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# DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTIFICATION OF METRONIDAZOLE IN PURE AND TABLET DOSAGE FORM

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## ABSTRACT

Three simple, rapid and accurate spectrophotometric methods were developed for quantification of metronidazole in pure and tablet dosage form. The methods involved measurement of absorbance at wavelengths 295, 345 and 350 nm for pH 1.2, 4.5 and 6.8 respectively. The methods were validated according to ICH guidelines with respect to different analytical parameters; linearity, precision, accuracy and percentage recovery, Limit of detection (LOD) and Limit of quantification (LOQ). Beer-Lambert's law was obeyed within the concentration range of 2-64µg/mL. The Limit of detection and quantification values were determined to be 0.0122 and 1.1154, 0.0185 and 1.6935, 0.0858 and 0.26026 for pH 1.2, 4.5, and 6.8 respectively. The proposed methods were found to be precise since coefficient of variation (% RSD) values were less than 15 % and recovery values were found to be in the range of 95.71-100.71 %. The developed methods were successfully used for *in-vitro* dissolution of Metronidazole.

**Keywords:** Metronidazole, UV Spectrophotometric, method development, method validation, *in-vitro* dissolution

## INTRODUCTION

Metronidazole (2-(2-methyl-5-nitro-lH-imidazol-l-yl) ethanol) is a representative antibacterial and antiprotozoal drug that has been synthesized in various laboratories throughout the world, which is one of the rare examples of a drug developed against a parasite and has since gained broad use as an antibacterial agent (BP, 2009; Samuelson, 1999). Metronidazole (figure 1) is one of the first line drugs in the WHO model list of essential medicine used for treating various infection (WHO,2005); protozoal infections, amoebiasis (intestinal and extra intestinal), giardiasis, and trichomoniasis, Gram-positive Bacilli; *Clostridium difficile*, Dracunculus (guinea worm), balantidiasis, blastocystitis, infections by *Entameba polecki*, prophylaxis of infections caused by anaerobic bacteria, GI strains of *Bacteroides fragilis* and vaginal infections caused by *Gardnerella vaginalis* (Thomas *et al.*, 2007). It has also been used successfully in the treatment of antibiotic-associated pseudomembranous colitis, useful in Crohn's disease and in combination is an alternative therapy for *Helicobacter pylori* infections (Ashutosh, 2004). There are many brands of

metronidazole in the Nigerian market from different manufacturers, some are manufactured locally and some imported. There is reliable evidence that some of them are fake, adulterated or substandard (Glass, 2014). Hence present study aims to develop and validate a precise, simple, rapid and cost effective UV spectrophotometric method for the quantification of metronidazole in a pharmaceutical dosage form in three different physiological media to ensure interchangeability.

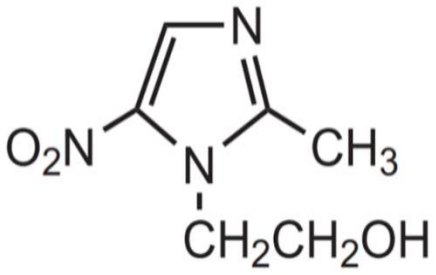


**Figure 1:** Chemical Structure of metronidazole

## MATERIALS AND METHODS

### Preparation of Simulated Physiological Media (pH 1.2, 4.5 and 6.8)

Simulated gastric pH, Acetate buffer and Phosphate buffer were prepared according to USP and were validated to have pH 1.2, 4.5 and 6.8 respectively

### Preparation of Stock Solutions

Stock solutions (l00µg/ml) were prepared by dissolving 10mg of standard metronidazole powder in 100 ml of each of the prepared simulated physiological media [pH 1.2, 4.5 and 6.8].

### Determination of Wavelength of Maximum Absorption

Working solutions (16µg) were prepared from each of the stock solutions and then scanned at 400-200 nm against their blank.

### Construction of Calibration Curve

A six points calibration curves were constructed by preparing solutions of concentration range 2-64µg/ml by serial dilution of each stock solution (100µg/ml).

