ejpmr, 2023, 10(9), 405-411

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF OFLOXACIN AND METRONIDAZOLE BY SIMULTANEOUS EQUATION METHOD

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Article Received on 24/06/2023 **Article Revised on 14/07/2023** **Article Accepted on 04/08/2023**

ABSTRACT

The UV spectrophotometer has successfully accomplished simultaneous estimation. The simultaneous equation approach uses the creation and solution of simultaneous equations in mathematics. The suggested approaches are straightforward, quick, affordable, and accurate for routine simultaneous measurement of ofloxacin and mertronidazole, and they have been effectively used in pharmaceutical formulation without excipient influence. Utilising the USP II dissolution test apparatus, the simultaneous equation approach was successfully used to conduct a dissolution analysis of a commercial tablet formulation. The primary goal of this study is to provide a straightforward, affordable, and trustworthy UV spectroscopic approach for the regular measurement of ofloxacin and metronidazole in suspension dose form. There is no literature available for the simultaneous UV spectroscopic assessment of ofloxacin and metronidazole in suspension dose form. In order to simultaneously estimate ofloxacin and metronidazole in suspension formulation, a spectroscopic approach was created. The created technique was approved in accordance with ICH norms.

KEYWORDS: Ofloxacin, Mertronidazole, Simultaneous equation, UV spectrophotometer.

INTRODUCTION

The Light of Knowledge is an often used phrase, but it is particularly appropriate in reference to spectroscopy. Most of what we know about the structure of atoms and molecules comes from studying their interaction with light (electromagnetic radiation). Different regions of the electromagnetic spectrum provide different kinds of information as a result of such interactions. Realizing that light may be considered to have both wave-like and particle-like characteristics, it is useful to consider that a given frequency or wavelength of light is associated with a "light quanta" of energy we now call a photon. As noted in the following equations, frequency and energy change proportionally, but wavelength has an inverse relationship to these quantities. The spectroscopic techniques described below do not provide a three-dimensional picture of a molecule, but instead yield information about certain characteristic features. A brief summary of this information follows:

- **Mass spectrometry:** Sample molecules are ionized by high energy electrons. The mass to charge ratio of these ions is measured very accurately by electrostatic acceleration and magnetic field perturbation, providing a precise molecular weight. Ion fragmentation patterns may be related to the structure of the molecular ion.
- **Ultraviolet-Visible spectroscopy:** Absorption of this relatively high-energy light causes electronic excitation. The easily accessible part of this region (wavelengths of 200 to 800 nm) shows absorption only if conjugated pi-electron systems are present.
- **Infrared spectroscopy:** Absorption of this lower energy radiation causes vibrational and rotational excitation of groups of atoms. Within the molecule. Because of their characteristic absorptions identification of functional groups is easily accomplished.
- **Nuclear magnetic resonance spectroscopy:** Absorption in the low-energy radio-frequency part of the spectrum causes excitation of nuclear spin states. NMR spectrometers are tuned to certain nuclei (e.g. ^{1}H, ^{13}C, ^{19}F & ^{31}P). For a given type of nucleus, high-resolution spectroscopy distinguishes and counts atoms in different locations in the molecule.

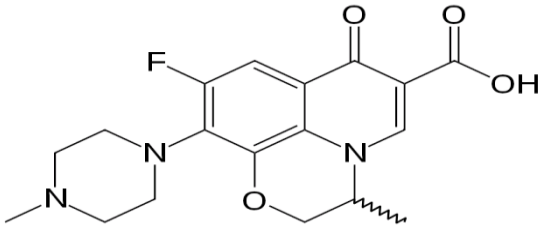
Applications

- Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the

colour of a dilute solution of copper sulfate is a very light blue; adding ammoniaintensifies the colour and changes the wavelength of maximum absorption (λ_{max}).

- Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, orethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.) Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases.

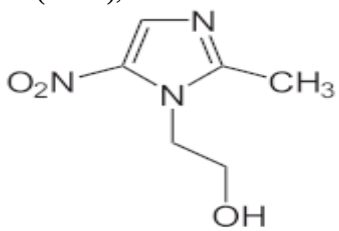
- **Ofloxacin**



- **IUPAC Name:** 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid

Molecular formula: $C_{18}H_{20}FN_{304}$
Molecular weight: 361.368g/mol
Category: Antibiotic

Physicochemical properties
Solubility: 1, 2 dichloromethane, chloroform, methanol, carbon tetrachloride and water
Pka: 5.97
Melting point: 254°C
Metronidazole (**MNZ**),



- **IUPAC Name:** 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol
- **Molecular formula:** $C_6H_9N_3O_3$
- **Molecular weight:**171.156g/mol
- **Category:** Antibiotic

- **Physicochemical properties**
- **Solubility:** water, Methanol and alcohol
- **Pka:** 2.38
- **Melting point:** 160.5°C

MATERIAL AND METHODS

Apparatus and Instruments of the experimentation

- Double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path.
- Electronic Analytical Balance (Model Shimadzu AUW-220D)
- Ultra Sonicator. (Life Care Equipment Pvt. Ltd)
- Pipettes – 1,2, 5, 10 ml.
- Volumetric flasks – 10,25, 50, 100 ml (Merck).
- Beaker – 250 ml.

