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# Direct spectrophotometric method and analytical method validation of metronidazole and ornidazole

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## ABSTRACT

A direct spectrophotometric method was developed for the detection and quantitative determination of Metronidazole and Ornidazole in pure form as well in pharmaceutical formulations in the form of tablets and capsules. The method was based on the formation of reddish-purple colour dye due to the diazotization reaction between the nitro group of the drug samples, sulphanilamide and NEDA. The drug samples dissolved in hot water followed by the addition of 2ml of each 0.5% Sulphanilamide and 0.3% NEDA. It exhibited a stable reddish purple colour. The colour compound showed a maximum absorbance at 540nm. Beer's law was obeyed in the range of 200-800 $\mu gmL^{-1}$ for Metronidazole and 100-600 $\mu gmL^{-1}$ for Ornidazole with a limit of detection of 0.01 $\mu gmL^{-1}$ and 0.02 $\mu gmL^{-1}$ respectively. The method was found to be simple, accurate and rapid.



## KEYWORDS

Metronidazole;
Ornidazole;
NEDA;
Sulphanilamaide;
Spectrophoto0metry;
Quantitative determination of drugs.

## INTRODUCTION

5-Nitroimidazoles such as metronidazole and ornidazole are extensively used as antiamoebic, antiprotozoal and antibacterial drugs. The discovery of the antibacterial and antitrichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as antiparasitic agents. The discovery of the antitri-chomonal properties of metronidazole revolutionised the treatment of disease. Although the amoebicidal properties of metronidazole were studied, it was not clinically tested until some years later. In laboratory tests, metronidazole is effective against intestinal amoebiasis in rats and hepatic amoebiasis in hamsters and is also active against *E. histolytica* in vitro. The initial clinical tests of metronidazole indicated that it was capable of curing invasive amoebic dysentery and amoebic liver abscess. Subsequent clinical tests have established metronidazole as the drug of choice in the treatment of all forms of amoebiasis in humans.

Variation of the structure of metronidazole, principally to improve trichomonacidal activity and metabolic stability, led to the discovery of various other antiamaeobic agents. Ornidazole fall into the same class of drugs. Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propan-2-ol, with chemical formula $C_7H_{10}ClN_3O_3$. Ornidazole is a drug that cures some protozoan infections. It is used by the poultry industry. It has been investigated for use in Crohn's disease after bowel resection. Crohn's disease, also

known as *regional enteritis* is an inflammatory disease of the intestines that may affect any part of the gastrointestinal tract from mouth to anus, causing a wide variety of symptoms. It primarily causes abdominal pain, diarrhea (which may be bloody if inflammation is at its worst), vomiting, or weight loss, but may also cause complications outside of the gastrointestinal tract such as skin rashes, arthritis, inflammation of the eye, tiredness, and lack of concentration.

Most of the spectrophotometric methods found in the literature for the determination of metronidazole and ornidazole in the visible region involve initial reduction by treatment with Zn and HCl followed by the diazotisation and coupling of the resulting amine. All these methods are less sensitive, involve tedious procedures such as heating and extraction, utilise costly reagents and involve an additional diazotisation step. The present method describes a direct, accurate, precise and reproducible spectrophotometric method for the detection and quantitative determination of metronidazole and ornidazole in pure form and in pharmaceutical formulations. The method was validated by using various parameters as per ICH guidelines.

## EXPERIMENTAL

### Reagents

The pure form of samples of metronidazole[1] was prepared in our laboratory and ornidazole was obtained as a gift sample. These pure crystalline products were standardized by the standard method[1,2].

### Metronidazole and ornidazole tablets

Ten tablets each of each of, metronidazole and ornidazole of different pharmaceutical companies under study were accurately weighed and ground into a fine powder. From this, a sample 500mg was weighed followed by the addition of 150ml of double distilled water, and this solution is heated at a temperature of 90° C for 90minutes. After complete dissolution, the cooled solution was filtered through a Whatmann No 40 filter paper, the solution was made up to the mark into a 100 volumetric flask and standardized[1,2].

### 0.5% sulphanilamide in 20% hydrochloric acid

A stock solution of 0.5% sulphanilamide was prepared by dissolving an accurate amount of 0.5g of sulphanilamide in 20% hydrochloric acid, and the solution is made up to the mark using 20% hydrochloric acid in 100ml volumetric flask.

### 0.3% NEDA solution in 1% hydrochloric acid

A stock solution of 0.3% NEDA was prepared by dissolving an accurate amount of 0.3g of NEDA in 1% hydrochloric acid, and then the solution was made up to the mark using 1% hydrochloric acid in 100ml volumetric flask.