### Validation of the Developed Methods

Each of the developed method was validated for linearity, precision, accuracy and percentage recovery, limit of detection (LOD) and limit of quantification (LOQ) in accordance with ICH guideline

### Linearity

This was established by least square methods using Microsoft excel 2016.

### Precision

The absorbance of a 16µg/ml solution of metronidazole in each of the simulated physiological media was carried out six times at an hour interval within the same day for intraday, while absorbance of the same solution was taken daily for three consecutive days for interday.

### Accuracy and Percentage Recovery

Five milliliters (5mL) of a 10 µg/ml solution of Metronidazole was quantitatively measured and transferred into four labeled 10 ml test

tubes (A-D). Test tubes B, C, and D were spiked with 2.6ml, 3ml and 3.4ml of the stock solution to obtain 18, 20 and 22 µg/ml respectively while test tube A was left unspiked. Absorbance was taken in triplicates and the mean percentage recovery was calculated as = (Theoretical Conc. – Actual Cone./Actual Conc.)*100. This was carried out for solutions of metronidazole in each of the simulated physiological media.

### *In-vitro* dissolution studies

One tablet from four samples was subjected to the dissolution medium (900ml) after preheating it to 37 ± 0.5°C. The basket speed was maintained at 100 revolutions per minute (rpm). Five (5ml) sample was then withdrawn and one (1 ml) of the aliquot solution was quantitatively taken in to 10 ml beaker and diluted to volume with the dissolution medium and absorbance was measured using the validated wavelength (295nm, 345nm and 350nm) for pH 1.2, 4.5 and 6.8 respectively against their blank.

## RESULTS AND DISCUSSION

The UV spectra of Metronidazole in different simulated physiological media were recorded. The drug showed good absorbance in all the three media with a wavelength of maximum absorption (λmax) of 295nm, 345nm and 350nm recorded for pH 1.2 (method 1), pH 4.5 (method 2) and pH 6.8 (method 3) respectively as shown in figure 2, 3 and 4 bellow.