The Reagents and Materials of the work

Ofloxacin (Lupin Research Park, Aurangabad, Maharashtra State)
Metronidazole (Lupin Research Park, Aurangabad, Maharashtra State)
Methanol - analytical grade (Astron Chemicals LTD., Ahmadabad)Marketed formulation (OLMESAR, Macleod Pharma)

Preparation of standard stock Solution & Calibration curve

Preparation of standard stock solutions: Accurately weighed portion of MET (10 mg) and OFL (10 mg) were transferred to two different 100 mL volumetric flask. The volume was made up to mark with the prepared diluents solution to obtain standard stock solution having concentration of 100µg/mL of MET and OFL each.

Simultaneous equation method: Working standard solutions (10µg/mL) each of Ofloxacin and Metronidazole were scanned in range of 200-400 nm to determine the λ-max of both drugs. The λ-max of Ofloxacin and Metronidazole were found to be 294.0 nm and 340.0 nm respectively fig.9.1 &9.2 five standard solutions having concentration 2,4,6,8 & 10µg/mL for Ofloxacin and 2,4,6,8 and 10µg/mL for Metronidazole were prepared by appropriate dilutions from their respective standard stock solutions. The absorbances of resulting solutions were measured at 294.0 nm and 340.0 nm and absorptivity coefficients were calculated using Beer Lambert law. The graph of absorbance Vs concentration was plotted at each wavelength and regression coefficients were calculated.

The concentrations of both drugs were calculated by solving these simultaneous equations.

C x = (A1 aY2 - A2 Ay1) / (aX1 aY2 - aX2 aY1)…….…... (1)

C y = (aX1 A2 - aX2 A1) / (aX1 aY2 - aX2 ay1)…… (2)

Where; Cx & Cy are concentrations of MET and OFL respectively in gm/100 ml in the sample solution.
A1 & A2 are the absorbances of the mixture at 340.0nm & 294.0 nm respectively

aX1 and aX2 =Absorptivity of MET at 318.0nm and 294.0nm aY1 and aY2=Absorptivity of OFL at 340.0nm and 294.0nm

Method of validation

1) The Linearity and Range

The Linearity is articulated in the terms of a correlation co-efficient of the linear regression analysis. The linearity response range was to a determined by analyzing five independent or does not depend levels of calibration curve in a range of a 5-25µg/ml for Ofloxacin and 2-10µg/ml for Metronidazole. The calibration curve of absorbance vs concentration was a plotted and correlation coefficient ratio and regression line equations of the Ofloxacin and Metronidazole were determined for such range. The result shown in table no. 9.1&9.2.

2) Precision of drugs

a) Intraday precision

The Intraday precision, it was carried or agreed out by prepare three replicates of three different concentrations, inside the linearity range and the measure the absorbance of each of the solutions on the same day same way. The % RSD (% relative standard deviation) it was calculated. The absorbance at 294nm and 340nm is taken into calculation and absorptive coefficients were calculated by using calibration curve. The result shown in table no. 9.

b) Interday precision

The Interday precision, it was carried or agreed out by prepare three replicates of three different concentrations, inside the linearity range and measure the absorbance of each solution on the three different days. The % RSD (% relative standard deviation) was calculated. The absorbance at 294nm and 340nm is taken into calculation and absorptive coefficients were calculated by using calibration curve. The result shown in table no. 9.4.

3) Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of Metronidazole and ofloxacin at three concentration levels of 80, 100 and 120 % were added. At each level %recovery was determined, which are in the range of 98-102%. The results are given in Table The result shown in table no. 9.5.

