon_1 A\lambda_2)/(\lambda_1\varepsilon_1\lambda_2\varepsilon_2 - \lambda_1\varepsilon_2\lambda_2\varepsilon_1) \quad (2)$$

First order derivative spectroscopy (Method II)

The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug. First order derivative spectra of both the drugs were recorded (Fig. 2). It was observed that metronidazole showed $dA/d\lambda$ zero at 323.4 nm in contrast to ofloxacin that has considerable $dA/d\lambda$. Further, ofloxacin has zero $dA/d\lambda$ at 300.8 nm while at this wavelength metronidazole has significance $dA/d\lambda$. Therefore these two wavelengths can be employed for the estimation of metronidazole and ofloxacin without any interference. The calibration curves were plotted at these two wavelengths using different concentrations against absorbance within the range mentioned above. The equations obtained to determine concentration of metronidazole and ofloxacin are as follows respectively:

$$C = 82.716 \times Abs + 0.0434 \quad (3)$$

$$C = 209.25 \times Abs - 0.0207 \quad (4)$$

Estimation from marketed preparation

An aliquot sample stock solution (2 mL) was transferred to 100 mL standard volumetric flask and volume was made up to the mark with DMF. This solution

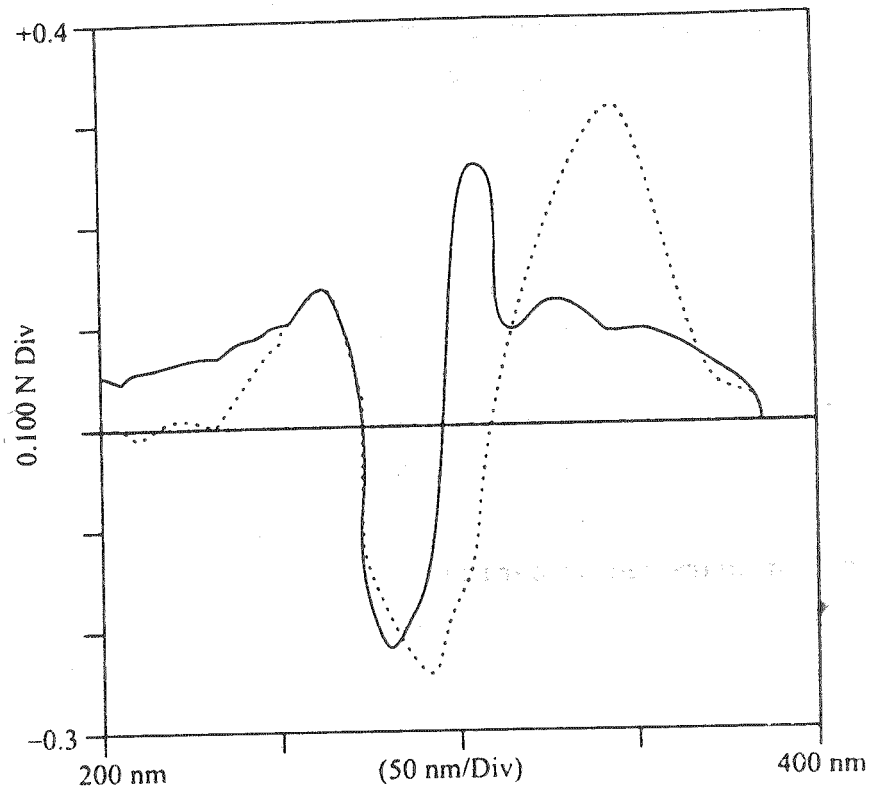

Fig. 2. Overlain first derivative spectra of metronidazole and ofloxacin. UV absorption spectra of metronidazole (——) (20 mcg/mL) and ofloxacin (·····) (20 mcg/mL) in DMF

was scanned in the range 200–400 nm against DMF as blank. Then the spectra were derivatized to first order derivative (n = 6), $dA/d\lambda$ were measured at wavelengths 323.4 nm and 300.8 nm. Using the equations (3) and (4), the concentration of each drug was calculated.

