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# UV DIFFERENTIAL SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF METRONIDAZOLE IN BULK AND PHARMACEUTICAL FORMULATION

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**ABSTRACT**

A simple, precise, economical and accurate UV differential spectroscopic method has been developed for the estimation of Metronidazole (MND) in bulk and in pharmaceutical dosage form. The proposed method was based on the principle that Metronidazole exhibits two different chemical forms that differs in the absorption spectra in equimolar acidic and basic solution. The absorptions were measured in equimolar acidic and basic solution separately against reagent blank. Metronidazole has exhibited maximum absorbance at 278 and 320 nm in acidic (0.1N HCl) and basic (0.1N NaOH) solution respectively. Difference in absorbance between these two maxima was calculated to find out the amplitude. The amplitude plotted against concentration showed linear response in the concentration range of 2-14µg/mL with linear co relation co efficient value 0.999. The proposed method was applied to pharmaceutical formulation and the common excipient present in the formulation does not interfere in the analysis of the drug. The method was validated as per ICH guidelines and results of statistical analysis were found to be satisfactory.

**Keywords:** Metronidazole (MND), UV differential spectrophotometry, overlay spectra, Tablet dosage form, and validation.



## INTRODUCTION

Metronidazole [2-(2-methyl-5-nitro-1H-imidazol-1yl) is an amaebicide, antiprotozoal and antibiotic effective against anaerobic bacteria and certain parasites[1]. It is the drug of choice for first episodes of mild-to-moderate clostridium difficile infection[2]. Metronidazole exerts rapid bactericidal effects against anaerobic bacteria, with a killing rate proportional to the drug concentration[3]. Concentration-dependent killing has also been observed with Entamoeba histolytica and trichomonas vaginalis. Metronidazole kills bacteroides fragilis and clostridium perfringens more rapidly than clindamycin[4].

Literature survey revealed that Metronidazole is officially determined by potentiometry, titrimetry, GC[5], spectrophotometric[6-8], polarography[9], HPLC[10] and UPLC[11] methods. IP[12] describes the non- aqueous titration method using perchloric acid as titrant. USP[13] describes HPLC and non aqueous titration method. BP[14] describes potentiometric titrations.

The objective of present investigation is to develop simple, precise, accurate and economical UV differential-spectrophotometric method for determination of metronidazole in bulk and in Tablet dosage form using equimolar acidic (0.1N HCl) and basic (0.1N NaOH) as a solvents. The developed methods were validated as per ICH guidelines.

## EXPERIMENITAL

### Chemicals and reagents

The working standard Metronidazole (purity - 99.85%) was obtained as a gift sample from Padmaja Laboratories Ltd, Vijayawada, India. The commercially available Tablet, Metrogyl 400mg containing 400mg of MND was procured from the local pharmacy and used for analysis. Freshly prepared 0.1N HCl (AR, Qualigens), 0.1N NaOH (AR, Qualigens) and distilled water were used in the present analysis, Whatmann filter paper no.41 used.

**Instruments**
A Shimadzu UV – 1800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV – probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Analytical balance Shimadzu (220h) was used for weighing purpose.

**Difference spectroscopy**
The selectivity and accuracy of spectrophotometric analysis of sample containing absorbing interference may be markedly improved by the technique of difference spectrophotometry. The essential feature of difference spectrophotometric assay is that the measured value is the difference in absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics. This is simplest and most economically employed for altering spectral properties of analyte by means of adjustment of pH by means of acid, alkali and buffer.

**Preparation of standard stock solution**
The working standard MND (100mg) was weighed accurately and transferred to two (100mL) volumetric flasks. It was dissolved in 30mL of 0.1N NaOH and 0.1N HCl separately and finally made up to volumes with the 0.1N NaOH and 0.1N HCl. The solutions are further diluted with suitable diluents to get the final concentration 100µg/mL.

**Determination of λmax**
From the above prepared stock solution test concentrations 10µg/mL were prepared separately using both solvents. The prepared solutions were scanned over the range of 400-200nm against reagent blank. From the spectrum obtained, the λmax was found to be 278 and 320 nm in acidic and basic solutions respectively. The spectra's are overlaid in order to get the isobestic point (Figures-2, 4 & 6). The intermediate wavelength 324 nm was found from the overlay spectra. The difference in absorbance was calculated to find out the amplitude.

**Construction of calibration curve**
From the above solution, series of aliquots of 2-14µg/mL concentrations were prepared using 0.1N NaOH and 0.1N HCl separately. These solutions were used to determine linearity. The calibration curve for metronidazole was plotted in the concentration v/s absorbance (figure-3, 5 & 7) and regression equation was calculated. This regression equation was used to estimate the drug content in Tablet dosage form.

