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RESEARCH ARTICLE

Development and Validation of a UV-Spectrophotometric Method for Simultaneous Quantification of Ofloxacin and Ornidazole in Pharmaceutical Formulations

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Abstract: A simple, rapid, and cost-effective UV-spectrophotometric method was developed and validated for simultaneous determination of Ofloxacin and Ornidazole in combined tablet formulations. The method utilized maximum absorption wavelengths of 294 nm for Ofloxacin and 319 nm for Ornidazole in a solvent system consisting of 0.1N acetic acid and water (10:90 v/v). The developed method had excellent linearity in the concentration range of 10-50 µg/mL for both drugs, with correlation coefficients of 0.9998 and 0.9999 for Ofloxacin and Ornidazole, respectively. Method validation following ICH guidelines confirmed high precision with relative standard deviation values below 2% for both intra-day and inter-day analyses. Recovery studies yielded excellent results ranging from 98.52% to 100.04%, indicating high accuracy. The limits of detection were determined to be 0.360 µg/mL for Ofloxacin and 0.488 µg/mL for Ornidazole, while the limits of quantification were 1.092 µg/mL and 1.481 µg/mL, respectively. The developed method showed robust performance across different analysts and experimental conditions. The validated method was successfully applied to commercial tablet formulations, providing accurate and reliable results. This method offers advantages of simplicity, accuracy, and cost-effectiveness for routine quality control of Ofloxacin and Ornidazole in pharmaceutical preparations.

Keywords: UV-Spectrophotometry; Ofloxacin; Ornidazole; Method validation; Pharmaceutical analysis.

1. Introduction

Pharmaceutical analysis plays a vital role in ensuring drug quality and safety, with particular emphasis on developing precise analytical methods for combination drug products. The concurrent quantification of multiple active pharmaceutical ingredients (APIs) in fixed-dose combinations presents unique analytical challenges that necessitate the development of specific, accurate, and reliable analytical methods. Ofloxacin, chemically designated as (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, belongs to the fluoroquinolone class of antibiotics [1]. It exhibits broad-spectrum antimicrobial activity by inhibiting bacterial DNA gyrase, making it effective against both gram-positive and gram-negative organisms [2]. The drug has demonstrated significant clinical efficacy in treating various bacterial infections, including respiratory tract infections, urinary tract infections, and skin infections [3]. Ornidazole, 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol, is a nitroimidazole derivative with potent antiprotozoal and antibacterial properties [4]. Its mechanism of action involves the reduction of its nitro group by bacterial nitroreductases, leading to the formation of toxic metabolites that interfere with bacterial DNA synthesis [5]. The drug shows particular effectiveness against anaerobic bacteria and various protozoal infections [6].

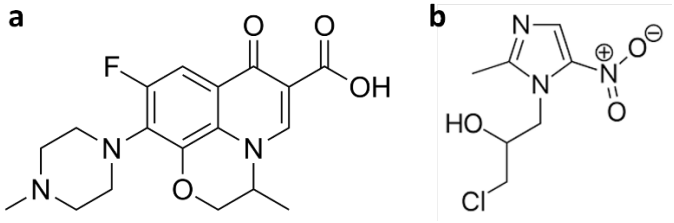

Figure 1. Structures of a. Ofloxacin and b. Ornidazole

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The fixed-dose combination of Ofloxacin and Ornidazole has emerged as a significant therapeutic option, particularly in treating mixed bacterial infections. This combination shows a broad-spectrum antibacterial activity of Ofloxacin with the antianaerobic and antiprotozoal properties of Ornidazole [7]. The synergistic action of these drugs provides enhanced therapeutic efficacy in various clinical conditions, particularly in gastrointestinal and gynecological infections [8]. Current analytical methods for this drug combination include high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and various spectrophotometric techniques [9]. While chromatographic methods offer high sensitivity and specificity, they often require expensive instrumentation, specialized expertise, and complex sample preparation procedures [10]. UV-spectrophotometric methods, by contrast, offer advantages of simplicity, cost-effectiveness, and rapid analysis, making them particularly suitable for routine quality control applications in pharmaceutical settings [11]. The development of a UV-spectrophotometric method for simultaneous estimation of Ofloxacin and Ornidazole requires careful consideration of various analytical parameters. These include the selection of appropriate wavelengths where both drugs show adequate absorption, optimization of the solvent system for maximum stability and sensitivity, and validation of the method according to current regulatory guidelines [12].

