# SPECTROPHOTOMETRIC DETERMINATION OF ORNIDAZOLE IN PURE AND PHARMACEUTICAL FORMULATIONS

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**ABSTRACT**

Two simple, sensitive, rapid and reproducible spectrophotometric methods have been developed for the determination of ornidazole in pure form or in their tablets. The proposed methods are based on the reduction of the nitro group to amino group of the drug. The reduction of Ornidazole is carried out with Zn powder and 5N HCl at room temperature in methanol. The resulting amine was then subjected to two methods. Method A is based on the extraction product with Potassium ferricynide - Fe (III) reagent to form bluish green colored choromogen exhibiting an absorption maximum at 570 nm with appearent molar absorptivity of $0.395x10^4$ L $mol^{-1}$ $cm^{-1}$ and obeyed Beer's law in the concentration range 5-55 µg/ml. Method B is based on the oxidation followed by complex with 2, 2-Bipyridyl – Fe (III) to from orange colored chromogen exhibiting absorption maxima 510 nm with apparent molarabsorptivity of $0.710x10^4$ L $mol^{-1}$ $cm^{-1}$ and obeyed Beer's law in the concentration range 5-50 µg/ml .The sandell's sensitivity, limit of detection (LOD) and quantification (LOQ) values have been reported for both the methods. The accuracy and precision of the methods were evaluated on intraday and inter- day basis. The methods were by the common pharmaceutical adjuvants. The reliability and the performance of the proposed methods are established through recovery studies.

**Key words:** Ornidazole; Ferric Chloride; Potassium ferricyanide; 2, 2-Bipyridyl; Zinc powder, Spectrophotometry;

**INTRODUCTION**

Ornidazole, chemically known as 1-chloro-3- (2-methyl-5-nitro-1*H*-imidazol-1-yl) -2 proponol (Fig.1). Ornidazole is a 5-Nitroimidazole derivative and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections. It has been used for amebic liver obscesses, duodenal ulcers, giardiasis, intestinal lambliasis and vaginitis[1-3]. Ornidazole has recently been used with success in patients with active chron's disease[4]. Ornidazole is one of the most frequently used antibiotics for curing Helicobacter pylori infection. Ornidazole has also been preferred for surgical prophylaxix because of its longer elimination half life and excellent penetration in to lipidic tissues versus other nitroimidazole derivatives theraphy [5-6]. Several methods have been reviewed in the literature for the analysis of ornidazole. Some techniques including revealsvoltametry[7,] Adsorptive stripping voltametry[8], HPLC [9-10], Chemiluminescence[11] and spectrophotometric methods[12,13] for its determination in dosage forms and biological fluids. In the current literature, there is no publication concering visible spectrophotometric determination. Purpose of this work is to present the development and validation of simple, sensitive, accurate and precise.

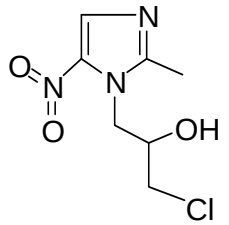


Fig.1: Structure of Ornidazole

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## MATERIALS AND METHODS

### Instrumentation

Shimadzu UV-Visible double beam spectrophotometer (model 2450) with 1cm matched quartz cells was used for all the spectral measurements. All the chemicals used were of A.R. grade.

### Reagents and chemicals

Freshly prepared Methanol, Potassium ferricynide, Ferric chloride, Zinc powder, 2,2-Bipyridyl, Orthophosphoric acid were used in the present investigation.

### Reduction of Nitro group in Ornidazole

100 mg of ornidazole pure or equivalent tablet powder was accurately weighed and dissolved in 20 ml of methanol. The methonolic solution ornidazole was treated with 10 ml of 5N HCl and 0.5g of Zn powder was added in the portions while shaking and refluxed at 80$^{o}$C for 10 min. The solution was filtered using a whattman filter paper 41 to remove the insoluble matter and the volume was made upto 100 ml, to get the final concentration of 1 mg /ml.

### Preparation of standard solution

The resulting amine from the above solution 10 ml was taken in 100 ml volumetric flask and made upto with methanol to get the concentration 100 µg/ml and dilution was carried out to the further working standards.

### Procedure

### Method A

Varying aliquots of standard ornidazole solution equivalent to 5-55 µg/ml (0.5-5.5 ml) were accurately measured by means of micro burette and transferred into a series of 10 ml volumetric flasks and the total volume brought to 10 ml by adding 0.5 % Ferric chloride and 0.5% potassiumferricynide was kept on water bath for 15 min for complete color development and cooled. Then transferred the colored solution in to 125 ml separating funnel. The mixture was extracted twice with 10 ml chloroform by shaking for 2 min, and then allowed to stand for clear separation of the two phases. The absorbance of the separated chloroform layer i.e bluish green colored chromogen was measured against the reagent blank at 570 nm. The colored species was stable for more than 14 hrs.