All the other reagents used were of AnalaR grade only.

### Apparatus

An ELICO made SL-177, Scanning Visible Spectrophotometer used for all absorbance measurements. Matched set of 1cm glass quartz cuvettes were used.

Shimadzu-AUX 220, digital electronic balance was used for all weighing procedures.

An ELICO made LI-127; pH-meter was used for all pH measurements.

### Recommended procedure for the determination of metronidazole and ornidazole

An aliquot of drug sample of both metronidazole and ornidazole (2.0ml) mixed with 2ml of each 0.5% Sulphanilamide and 0.3% NEDA solution, to give stable reddish- purple coloured product. The mixture was made up to 50ml in a volumetric flask and the spectra were taken for an aliquot of the solution showed a $\lambda_{max}$ at 540nm (Figure 1 and Figure 3)

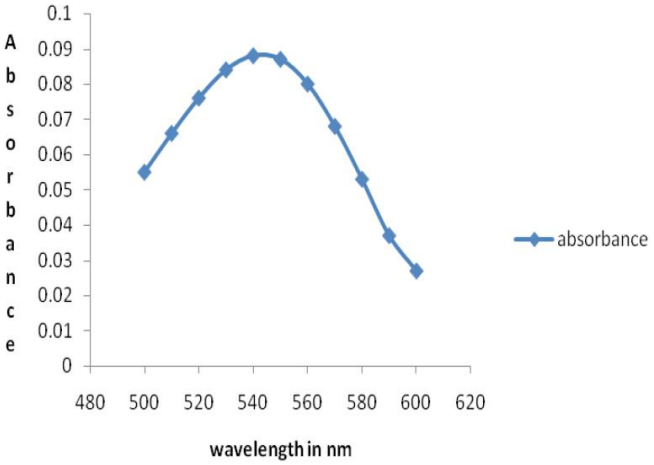


**Figure 1 : Absorption spectrum of the reddish purple coloured product obtained by reaction between metronidazole, sulphanilamide and NEDA. The $\lambda_{max}$ is 540 nm.**

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For the determination of metronidazole and ornidazole, an aliquot volume of the sample solution was mixed with 2ml of each 0.5% Sulphanilamide and 0.3% NEDA solutions, to give stable reddish- purple coloured product. The mixture was made up to 50ml in a volumetric flask. The solution was taken in an optically matched cuvette of ELICO SL-177 spectrophotometer and the absorbances are measured at 540nm. The absorbance was compared with the standard curve (Figure 2 and Figure 4). Beer's law was valid over the range between 100-600µgmL$^{-1}$ for metronidazole (Figure 2) and 100-300µgmL$^{-1}$ for ornidazole (Figure 4).

## RESULTS AND DISCUSSION

The reddish purple colour obtained for metronidazole (MZ) and ornidazole with sulphanilamide and NEDA was determined at a $\lambda_{max}$ of 540nm. This maximum was the only one obtained for the absorption spectra run in the range of 400-600nm. It was observed that the reaction was dependent on the pH as well as the concentration of the reagents. Below and above the pH values of 3.5, the colour produced was found to be unstable and fades. The reddish-purple colour of the product was obtained and stable with 0.5%sulphanilamide and 0.3% NEDA solutions. Below the concentration level, the colour developed was found to be unstable, and above the concentration level colour formation not observed. And hence the concentrations of the reagents were fixed as 0.5% and 0.3% for sulphanilamide and NEDA respectively. For each of the standard solution prepared, the absorbance measurements were recorded for every 30minutes and continued to 3 hours. The reaction product obtained absorbance maximum within 30minutes and was found to be stable more than 24 hours. Measurements taken earlier than 30minutes were found to be in accurate.