The main aim of this work was to develop and validate a UV-spectrophotometric method for the simultaneous determination of Ofloxacin and Ornidazole in pharmaceutical formulations. The method utilizes the principle of simultaneous equations (Vierordt's method) and has been thoroughly validated according to International Conference on Harmonisation (ICH) guidelines [13]. The validated parameters include linearity, precision, accuracy, limits of detection and quantification, and ruggedness.

2. Materials and Methods

2.1. Instrumentation and Materials

A double-beam UV-Visible spectrophotometer (Systronics 112) equipped with matched 1-cm quartz cells and automatic wavelength correction capability was employed for spectral measurements. A high-precision digital analytical balance (Shimadzu LIBROR AEG-45SM) was used for weight measurements, and an ultra-sonicator (Kshitij Innovations) facilitated sample preparation [14].

Reference standards of Ofloxacin and Ornidazole were obtained as gift samples from Seldom Pharma Pvt Limited, Yanam. All chemicals used were of analytical reagent (AR) grade. Acetic acid (0.1N) was prepared using distilled water. The commercially available tablet formulation Angloff-OZ (containing Ofloxacin 200 mg and Ornidazole 500 mg) was procured from local pharmaceutical outlets [15].

2.2. Method Development

2.2.1. Solvent Selection

The selection of an appropriate solvent system was based on several criteria including solubility profiles of both drugs, stability considerations, and cost-effectiveness. After extensive trials, a binary solvent system comprising 0.1N acetic acid and water (10:90 v/v) was selected as the optimal medium, providing excellent solubility for both active ingredients while maintaining their stability [16].

2.2.2. Wavelength Selection

The absorption spectra of both drugs were recorded individually in the wavelength range of 200-400 nm. The wavelength maxima were determined to be 294 nm for Ofloxacin and 319 nm for Ornidazole. These wavelengths were selected for simultaneous quantification as they provided optimal absorption characteristics with minimal interference [17].

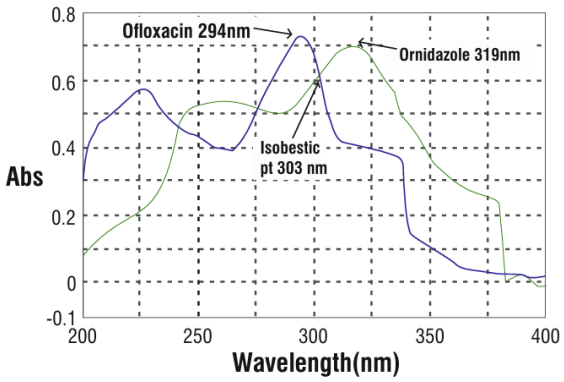

Figure 2. Overlay UV Absorption Spectra of Ofloxacin and Ornidazole

2.3. Preparation of Solutions

2.3.1. Standard Stock Solutions

Ofloxacin Stock Solution: A precisely weighed quantity of 10 mg Ofloxacin was transferred to a 100 mL volumetric flask. The drug was dissolved in the selected solvent system using sonication for 15 minutes, followed by volume adjustment to the mark with the same solvent, yielding a concentration of 100 µg/mL [18].

Ornidazole Stock Solution: Similarly, 10 mg of Ornidazole was accurately weighed and transferred to a 100 mL volumetric flask. The solution was prepared following the same procedure as Ofloxacin, resulting in a 100 µg/mL stock solution [19].

Sample Solution: Twenty tablets of Angloff-OZ were weighed and finely powdered. A quantity of powder equivalent to 30 mg Ofloxacin (containing 75 mg Ornidazole) was accurately weighed and transferred to a 100 mL volumetric flask. The powder was dissolved in the solvent system using 15 minutes of sonication, followed by filtration through Whatman filter paper. Appropriate dilutions were made to achieve working concentrations within the linear range [20].

2.4. Analytical Method

2.4.1. Simultaneous Equation Method

The method employs Vierordt's simultaneous equations for calculating the concentrations of both drugs in the mixture. The absorbance values were measured at both selected wavelengths (294 nm and 319 nm), and concentrations were calculated using the following equations:

$$Cx = (A2ay1 - A1ay2)/(ax2ay1 - ax1ay2)$$
$$Cy = (A1ax2 - A2ax1)/(ax2ay1 - ax1ay2)$$

Where:

Cx = Concentration of Ofloxacin
Cy = Concentration of Ornidazole
A1 = Absorbance at 294 nm
A2 = Absorbance at 319 nm
ax1, ax2 = Absorptivities of Ofloxacin at respective wavelengths
ay1, ay2 = Absorptivities of Ornidazole at respective wavelengths [21]

2.5. Method Validation

2.5.1. Linearity and Range

Calibration curves were constructed using five concentration levels ranging from 10-50 µg/mL for both Ofloxacin and Ornidazole. Working standard solutions were prepared by appropriate dilution of stock solutions with the selected solvent system. Each concentration was analyzed in triplicate, and absorbance values were recorded at both wavelengths. The linearity was evaluated through linear regression analysis, calculating the correlation coefficient, slope, and y-intercept [22].