4) Limit of detection

It is the lowest amount of analyze in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3×N/S Where,

N = Standard deviation of the response and

S = Slope of the corresponding calibration curve. Results are shown in the table no. 9.6

5) Limit of quantification

It is the lowest concentration of analyze in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines. The result shown in table no. 9.6

6) Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Dronedarone hydrochloride using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to be reproducible, since there was no significant difference b/w analyst. Thus, the proposed methods could be considered rugged. The results are shown in table no. 9.7

Analysis of OFLO and METRO in pharmaceutical formulations

Tablets were weighed and powdered. An average weight of the tablet containing the two drugs ofloxacin and metronidazole in the ration of 2:3 and the amount of 580 mg was dissolved in 30 mL methanol by vigorously shaking and the volume was made up to the mark. The solution was then filtered through Whatmann filter paper No. 41 and the solution was diluted to get a final concentration of 20 µg/mL of Ofloxacin and 50 µg/mL of Metronidazole. The sample solutions were measured at 294nm for Ofloxacin 340nm for Metronidazole in the Spectronic 1001, spectrophotometer. The results are represented in the Table no.9.8

RESULT AND DISCUSSION

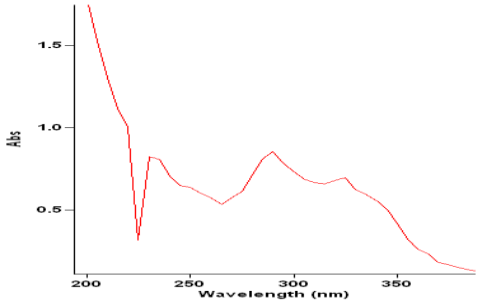


Fig. 9.1: Identification of λmax of Ofloxacin.

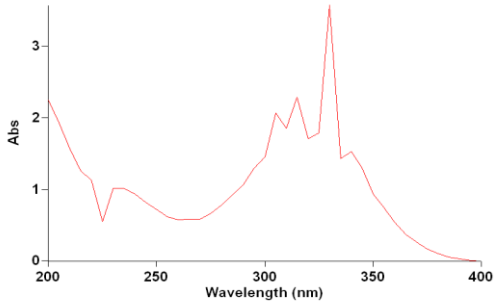


Fig. 9.2: Identification of λmax of metronidazole.

Table 9.1: Linearity table of ofloxacin.

Sr. No	Concentration (µg/ml)	Absorbance
1	2	0.251
2	4	0.568
3	6	0.861
4	8	1.210
5	10	1.502

Table 9.2: Linearity table of metronidazole.

Sr. No	Concentration (µg/ml)	Absorbance
1	2	0.065
2	4	0.135
3	6	0.230
4	8	0.310
5	10	0.384

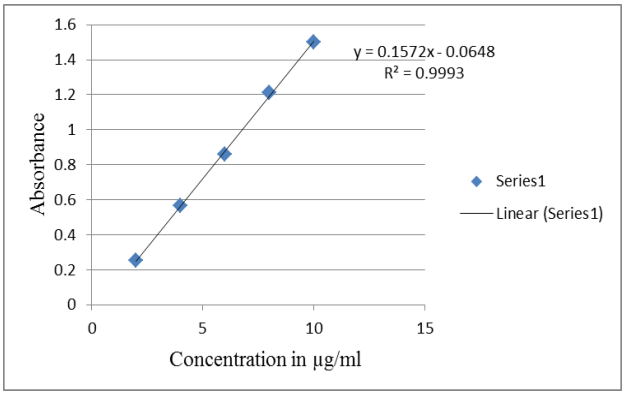


Fig. 9.2: Linearity curve of ofloxacin.

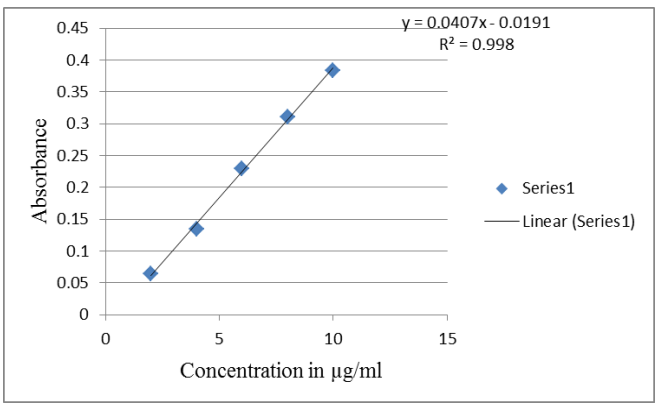


Fig. 9.3: Linearity curve of metronidazole.

Table 9.3 Linearity regression data for Ofloxacin and Metronidazole.