**Analysis of Tablet formulation**
For the preparation and analysis of sample solution, each Tablet containing 400mg of MND, 20 Tablets were accurately weighed and average weight per Tablet was determined. The Tablets were powdered and the powder equivalent to100mg drug was taken and treated in similar manner as that of standard. In both acidic and basic solvents. These solutions were then filtered through Whatmann filter paper no. 41. From the stock solution Further dilutions were made with 0.1N HCl and 0.1N NaOH to get required concentration of 4µg/mL solutions. In this method, the concentrations were determined by measuring absorbance of sample solutions at isobestic point. The nominal contents were determined either from the previously plotted calibration graphs or using the corresponding regression equations as shown in the Table 1.

**Method validation**
The method was validated for different parameters like linearity, accuracy and precision according to ICH guidelines(Q2).

**Linearity**
The linearity of the method is the ability to elicit the results that are directly proportional to the concentration of the analyte in samples. From the working standard, series of dilutions were made to

10mL volumetric flask with 0.1N NaOH and 0.1N HCl separately to get concentration range of 2-14µg/mL. The absorbance was measured at ISOBESTIC POINT in acidic and basic solutions respectively. Calibration curve was prepared by plotting concentration vs. difference in absorbance and found to be linear in the concentration range of 2-14µg/mL.

**Accuracy**

Accuracy of the proposed method was determined by calculating the recoveries of MND by standard addition method. It was studied by preparing different solutions at different concentrations of 50%, 100 %, 150% in which the amount of marketed formulation(pre analysed) was kept constant and the amount of pure drug was varied that is 2, 4, 6 for 50%, 100%, 150% in equimolar acidic (0.1N HCl) and basic (0.1N NaOH) solutions respectively. The amount of MND recovered estimated by applying obtained values to the regression line equation. The results were given in the Table 2.

**Precision**

Precision is the degree of repeatability of an analytical method under normal operation conditions, repeatability was done in two ways that is-

1. System precision
2. Method precision

Method precision was achieved by repeating the same procedure of preparation of solution six times, measures the absorbance and calculates the %RSD. In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. The results are shown in Table 3.

**LOD and LOQ**

In this study, LOD and LOQ were based on the standard deviation of the response (σ) and the slope of the corresponding curve (S) using the following equation-

LOD = 3.3σ/S and LOQ = 10σ/S

The results of validation parameters are shown in Table 4.

## RESULTS AND DISCUSSION

The optical characteristics such as Beer's limits within the concentration range of 2-14µg/mL, with correlation coefficient of 0.999. The % Assay was found to be 97.14% (Table-1). No interference was observed from the pharmaceutical excipients. The proposed methods are very precise; the %RSD is less than 2. All of the validation parameters for the proposed method were determined according to ICH guidelines. The method was found to provide high degree of precision (Table-3) and reproducibility (Table-2). The recovery studies showed that the results were within the limit indicating no interference with the marketed formulation. The LOD & LOQ value of the proposed method was found to be 0.15µg/ml and 0.46µg/ml (Table-4). The proposed method is simple, accurate, precise and economic and can be successfully employed for the routine analysis of the Metronidazole in pharmaceutical formulations.