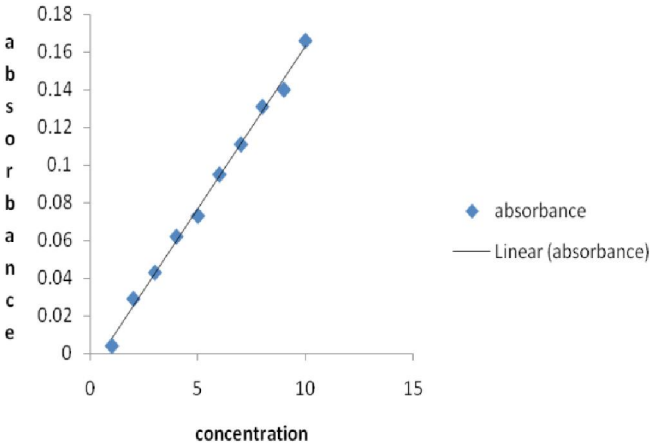


**Figure 2 : Calibration plot for estimation of metronidazole. Beer's lwaw obedience is 200-600µgmL$^{-1}$**

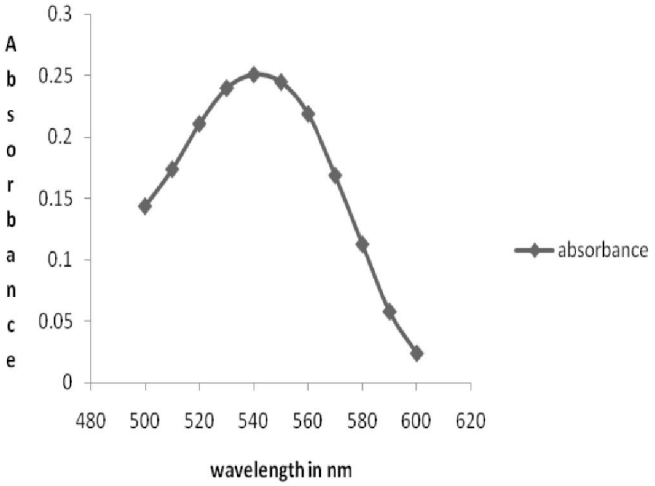


**Figure 3 : Absorption spectrum of the reddish purple coloured product obtained by reaction between metronidazole, sulphanilamide and NEDA. The $\lambda_{max}$ is 540 nm**

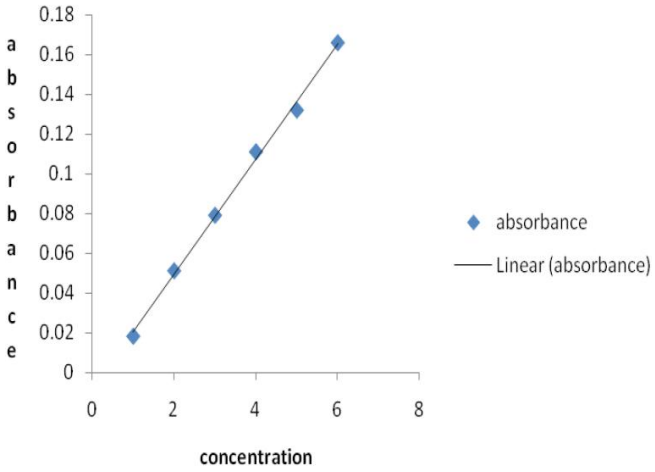


**Figure 4 : Calibration plot for estimation of metronidazole. Beer's lwaw obedience is 100-600µgmL$^{-1}$**

**TABLE 1 : Optical characteristics and validation data**

| Parameters | metronidazole | ornidazole |
|---|---|---|
| $\lambda_{max}$ (nm) | 540 | 540 |
| Beer's law limit (µgmL$^{-1}$) | 200-600 | 100-300 |
| Molar absorptivity (cm$^{-1}$ lit mole$^{-1}$) | 72.68 | 91.14 |
| Stability (h) | > 24 | >24 |
| Correlation coefficient, r | 0.9978 | 0.9985 |
| Relative standard deviation RSD* | 1.4% | 1.8% |
| Limit of detection (µgmL$^{-1}$) | 0.01 | 0.005 |
| Limit of quantification (µgmL$^{-1}$) | 0.04 | 0.015 |

***10 replicate analysis of 200µgmL$^{-1}$**

**TABLE 2 : Analysis for metronidazole and ornidazole formulations**

| Commercial formulations analyzed | PM# | SM@ | RSD** |
|---|---|---|---|
| Metrogyl 500mg | 100.2 | 100.0 | 1.5 |
| Flagyl-500mg | 99.8 | 99.9 | 1.8 |
| Ornidazole -300mg | 98.7 | 99.8 | 1.9 |
| Ornidazole 500mg | 99.5 | 99.9 | 1.4 |

