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Simple Spectrophotometric Methods for the Determination of Metronidazole and Spiramycin in Tablets

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ABSTRACT

A straightforward, precise, and accurate method was developed for determining metronidazole and spiramycin combination in tablets. The analysis was achieved using the absorbance subtraction method; utilizing the absorbance at 232 nm where both compounds absorb and 311 nm where only metronidazole is absorbing. The linear range of the method was 6.25 - 31.25 and 7.95 - 39.75 µg/mL for metronidazole and spiramycin, respectively. The method exhibited good precision in terms of repeatability and intermediate precision, with relative standard deviations below 2% in both cases. The method's accuracy was verified by the close agreement between the theoretical and actual concentrations of the analytes in nine laboratory-prepared synthetic mixtures with relative standard deviations below 2%. The validity of the method was further confirmed by the statistical comparison made between the results obtained and those from a previously reported high-performance liquid chromatographic method, t-calculated less than t-critical, p> 0.05.

Keywords: *UV spectrophotometry; Absorbance subtraction; Metronidazole; Spiramycin; Tablets*

INTRODUCTION

Spiramycin (SPI) belongs to the macrolide class of antibiotics and has demonstrated efficacy in addressing various periodontal infections such as marginal gingivitis, periodontal abscesses, necrotic ulcerative gingivitis, and Periodontitis. Similar to other macrolide antibiotics, spiramycin primarily targets gram-positive bacteria and exhibits less activity against gram-negative strains [1-3].

Metronidazole (MET), a synthetic nitroimidazole compound, exhibits bactericidal activity against a wide range of anaerobic bacteria and has been effective in treating acute necrotic ulcerative gingivitis [4]. Additionally, it has been explored experimentally for its potential in addressing periodontitis [5].

The chemical structures of metronidazole and spiramycin are given in Figure 1a and 1b, respectively.

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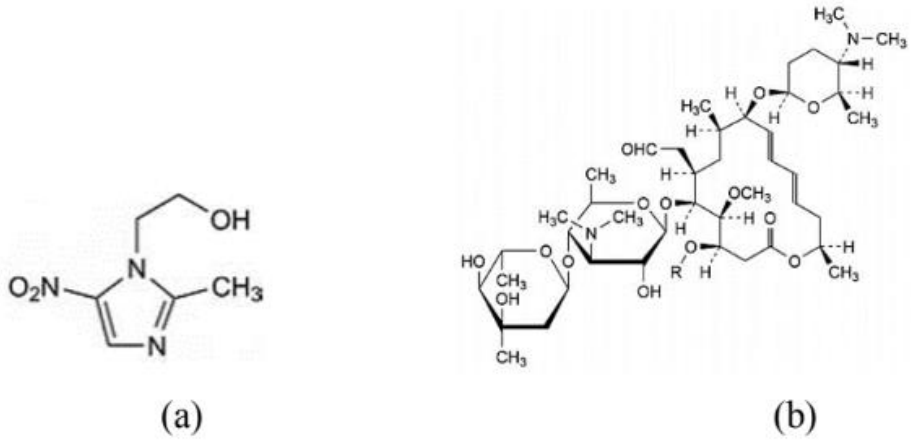

Figure 1: Chemical structure of (a) metronidazole and (b) spiramycin

Studies have shown that combining spiramycin and metronidazole results in a synergistic effect, enhancing their effectiveness against various infections and broadening their spectrum of activity [6,7]. As far as our current knowledge extends, there hasn't been the establishment of a pharmacopoeial monograph for SPI-MET combined tablets. However, literature review revealed that only a limited number of chromatographic methods [8-10] and spectrophotometric techniques [11-14] have been reported for the quantification of the two active ingredients in tablet formulations. The aim of this study was to devise a straightforward and dependable UV-spectrophotometric method as an alternative to costly separation-based techniques or complex spectrophotometric methods that demand advanced mathematical understanding and specialized computer programs.

Theoretical background [15]

The proposed method allows for the analysis of a mixture containing two drugs, X and Y, even if their spectra overlap at one wavelength (λ_1) and Y extends over X, while X does not contribute significantly at another wavelength (λ_2). In this method, the wavelength where the overlapping occurs (λ_1) can be utilized for the separate quantitative estimation of both X and Y in their mixture (X + Y). By employing mathematically calculated factors for one of the components, the determination can be achieved. Through simple manipulation steps, the absorbance values corresponding to X and Y can be obtained separately. Consequently, the concentration of each component can be determined via regression equations established at the overlapping wavelength, eliminating the need for a complementary method. The absorbance values corresponding to X and Y at (λ_1) are calculated by using absorbance factor which is a constant for pure Y representing the average of the ratio between the absorbance values of different concentrations of pure Y at λ_1 to those at λ_2 i.e. (abs λ_1/ abs λ_2)

$$\text{Absorbance of Y in the mixture at } \lambda_1 = \text{abs}_{\lambda 1}/\text{abs}_{\lambda 2} * \text{abs}_{\lambda 2}\,(X+Y) \quad (1)$$

$$\text{Absorbance of X in the mixture at } \lambda_1 = \text{abs}_{\lambda 1} - (\text{abs}_{\lambda 1}/\text{abs}_{\lambda 2} * \text{abs}_{\lambda 2}\,(X+Y)) \quad (2)$$