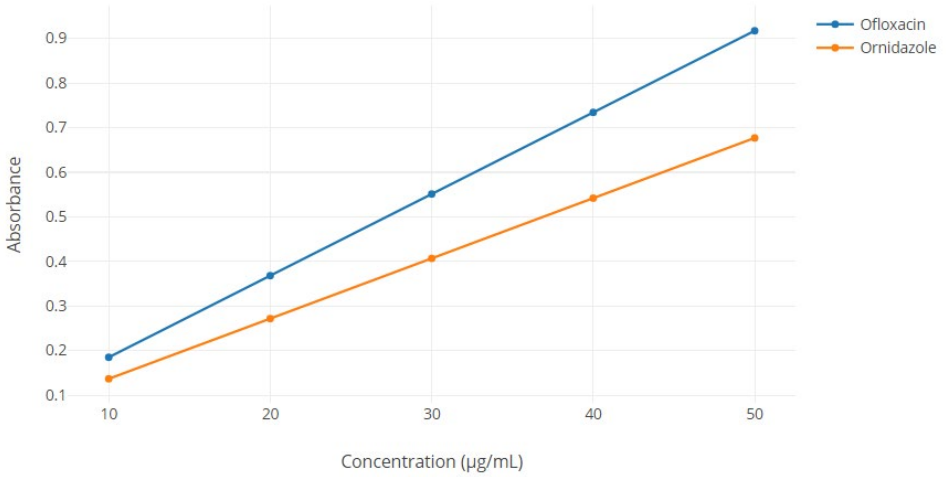

Figure 3. Calibration Curves for a. Ofloxacin b. Ornidazole

2.5.2. Accuracy

The accuracy of the method was determined through recovery studies using the standard addition technique. Known amounts of pure drug standards were added to pre-analyzed tablet powder at three different levels (50%, 100%, and 150% of the labeled claim). The resulting mixtures were analyzed according to the proposed method. The percentage recovery and relative standard deviation were calculated for each concentration level [23].

2.5.3. Precision

Repeatability: Repeatability was assessed by analyzing six replicate measurements of a sample solution containing 30 µg/mL of both drugs. The relative standard deviation (%RSD) was calculated to evaluate the precision of the method [24].
Intermediate Precision: Intermediate precision was evaluated through intraday and interday variation studies. For intraday precision, samples were analyzed at different time intervals within the same day. Interday precision was determined by analyzing samples on three consecutive days. The %RSD values were calculated for both studies [25].

2.5.4. Sensitivity

Limit of Detection: The LOD was calculated using the formula:
$LOD = 3.3 \times \sigma/S$
where σ represents the standard deviation of the response and S is the slope of the calibration curve [26].
Limit of Quantification: The LOQ was determined using the formula:
$LOQ = 10 \times \sigma/S$
utilizing the same parameters as in LOD calculation [27].

2.5.5. Ruggedness

Method ruggedness was evaluated by having three different analysts perform the analysis using the same instrumentation and experimental conditions. The results were compared to assess the method's reproducibility across different operators [28].

2.6. Assay of Pharmaceutical Formulation

The developed method was applied to analyze commercially available Angloff-OZ tablets. Twenty tablets were weighed and powdered. The powder equivalent to 30 mg of Ofloxacin and 75 mg of Ornidazole was accurately weighed and processed according to the sample preparation procedure. The content uniformity was calculated using the simultaneous equation method [29].

3. Results And Discussion

3.1. Method Development and Optimization

The development of this UV spectrophotometric method focused on establishing optimal conditions for simultaneous quantification of Ofloxacin and Ornidazole. The selected solvent system (0.1N acetic acid:water, 10:90 v/v) demonstrated excellent solvating properties for both drugs while maintaining their chemical stability. The absorption maxima at 294 nm for Ofloxacin and 319 nm for Ornidazole showed minimal spectral interference, enabling accurate simultaneous determination [30].

3.2. Method Validation

3.2.1. Linearity

The method demonstrated excellent linearity for both drugs in the concentration range of 10-50 µg/mL. The calibration curves showed strong linear relationships with correlation coefficients (r^2) of 0.9998 for Ofloxacin and 0.9999 for Ornidazole. The linear regression equations were determined as $y = 0.0183x + 0.0021$ for Ofloxacin and $y = 0.0135x + 0.0018$ for Ornidazole. The high correlation coefficients indicate strong linear relationships between concentration and absorbance for both drugs [31].