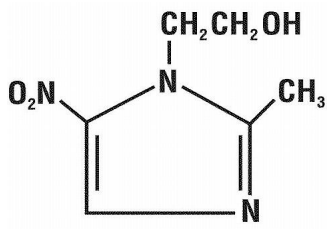


Fig.-1: Structure of Metronidazole

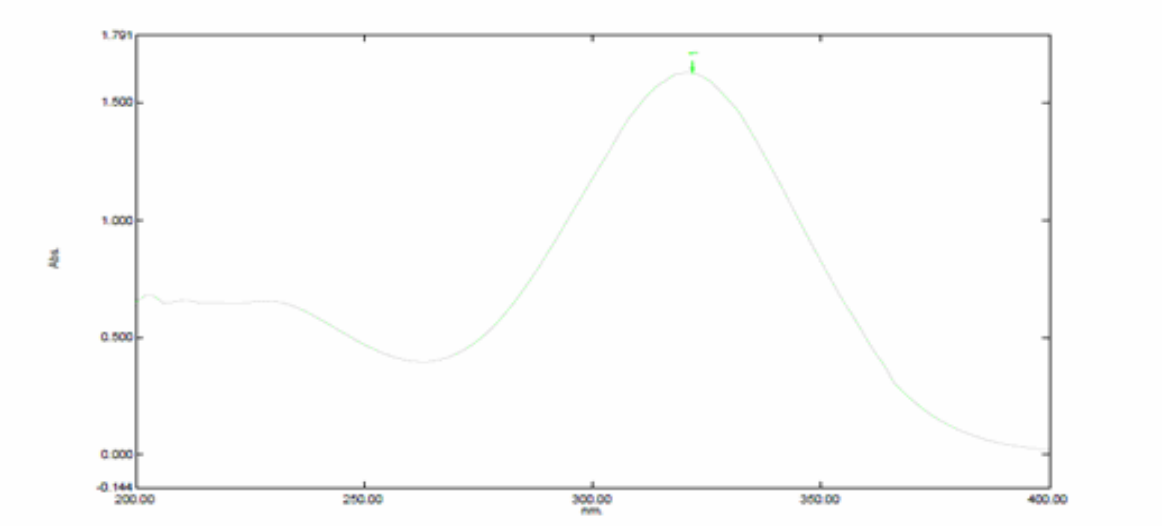


Fig.-2: Differential Spectrum of 0.1 N NaOH

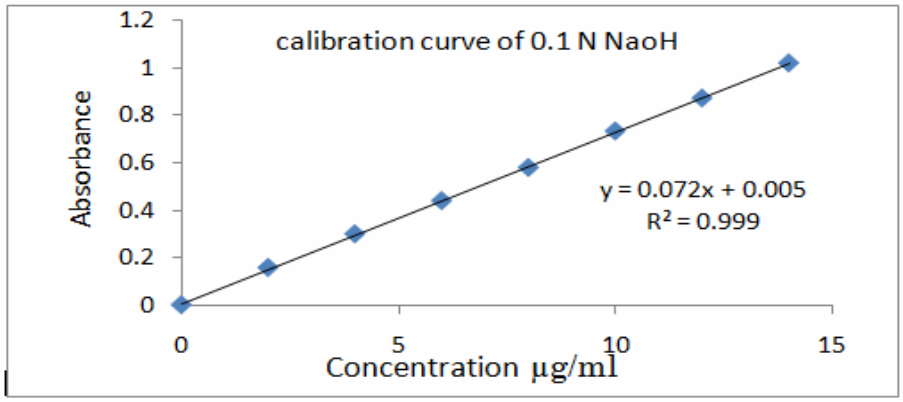


Fig.-3: Calibration curve of 0.1 N NaOH

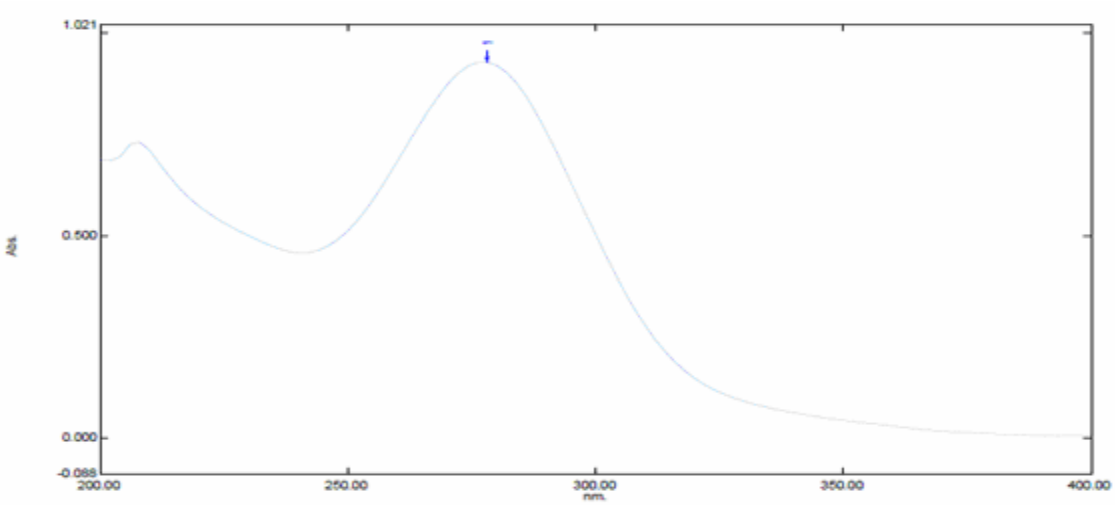


Fig.-4: Differential Spectrum of 0.1 N HCl

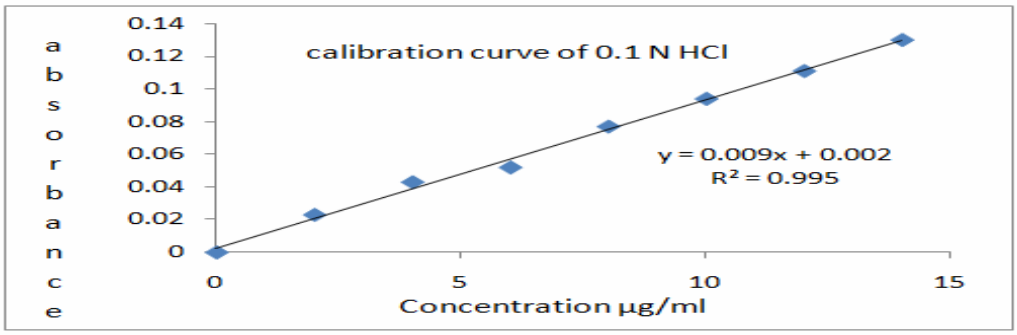


Fig.-5: Calibration curve of 0.1 N HCl

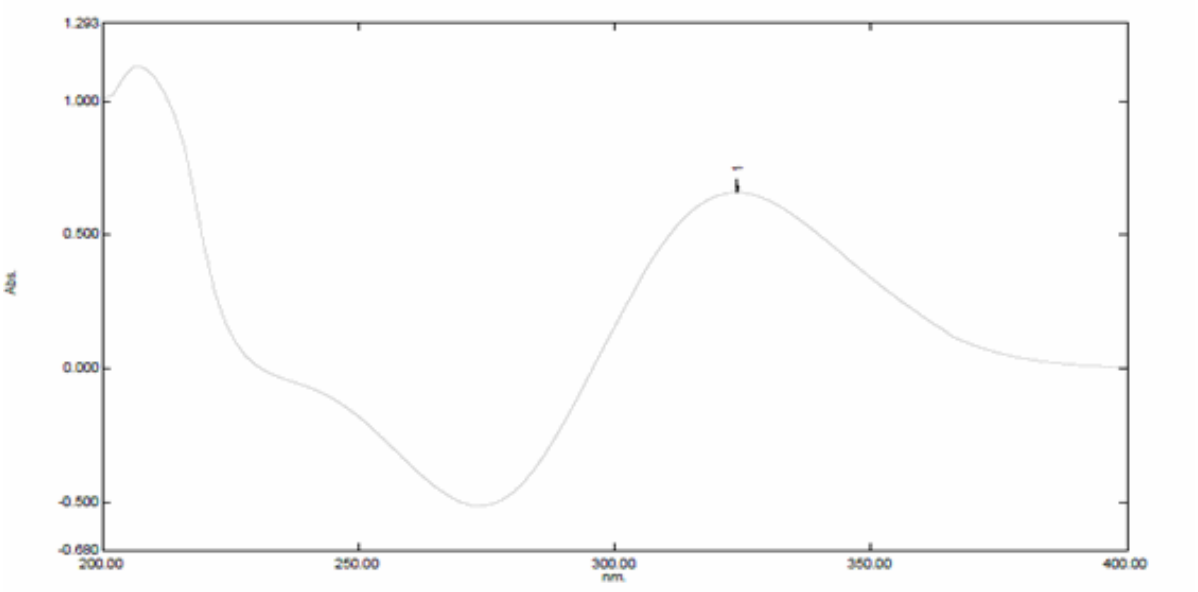


Fig.-6: Differential overlay spectrum

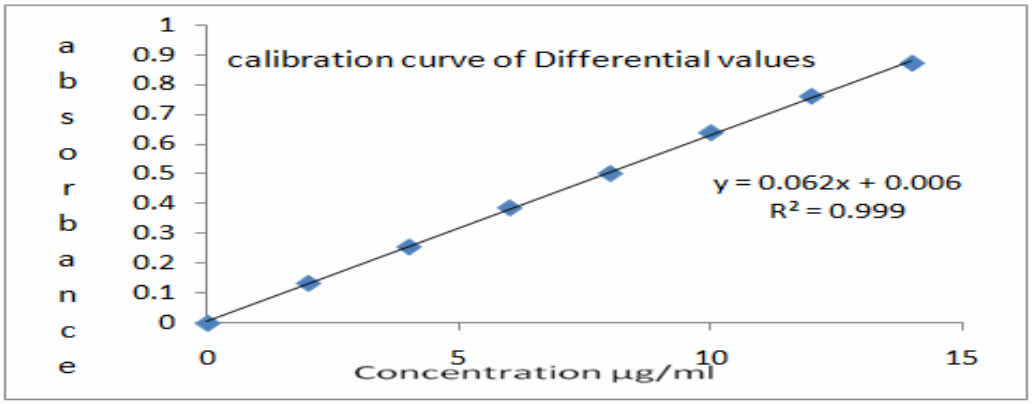


Fig.-7: Calibration curve of Differential values

Table-1: Estimation of marketed formulation

| Label claim (mg) | Amount taken(µg/ml) | Amount found (n=6)(µg/ml) | % recovery | %RSD |
|---|---|---|---|---|
| 400 | 4 | 3.8 | 97.14 | 0.1 |

Table-2: Recovery studies of proposed method

| S. No | Level of recovery | Pre analyzed conc. (µg/ml) | Amount added(µg/ml) | Amount found(µg/ml) | %Recovery |
|---|---|---|---|---|---|
| 1 | 50 | 4 | 2 | 5.98 | 99.66 |
| 2 | 100 | 4 | 4 | 7.90 | 98.75 |
| 3 | 150 | 4 | 6 | 10.12 | 101.2 |

Table-3: Precision studies of proposed methods

| S.NO | Concentration taken(µg/ml) | Method Precision | | System Precision | |