The corrected absorbance values at λ_1 are then used to calculate the concentration of each analyte from its calibration curve.

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EXPERIMENTAL

Apparatus and Software

Spectrophotometric measurements were conducted utilizing a double-beam UV-1800 spectrophotometer (Shimadzu, Japan) equipped with a 1-cm pair of quartz cuvettes. Data analysis was performed using Microsoft Excel Spreadsheet 2013.

Materials and Reagents

Metronidazole and spiramycin working standards were obtained from Azal Pharmaceutical Industries – Sudan. Dentacare tablets labeled to contain Metronidazole 125 mg and Spiramycin 750000 I.U per tablet were manufactured by Azal Pharmaceutical Industries. Methanol used was obtained from (Loba Chemie, India).

Preparation of Stock Standard Solutions

Accurately weighed about 12.5 mg of Metronidazole and 16 mg spiramycin working standard were individually weighed and transferred into two separate 100 ml volumetric flasks. To each flask, 70 ml of methanol were added and the dissolution was aided by sonication for 10 minutes, after cooling the volume was completed to mark with methanol.

Sample Preparation

Twenty tablets were accurately weighed and crushed to fine powder, an amount equivalent to one tablet was transferred to 100 ml volumetric flask, 70 ml of methanol were added and the sample was sonicated for 10 minutes, after cooling the volume was completed to mark with methanol. 1 ml of the filtrate obtained after passing the solution 202 ashless filter paper (Whatman No. 202) was diluted to 100 ml with methanol.

Preparation of Synthetic Mixtures

Nine solutions containing various concentrations of metronidazole and spiramycin, covering a range of concentrations bracketing those present in the sample, were prepared according to a multilevel multifactor design [16]. This was achieved by mixing different volumes from the two analytes stock solutions in nine separate 20 mL volumetric flasks and completing the volume to the mark with methanol.

Preparation of the Calibration Standards

Aliquot volumes ranging from 1 to 5 mL from the stock solution of each analyte were transferred into a separate set of five 20 ml volumetric flasks and then made up to the volume with methanol; to obtain concentrations of metronidazole in the range of 6.25-31.25 mg/L and spiramycin in the range of 7.95-39.75 mg/L.

Method Validation

The proposed method was validated according to the ICH guideline requirements [17] for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The absorbance values of these solutions was measured at the pre-selected wavelengths, and regression analysis data was obtained using the method of least squares. The slope, intercept, the standard deviation of the slope and intercept and confidence interval at 95% confidence level were reported.

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Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the two analytes were calculated from calibration regression analysis data; using the following equations (ICH Harmonized Tripartite Guideline, 2005).

$$LOD = 3 \times S_{x.y} / S$$

$$LOQ = 10 \times S_{x.y} / S$$

where $S_{x.y}$ = standard deviation of the residuals.

S = slope of the calibration curve

Precision

The repeatability precision (intra-day) of the method was assessed by analyzing six independent sample solutions containing analytes at 100% concentrations within the same day. The intermediate precision (inter-day) was evaluated by repeating the same procedure on a different day using fresh reagents and samples. The results were expressed as percentage content and relative standard deviation (%RSD).

Accuracy

The accuracy was accessed by analyzing the nine synthetic mixtures, the results were expressed as percentage content and relative standard deviation (%RSD).

RESULTS AND DISCUSSION

The overlain spectra of MET and SPI obtained over the wavelength range of 200-400 nm shown that MET has two absorbance maxima at 232 nm and 311 nm, while SPI is absorbing at 232 nm and has absorbance at 311nm, hence these two wavelengths (232 nm and 311 nm) were chosen as the analytical wavelength for the application of the proposed method (Figure 2).