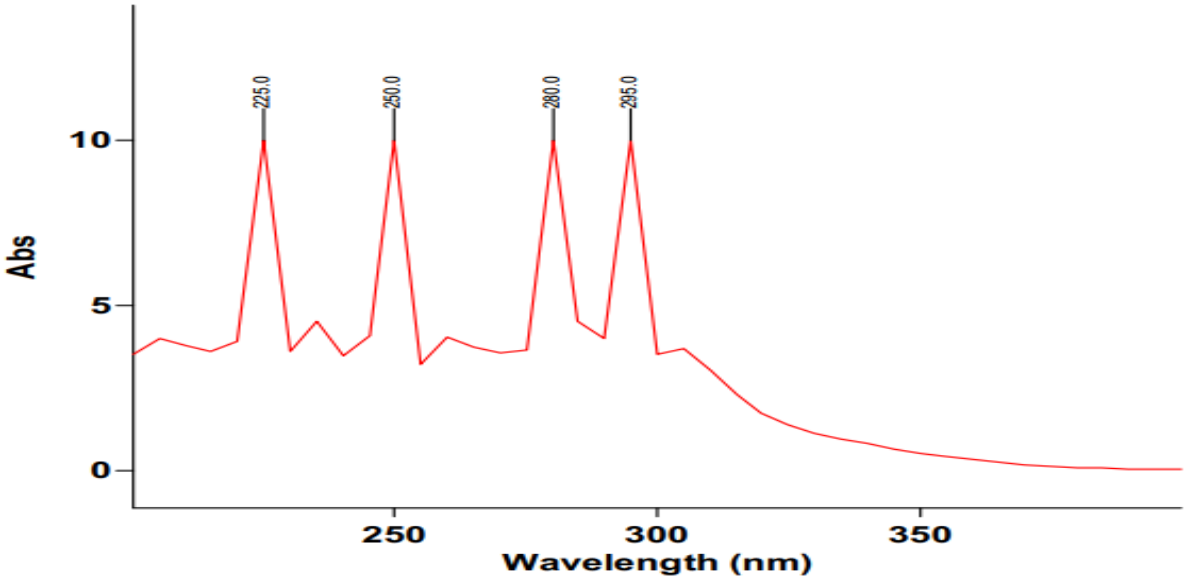


**Figure 2:** UV spectrum of metronidazole in pH **1.2**

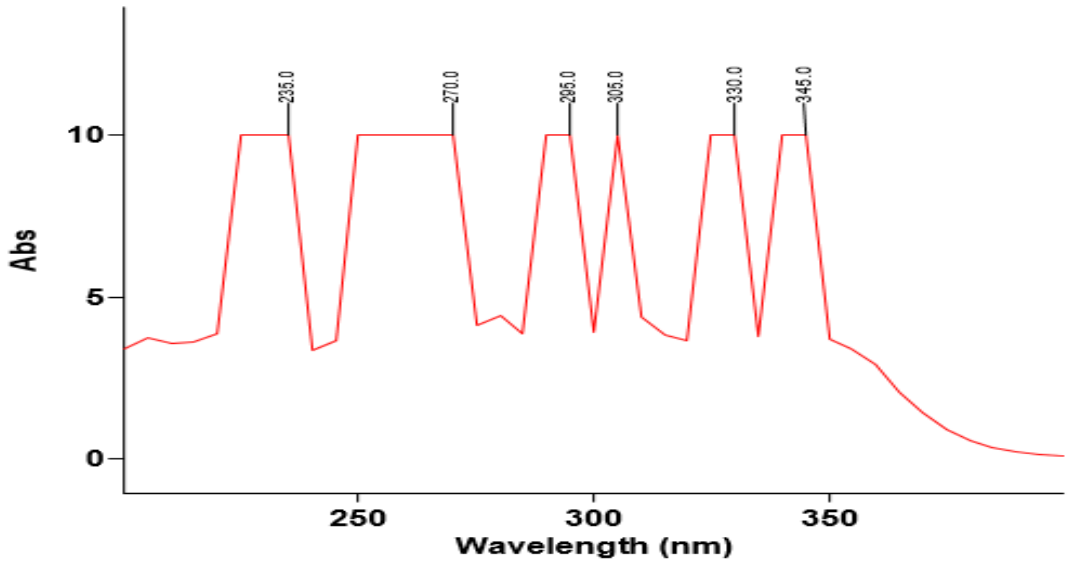


**Figure 3:** UV spectrum of metronidazole in pH 4.5

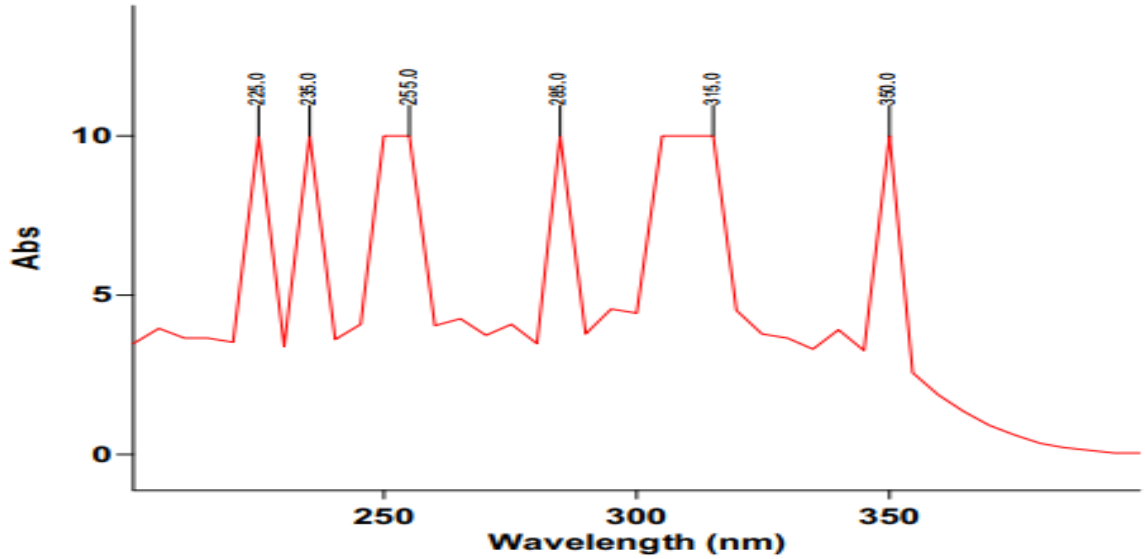


**Figure 4:** UV spectrum of metronidazole in pH 6.8

Beer lambert's law was obeyed at concentration range of 2-64µg/mL in all the methods as their correlation coefficient were closed to unity as seen in the graphs below. The acceptable limit is it should be linear in the specified range and the correlation coefficient should not be less than 0.99 (Joyani and Manabendra, 2014). Hence the relationship between the concentrations and the absorbance of metronidazole in all the media showed linearity.