### Method B

Into a series of 10 ml graduated tubes, aliquots of standard ornidazole solution equivalent to 5-50 µg/ml (0.5 -5 ml) were accurately transferred, and to each tube 1 ml of 0.2% 2, 2-Bipyridyl was added followed by 1ml of 0.2% $Fecl_3$ solution and the resulting solution was heated for 15 min at 80$^{0}$C and finally 2 ml of 0.1N ortthophoric acid was added. The volume was made upto 10 ml with distilled water and the absorbance of the orange colored chromogen was measured at 510 nm against reference blank.

Standard graph was prepared in each case by plotting the absorbance Vs ornidazole concentration and the concentration of the unknown read from the calibration graphs or computed from the respective regression equation derived using the absorbance – concentration data.

### Application for Pharmaceutical formulations

Accurately weighed amounts of the powdered ornidazole 250 mg and ornidazole 500 mg tablets equivalent to 100 mg was transferred into separate 100 ml volumetric flasks, and made upto the mark with methanol. Then the solution was sonicated for 10 min to give a working solution of 100 µg/ml. Aliquots in the concentration range cited in Table 2 and Table 3 was transferred in to 10 ml volumetric flasks. The general procedure was then applied as under construction of calibration graph, and the nominal contents of tablets were determined either from previously plotted calibration graphs or using the corresponding regression equations.

## RESULTS AND DISCUSSIONS

The proposed methods are simple, rapid and precise and do not suffer from any interference due to excipients of tablet. The proposed spectrophotometric methods were found to be linear in the range of 5-55

µg/ml and 5- 50 µg/ml with correlation co – efficient ($R^2$) of 0.994 and 0.997. The methods were validated in terms of accuracy; precision, reproducibility and the results are recorded in Table 2 and Table 3. The accuracy of the methods was determined by performing recovery studies by standard addition method in which pre analysed samples were taken and standard drug was added at three different levels. The precision of the proposed methods were estimated in terms of interday precision and intra day precision where in the methods were repeated on three different days and repeated for three different time periods in the same day respectively. The selectivity of the methods were checked by monitoring a standard solution of ornidazole in presence of excipients at the same concentration level as used in tablet using the methods described in the procedure for calibration curve in pharmaceutical tablets.

## CONCLUSION

Proposed methods are simple, sensitive and reproducible. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of ornidazole in pure form and in pharmaceutical preparations.

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Table.1: Optical Characteristics of proposed methods

| Parameters | Method A | Method B |
|---|---|---|
| λmax nm | 570 | 510 |
| Beer's Law limit (μg $ml^{-1}$) | 5-55 | 5-50 |
| Molar absorptivity | $0.395x10^4$ | $0.71x10^4$ |
| (L. $mol^{-1}$ $cm^{-1}$) | | |
| Specific absorptivity | 0.0179 | 0.0323 |
| Sandell's sensitivity (μg.$cm^{-2}$/0.001 A U) | 0.0558 | 0.0309 |
| Correlation coefficient ($r^2$) | 0.994 | 0.997 |
| Regression equation (Y = mX + C) | | |
| Slope (m) | 0.017 | 0.032 |
| Intercept (C) | 0.009 | 0.011 |
| % Relative Standard deviation | 1.13 | 1.18 |
| Color | Bluish green | Orange |
| Limit of detectiion ((μg $ml^{-1}$) | 0.9 | 0.98 |
| Limit of quantification ((μg $ml^{-1}$) | 1.2 | 3.1 |

Table.2: Assay of Ornidazole in Pharmaceutical formulations

| Pharmaceutical formulations | Labeled amount mg | Amount found by proposed method* mg | | % Recovery by proposed methods | |
|---|---|---|---|---|---|
| | | Method A Mean ± S.D | Method B Mean ± S.D | Method A | Method B |
| Tablet-I | 250 | 249.7±0.07 | 249.97±0.09 | 99.89 | 99.90 |
| Tablet-II | 500 | 499.2±0.22 | 499.3 ±0.12 | 99.84 | 99.86 |

* Average of five determinations

Table.3: Determination Ornidazole in its dosage forms

| Method | Amount added (µg/ml) | Amount found* (µg/ml) | % Found ± SD | RSD % |
|---|---|---|---|---|
| | Intraday | | | |
| | 15 | 14.909 | 99.39 ± 0.028 | 0.190 |
| **A** | 30 | 29.865 | 99.55 ± 0.019 | 0.064 |
| | Inter day | | | |
| | 15 | 14.866 | 99.11 ± 0.004 | 0.031 |
| | 30 | 29.812 | 99.37 ± 0.074 | 0.250 |
| | Intraday | | | |
| | 15 | 14.919 | 99.46 ± 0.023 | 0.154 |
| **B** | 30 | 29.916 | 99.72 ± 0.011 | 0.039 |
| | Inter day | | | |
| | 15 | 14.911 | 99.40 ± 0.029 | 0.201 |
| | 30 | 29.912 | 99.70 ± 0.016 | 0.055 |

* Average of five determinations