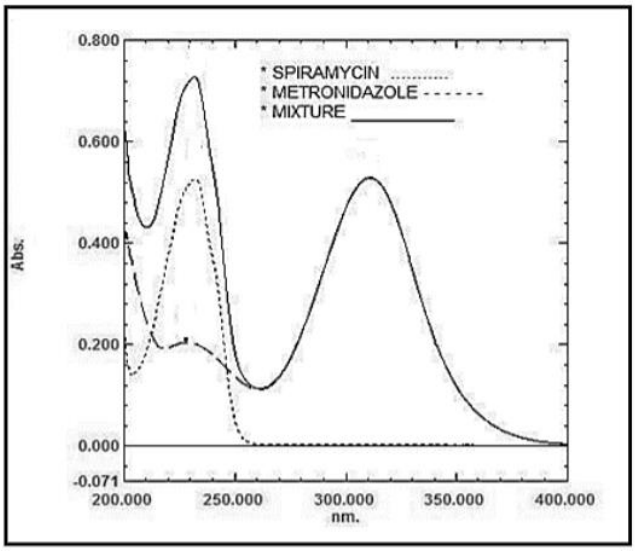

Figure 2: UV absorption spectra of spiramycin, metronidazole and their mixture at working concentrations

The absorbance of MET different concentrations was measured at these two wavelengths. The value of absorbance factor of MET representing the average of the ratio between absorbance at two wavelengths, one of them showing interference from SPI (λ_1) while SPI

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has no contribution at the other wavelength (λ_2) was found to be 0.371. The calibration curves were established for SPI and MET using absorbance measurements at the specified wavelengths, with a determination coefficient R^2 exceeding 0.990. This suggests a robust correlation between concentration and absorbance across the concentration range of 6.25-31.25 mg/L and 7.95-39.75 mg/L for MET and SPI, respectively. Statistical analysis of the calibration data was conducted to determine the Limit of Detection (LOD) and Limit of Quantification (LOQ) for both substances. These values, expressed in mg/L, represent the minimum concentrations at which SPI and MET can be reliably detected and quantified using the UV spectrophotometric methods proposed, as detailed in Table 1.

Table 1: Regression Analysis Data

Parameter	**Analyte**		
	MET		**SPI**
Wavelength	232 nm	311 nm	232 nm
Concentration (µg/mL)	6.25 - 31.25		7.95 - 39.75
slope	0.0209	0.0548	0.0334
intercept	-0.0017	0.0172	-0.0008
correlation coefficient (r^2)	0.9999	0.9999	1.0000
standard deviation of the slope	0.0001	0.0003	0.0001
standard deviation of the intercept	0.0026	0.0070	0.0013
limit of detection (µg/mL)	0.4046	0.4020	0.1249
limit of quantitation (µg/mL)	1.2262	1.2200	0.3786
residuals standard deviation ($S_{y.x}$)	0.0026	0.0067	0.0013

Accuracy was evaluated by examining nine laboratory-prepared synthetic mixtures with different concentrations of the two analytes. The analytical findings presented in Table 2 indicate the excellent precision of our methods, with Relative Standard Deviation (RSD) values below 2%. Furthermore, the actual content of both drugs ranged between 100.39% - 101.92% for SPI and 99.92% -101.83% for MET, when compared to the label claim.

The repeatability and intermediate precision studies yielded Relative Standard Deviation (RSD%) values below 2% along with satisfactory percentage recoveries. Statistical examination of the precision data using the Student t-test verified the absence of day-to-day variation in the outcomes Table 2.

Table 2: Assay Precision Results

Day	% of Label Claim (mean ± SD, = 6)	
	MET	SPI
Day 1	100.27 ± 0.658	100.52 ± 0.530
Day 2	100.69 ± 0.570	100.51 ± 0.820
t – calculated (t - tabulated)	1.17 (2.23)	0.021(2.23)

The further validate the method, a statistical comparison was made between the results obtained and those from a previously reported high-performance liquid chromatographic method [11]. Analysis showed no significant difference between the two methods at a

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significance level of p = 0.05 with n = 6 samples. Consequently, the developed method can be deemed as accurate and precise as the reported liquid chromatographic method, as shown in Table 3.

Table 3: Comparison of the proposed method to the Reference Method

Method	% of label claim (mean ± SD, = 6)	
	MET	SPI
Proposed method	100.27 ± 0.658	100.52 ± 0.530
Reference (HPLC)	100.51 ± 0. 815	100.86 ± 0.486
t - calculated (t - tabulated)	0.56 (2.23)	1.14 (2.23)

CONCLUSIONS

The results suggest that the proposed UV-spectrophotometric method is a viable and cost-effective alternative for the analysis of MET and SPI, offering simplicity, reliability, and comparable performance to more complex techniques. Statistical comparison with a previously reported high-performance liquid chromatographic method demonstrated no significant difference between the two methods, confirming the accuracy and precision of the developed UV-spectrophotometric method.

Conflict of Interest

The author declares no conflict of interest.

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