Table 1. Results of Linearity and Sensitivity

Parameter	Ofloxacin	Ornidazole
Wavelength (λmax, nm)	294	319
Linearity range (µg/mL)	10-50	10-50
Regression equation	y = 0.0183x + 0.0021	y = 0.0135x + 0.0018
Correlation coefficient (r^2)	0.9998	0.9999
LOD (µg/mL)	0.360	0.4888
LOQ (µg/mL)	1.092	1.481

3.2.2. Accuracy

Recovery studies yielded excellent results across all concentration levels. For Ofloxacin, the recovery at 50% level was 99.32%, at 100% level was 99.68%, and at 150% level was 100.04%, with a mean recovery of 99.68%. For Ornidazole, the recovery values were 98.52%, 99.64%, and 99.28% at 50%, 100%, and 150% levels respectively, with a mean recovery of 99.14%. These results indicate high accuracy and absence of interference from excipients [32].

Table 2. Recovery Studies

Drug	Level of Addition (%)	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Mean Recovery (%)
Ofloxacin	50	15	15.43	99.32	99.68
	100	30	29.68	99.68	
	150	45	44.96	100.04	
Ornidazole	50	15	14.77	98.52	99.14
	100	30	29.89	99.64	
	150	45	44.6	99.28	

3.2.3. Precision

Intraday and Interday Precision: The precision studies for Ofloxacin revealed intraday precision with %RSD of 0.00496 and interday precision with %RSD of 0.004222. For Ornidazole, the intraday precision showed %RSD of 0.007928 and interday precision demonstrated %RSD of 0.00509. The low %RSD values (<2%) demonstrate excellent precision of the method [33].

Table 3. Results for Precision

Parameter	Ofloxacin (%RSD)	Ornidazole (%RSD)
Intraday precision	0.00496	0.007928
Interday precision	0.004222	0.00509

3.2.4. Sensitivity

The method showed high sensitivity with low detection and quantification limits. For Ofloxacin, the LOD and LOQ values were determined to be 0.360 µg/mL and 1.092 µg/mL respectively. Ornidazole demonstrated LOD of 0.4888 µg/mL and LOQ of 1.481 µg/mL. These values indicate the method's capability to detect and quantify both drugs at low concentrations [34].

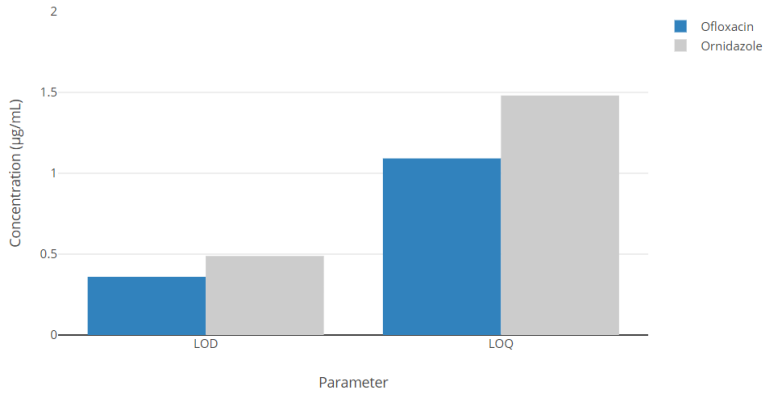

Figure 4. Results of Sensitivity

3.2.5. Ruggedness

The method demonstrated good ruggedness across three different analysts. For Ofloxacin, the %RSD values were 0.035642, 0.035203, and 0.033466 for analysts 1, 2, and 3 respectively. Similarly, for Ornidazole, the %RSD values were 0.040937, 0.040925, and 0.033669 across the three analysts. The consistent %RSD values across different analysts confirm the method's ruggedness [35].

Table 4. Results of Ruggedness

Analyst	Ofloxacin (%RSD)	Ornidazole (%RSD)
Analyst 1	0.035642	0.040937
Analyst 2	0.035203	0.040925
Analyst 3	0.033466	0.033669

4. Conclusion

The developed UV spectrophotometric method for simultaneous estimation of Ofloxacin and Ornidazole demonstrates showed high precision, accuracy, and reliability. The simplicity, cost-effectiveness, and rapid analysis time of the developed method make it particularly suitable for routine quality control analysis in pharmaceutical laboratories. The method validation results meet all ICH guidelines requirements and can be successfully applied for the simultaneous estimation of Ofloxacin and Ornidazole in combined pharmaceutical formulations without any interference from common excipients.

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