|---|---|---|---|---|---|
| | | Mean±SD | %RSD | Mean±SD | %RSD |
| 1 | 10µg/ml | 0.47±0.0085 | 1.2% | 0.48±0.0004 | 0.10% |
| 2 | 10µg/ml | 0.47±0.0048 | | 0.48±0 | |
| 3 | 10µg/ml | 0.47±0.0069 | | 0.48±0 | |
| 4 | 10µg/ml | 0.47±0.0060 | | 0.48±0.0004 | |
| 5 | 10µg/ml | 0.47±0.0012 | | 0.48±0.0004 | |
| 6 | 10µg/ml | 0.47±0.002 | | 0.48±0.0008 | |

Table-4: Summary of validation parameters of Metronidazole

| Parameter | Metronidazole |
|---|---|
| $\lambda_{max}$ | 324 nm |
| Beer's limit (µg/mL) | 2-14 |
| Linearity indicated by correlation coefficient | 0.999 |
| LOD(µg/mL) | 0.15 |
| LOQ(µg/mL) | 0.46 |
| Precision indicated by % RSD | 0.10% |
| Accuracy indicated by % recovery | 99.93 % |

## CONCLUSION

The proposed method was accurate, precise and rapid as it does not require any sophisticated instruments for chromatographic method. This method was suitable for the routine analysis of Metronidazole as upon altering the spectral characteristics, there was no interference with the common excipients. The preparations of solvents for this method were simple and economical.

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## REFERENCES