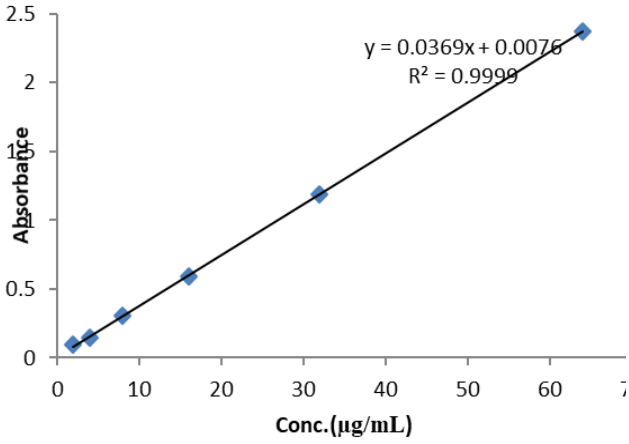


**Figure 5:** Calibration curve of metronidazole in pH 1.2

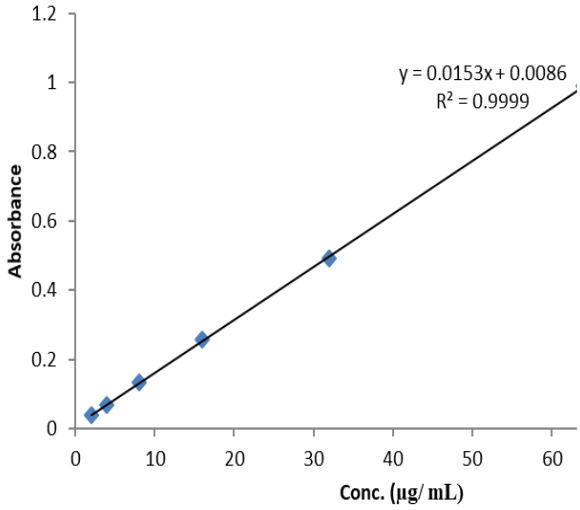


Figure 6: Calibration curve of metronidazole in pH 4.5

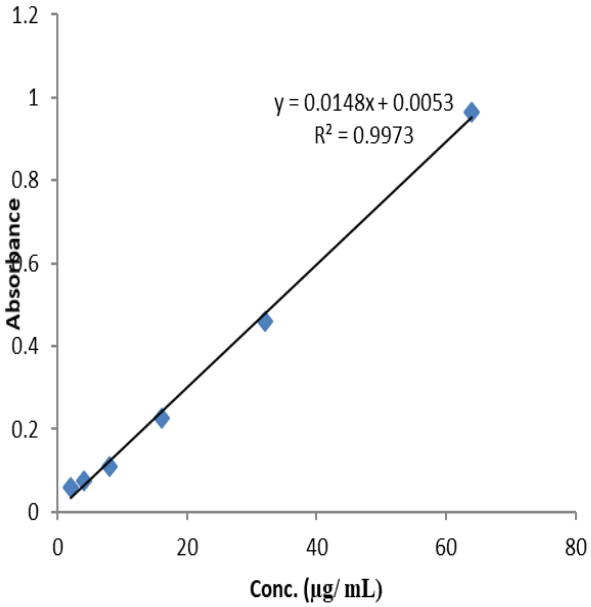


**Figure 7:** Calibration curve of metronidazole in pH 6.8

**Table 1:** Summary of the calibration curve parameter of the developed method

| S/N | Parameter | pH 1.2 | pH 4.5 | pH 6.8 |
|---|---|---|---|---|
| **1** | ʎmax (nm) | 295 | 345 | 350 |
| **2** | Concentration range (µg/mL) | 2-64 | 2-64 | 2-64 |
| **3** | Regression equation | Y=0.0369X+0.0076 | Y=0.0153X+0.0086 | Y=0.0148X+0.0053 |
| **4** | Coefficient of correlation | 0.9999 | 0.9999 | 0.9973 |
| **5** | Intercept | 0.0076 | 0.0085 | 0.0053 |
| **6** | LOD (µg/mL) | 0.0122 | 0.0185 | 0.0858 |
| **7** | LOQ (µg/mL) | 1.1156 | 1.6935 | 0.26026 |

Meanwhile, the LOD and LOQ revealed that the methods were sensitive enough to detect and quantify as little as 0.0122 and 1.1154, 0.0185 and 1.6935, 0.0858 and 0.26026 for pH 1.2, 4.5, and 6.8 respectively.

**Table 2:** Results for precision of the method

| Medium | pH 1.2 | pH 4.5 | pH 6.8 |
|---|---|---|---|
| Intraday (%RSD) | 1.35 | 2.95 | 3.38 |
| Interday (%RSD) | 9.42 | 6.70 | 4.08 |

Unlike some results reported in (Arun *et al 2010*, Sireesha *et al 2016*, Thulasamma P. and Venkateswarlu, P. 2009) the developed methods showed good precision at less than 15% Coefficient of variations (Table 2) which can be due to higher concentration range used.

**Table 3:** Results for accuracy and recovery

| pH | Amount added | Amount obtained | Mean % recovered | Mean % Relative error |
|---|---|---|---|---|
| **1.2** | 8 | 7.89 | | |
| | 10 | 10.05 | 99.42 | 0.91 |
| | 12 | 11.89 | | |
| **4.5** | 8 | 8.20 | | |
| | 10 | 10.18 | 100.71 | 2.71 |
| | 12 | 11.75 | | |
| **6.8** | 8 | 7.68 | | |
| | 10 | 9.61 | 95.71 | 4.29 |
| | 12 | 11.39 | | |

Also, the methods were found to be accurate as indicated by their high mean percentage recovery and low mean percentage relative error.

**Table 4:** Percentage release of metronidazole in all the media at 60minutes

| Brands | Percentage release | | |
|---|---|---|---|
| | pH 1.2 | pH 4.5 | pH 6.8 |
| MA | 101.64 | 99.94 | 98.77 |
| MB | 103.94 | 106.03 | 100.38 |
| MC | 103.12 | 99.34 | 102.42 |
| MD | 101.45 | 90.43 | 89.24 |

From the results of *in-vitro* studies, not less than 85% of the label amount was released by all the brands at 60minutes as specified by USP. Hence, the developed methods were simple, cheap, precise, accurate and reproducible. Therefore, can be used for *in-vitro* analysis of metronidazole in a pharmaceutical dosage form**.**

## CONCLUSION

From the results obtained. the developed UV Spectrophotometric methods provided simple, precise, rapid and accurate analytical method for quantification of Metronidazole in pharmaceutical dosage form. Correlating the obtained results with the standard values, the methods were found to be valid and hence can be easily and conveniently adopted for quantification of Metronidazole in pharmaceutical dosage form.

**Acknowledgment**

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