Sr. No.	Parameter	Result	
		Oflo	Metro
1.	Absorption maxima(nm)	294nm	340nm
2.	Linearity range(µg/ml)	2-10	2-10
3.	Standard regression equation	Y=0.157x-0.064	Y=0.040x-0.019
4.	Correlation coefficient(r_2)	0.999	0.998
5.	Accuracy(%recovery)		
6.	Precision		
7.	LOD and LOQ		

Table 9.4: Precision data for proposed method

a) Intraday precision

Sample	Absorbance		Concentration obtained		Standard deviation		% Relative standard deviation	
	340nm Metro	290nm Oflo	C_x (µg/ml)	C_y (µg/ml)	C_x	C_y	C_x	C_y
1	0.3758	1.488	9.87	9.89	0.0752	0.0701	0.7557	0.7039
2	0.3798	1.501	9.97	9.97				
3	0.3818	1.553	10.02	10.03				

b) Interday precision

Sample	Absorbance		Concentration obtained		Standard deviation		% Relative standard deviation	
	340nm Metro	290nm Oflo	C_x (µg/ml)	C_y (µg/ml)	C_x	C_y	C_x	C_y
1	0.3790	1.195	9.97	9.92	0.1137	0.0842	1.155	0.8547
2	0.3734	1.491	9.81	9.91				
3	0.3710	1.469	9.75	9.77				

Table 9.5: Excellent recovery at each added concentration.

Recovery Level	Drug	Amount present (µg/ml)	Spiked Drug(µg/ml)	Total Concentration (µg/ml)	Total amount recovered	% Recovery (n=3)	%RSD
80%		6	4	10	3.93	98.25%	0.72%
100%	Metronidazole	6	6	12	5.89	98.16%	0.83%
120%		6	8	14	7.69	96.12%	1.13%
80%		6	4	10	3.86	96.5%	0.64%
100%	Ofloxacin	6	6	12	5.78	96.33%	0.59%
120%		6	8	14	7.78	97.25%	0.32%

Table 9.6 LOD and LOQ for drug in Solvent.

Sr. No.	Conc. (µg/ml)	Absorbance		S.D		Slope		LOD (µg/ml)		LOQ (µg/ml)	
		Metro	Oflo	Metro	Oflo	Metro	Oflo	Metro	Oflo	Mtro	Oflo
1	10 (µg/ml)	0.3840	1.502	0.00653	0.0736	0.040	0.157	0.5387	1.547	16.32	46.87
2		0.3921	1.603								
3		0.3829	1.421								
4		0.3915	1.515								
5		0.3808	1.430								

Table 9.7 Ruggedness data at 10 µg/ml by two analysts at different days.

Test conc (µg/ml)	Analyst -1		Analyst-2	
	Metro	Oflo	Metro	Oflo
10	0.3840	1.502	0.3842	1.505
10	0.3921	1.603	0.3925	1.615

10	0.3829	1.421	0.3830	1.425
10	0.3916	1.515	0.3915	1.516
10	0.3808	1.430	0.3809	1.435
Mean	0.3862	1.494	0.3864	1.499
S.D.	0.0065	0.0736	0.0035	0.25
% RSD	1.69	4.926	0.9083	13.89

Table 9.8: Estimation of Ofloxacin and Metronidazole in pharmaceutical formulations.

Sample	Label claim (mg/tab)		Amount found by proposed method (mg/tab)		% recovery by proposed method	
	OFLO	METRO	OFLO	METRO	OFLO	METRO
Tablet 1	50	100	49.96	99.98	99.92	99.98
Tablet 2	50	100	49.96	99.86	99.92	99.86
Tablet 3	50	100	49.86	99.92	99.72	99.92

Simultaneous Equation Method			
Validation and parameters		**Metronidazole**	**Ofloxacin**
	Analytical Wavelength	340nm	290nm
Linearity and Range	Linearity range	2-10µg/ml	2-10µg/ml
	Regression Equation	Y=0.157x-0.064	Y=0.040x-0.019
	Correlation Efficient	0.998	0.999
Precision	Intraday Precision (%RSD)	0.7557	0.7039
Accuracy	80%	0.72	0.64
	100%	0.83	0.59
	120%	1.13	0.32
LOD		0.5387	1.547
LOQ		16.32	46.87
Ruggedness	Analyst-1 (%RSD)	1.69	4.926
	Analyst-2 (%RSD)	0.908	4.745

READINGS AND CONCLUSIONS

The goal of the current study was to create a spectrophotometric approach that was easy to use, quick, sensitive, precise, repeatable, and accurate for estimating simultaneous measurements of ofloxacin and metronidazole in pharmaceutical dosage forms. It was discovered that the suggested absorbance difference approach was one of the most flexible analytical techniques used for regular analysis purposes such assay and pharmaceutical formulations. It was also straightforward, quick, inexpensive, and easy to use. There is no method described in the literature for the simultaneous analysis of ofloxacin and metronidazole in binary tablet formulations. The findings produced by the suggested procedure are quite consistent with what the tablet's label claims. Tablet excipients and additives, which are often present, do not interact. Recovery experiment was carried out to test the method's accuracy, and % recovery figures are recorded. The suggested approach was used to conduct the statistical analysis. Standard deviation and coefficient of variation readings were both adequately low, demonstrating the method's accuracy and repeatability.

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