Two wavelengths method (Method III)

In the third method, further dilutions have been made from the standard stock solution to give 20 µg/mL of each MT and OF separately. The absorbance of ofloxacin was measured at 324.8 nm (λ_1 which is λ_{max} for metronidazole) and the wavelength at which the same absorbance was found to be same with that of 324.8 nm has been recorded and was found to be 282.8 nm (λ_2 for metronidazole). The same method is applied for the determination of λ_1 and λ_2 for ofloxacin which was found to be 301.4 and 344.8 nm respectively. The suitable mixed standards were scanned at the respective λ_1 and λ_2 for MT and OF respectively and A_1-A_2 values *vs.* corresponding concentrations were plotted to get the calibration curve. The equations obtained for the estimation of metronidazole and ofloxacin concentration are as follows respectively:

$$C = 32.103 \times Abs + 0.2490 \quad (5)$$

$$C = 16.7 \times Abs - 0.0578 \quad (6)$$

Estimation from marketed preparation

An aliquot of sample stock solution (2 mL) was transferred to 100 mL standard volumetric flask and volume was made up to the mark with DMF. This solution

was scanned in the range 200–400 nm against DMF as blank. Absorbances were recorded at wavelengths 324.8, 282.8, 301.4 and 344.8 nm. The concentration of each drug was then calculated by using eqns. (5) and (6).

Graphical absorbance ratio method (Method IV)

In the fourth method, the isoabsorptive point for both the drugs has been determined from the standard stock solution which is found to be 266.0 nm for A_1 and 324.8 nm for A_2, which is the λ_{max} for metronidazole as selected. The absorbances at 266.0 nm were noted and the absorbance ratio (A_1/A_2) was calculated in the photometric mode from the two values. The equations obtained for the estimation of metronidazole and ofloxacin concentrations are as follows respectively:

$$C = -4.6830 \times Abs + 2.4214 \quad (7)$$

$$C = 4.6849 \times Abs - 1.4221 \quad (8)$$

Estimation from marketed preparation

An aliquot of sample stock solution (2 mL) was transferred to 100 mL standard volumetric flask and volume was made up to the mark with DMF. This solution was scanned in the range 200–400 nm against DMF as blank. Absorbances were recorded at wavelengths 324.8 nm and 266.0 nm. The concentration of each drug was then calculated by using eqns. (7) and (8).

RESULTS AND DISCUSSION

Results of analysis for all methods are given in Table-1. To determine the precision and accuracy of the method, recovery experiments were performed using the proposed methods. A fixed volume of standard solution was added to different concentrations of sample solutions. The total amount of drug was then determined by these methods and the amount of added drugs was found by difference. The results of recovery are given in Table-2. All the four methods were found to be accurate, simple and rapid for routine simultaneous analysis of the drugs from the formulations without prior separation. In the first method, once absorptivity coefficients were determined, very little time is required for analysis, as it would only require determination of absorbances of the sample solutions at the selected wavelengths and a few calculations. The second method is used to eliminate the spectral interference from one of the two drugs while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug at the selected wavelengths. The third method is based on the fact that 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 components. The fourth method involved only the ratio of observed absorbances at two selected wavelengths, one being the isobestic point and the other being the λ_{max} of any one of the drugs. All the above methods are less time consuming and can be easily applied to routine analysis.

TABLE-1
RESULTS OF COMMERCIAL SAMPLE ANALYSIS

Drug/Label claim (mg/5 mL of suspension)	Method I		Method II		Method III		Method IV	
	Amount* found (mg)	± Standard deviation	Amount* found (mg)	± Standard deviation	Amount* found (mg)	± Standard deviation	Amount* found (mg)	± Standard deviation
Metronidazole/100	99.893	0.041956	99.968	0.016005	99.981	0.0050	99.989	0.0020
Ofloxacin/50	49.874	0.097700	49.958	0.026624	49.971	0.0220	49.957	0.0386

Asterik (*) denotes mean of five determinations.

TABLE-2
RECOVERY STUDY OF COMMERCIAL SAMPLE

Drug	Concentration of amount added in final solution (μg/mL)	Recovery (%)			
		Method I	Method II	Method III	Method IV
Metronidazole	5	99.944	99.940	99.989	99.967
	5	99.953	99.960	99.909	99.945
	5	99.953	99.967	99.953	99.957
Ofloxacin	10	99.944	99.930	99.850	99.858
	10	99.939	99.942	99.939	99.945
	10	99.945	99.860	99.860	99.841

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