**#Proposed method; @Standard method; **10 replicate analysis**

Beer's law was valid over the range between 200-600μgmL$^{-1}$ for metronidaziole and 100-600μgmL$^{-1}$ for ornidazole. The molar absorptivity (€) of metronidazole was 72.68 cm$^{-1}$ lit mole$^{-1}$ and that of ornidazole was 91.14 cm$^{-1}$ lit mole$^{-1}$. Detection limits (LOD) of metronidazole were 0.01μgmL$^{-1}$ and that of ornidazole was 0.005μgmL$^{-1}$. The limit of quantitation (LOQ) for metronidazole was 0.04μgmL$^{-1}$ and that of ornidazole was 0.015μgmL$^{-1}$. The correlation factor for metronidazole was 0.9979 and 0.9985 for ornidazole. Relative standard deviation calculated for 10 measurements for each of the sample of drug was found to be in the limit prescribed, such as 1.4% for metronidazole and 1.8% for ornidazole. The lower values of RSD indicate the good precision and reproducibility of the method. From the data it was found that the LOQ values were 3.3 times greater than the LOD values. LOD is well below the lower limit of the Beer's law range. The results were accurate, precise and reproducible. To study the potential interference problems from the commonly used excipients and other additives such as glucose, dextrose, lactose, starch, sodium alginate, talc, magnesium alginate, and magnesium stearate, and ascorbic acid, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (tinidazole and secinidazole10 μg mL$^{-1}$), excipi-

**TABLE 4 : Literature survey of the spectrophotometric determination of tinidazole and metronidazole.**

| Reagents used | λmax in nm | Beer's law range in □g mL$^{-1}$ | Critical experimental conditions involved | Reference |
|---|---|---|---|---|
| p-Dimethyl amino cinnam aldehyde | 510 | 50 – 400 for MZ | Involves reduction with Zn-HCl and low sensitivity. Analysed only MZ. | 3 |
| 4-Dimethyl amino benzaldehyde | 550 | 10 – 100 for TZ | Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ. | 4 |
| β-Naphthol | 480 | 10 – 80 for MZ | Involves reduction with Zn-HCl and diazotisation and coupling with the cited reagent. Low sensitivity. Analysed only MZ. | 5 |
| Metol and $K_2Cr_2O_7$ | 720 | 2.4 – 24 for TZ<br>1.6 – 16 for MZ | Involves reduction with Zn-HCl and the use of buffer of pH 2.9 and colour formation, and its stability is pH dependent. | 6 |
| NN-dimethyl-p-phenylenediamine and chloramine-T | 540 | 4 – 36 for TZ<br>3 – 24 for MZ | Involves reduction with Zn-HCl and the use of buffer of pH 7 and colour formation and its stability is pH dependent. | 7 |
| Vanillin | 412 | 10 – 50 for TZ | Involves reduction with Zn-HCl and heating for20 min with the reagent and cooling before absorbance measurement. Analysed only TZ. | 8 |
| Salicylaldehyde | 380 | 20 – 70 for TZ | Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ. | 9 |
| Bromocresol purple | 618 | 2 – 24 for MZ | Involves extraction with $CHCl_3$ and use of buffer of pH 10. | 10 |
| Bromocresol green | 654 | 2 – 22 for MZ | Involves extraction with $CHCl_3$ and use of buffer of pH 9.5. | 11 |
| NaOH and KCl | 368 | 10 – 30 for TZ | Low sensitivity and involves heating at 100 $^oC$ for 10 min. | 12 |
| Bromothymol blue | 440 | not given | Involves extraction with $CHCl_3$ and use of buffer of pH 4.4. | 13 |
| Methylbenzothiazolin-2-onehydrazine (MBTH) | 500 and 490 | 1-32 for MZ<br>4-36 for TZ | Involves reduction with Zn-HCl and MBTH is a costly reagent. | 14 |
| N(1-naphthyl) ethylene diamine dihydrochloride (NEDA) | 520 and 505 | 0.5 – 18 for MZ and TZ | Involves reduction with Zn-HCl and an additional step of diazotisation. Beer's law valid for low range of concentration. | 15 |

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ents in different concentrations were added and analyzed. Excipients up to the concentration of 50mg $ml^{-1}$ do not interfere in the assay. The results were accurate, precise and reproducible.

## CONCLUSIONS

The solutions of metronidazole and ornidazole gave a stable reddish-purple coloured product with 0.5% sulphanilamide and 0.3% NEDA solutions. The $\lambda_{max}$ for the reddish-purple colour product was 540nm, with molar absorptivitites of 72.68 $M^{-1}cm^{-1}$ and91.14$M^{-1}cm^{-1}$ at 540nm. Beer's law was valid over the range between 200-600µgm$L^{-1}$ for metronidaziole and 100-600µgm$L^{-1}$ for ornidazole. The determination of the drug samples was rapid and accurate and hence recommended.

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