1. Arun K. Mishra, Rajdhar Yadava, *IJPRD*, **2**, 6 (2010).
2. Joyani Das, Manabendra Dhua, *Journal of Pharmascitech***, 3,** 2 (2014).
3. Arun K. Mishra, Aravind Kumar, Amrita Mishra, *J. Nat. Sci. Biol. Med*., **5(2)**, 261(2014).
4. P. Thulasamma, P. Venkateswarlu, *Rasayan J. Chem***, 2(4)**, 865(2009).
5. S. Asthour, N. Kattan, *International Journal of Bio-medical Sciences*, **6**,13(2010).
6. P. D. Panzade, K. R. Mahadlik, *East Pharm*, **43** ,115 (2000)
7. P. Nagaraja, K. R. Sunitha, R. A. Vasantha, H. S. Yathirajan, *J. Pharm. Biomed. Anal*, **28** ,527 (2002)
8. T. Saffaj, A. Charrouf, Y. Abourriche, A. Abboud, M. B. Bennamara, *Formaco*, **59**, 843 (2004)
9. D. M. Joshi, A. P. Joshi, *J. Indian. Chem. Soc.*,**74**, 585 (1997)

10. C. M. Kaye, M. G. Sankey, L. A. Thomas, *Br. J. Clin. Pharmacology,* **9**, 528 (1980)
11. R. Yang, *Chinese Journal of Chromatography,***27**, 50 (2009)
12. Indian pharmacopoeia, Controller of publications, New Delhi, **2**, 764 (1996)
13. British pharmacopoeia, Her Majesty's Stationary Office, London, **2**, 1257(2003)
14. The USP Convention, Rockville, MD,The USP 24th edn., The NF,**19**,1104(2000 )

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