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Visible Spectrophotometric Estimation of Ornidazole in Pure and Pharmaceutical Formulation

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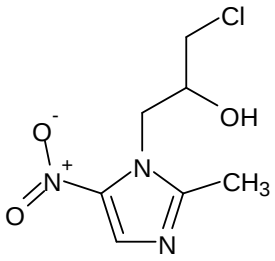
Abstract: The main objective was to develop and validate a simple, accurate, precise and sensitive ion-pair spectrophotometric extraction method for the assay of Ornidazole in pure drug and tablets. The method is based upon reduction of ornidazole and reaction of reduced ornidazole with orcinol to form yellow coloured ornidazole-orcinol complex. The colored complex obeyed Beer's law in the concentration range of 10-60 µg/ml at λ_{max} of 420 nm. The proposed method was validated as per ICH guidelines Q 2. The recovery studies confirmed the accuracy and precision of the method. The above method was a rapid tool for routine analysis of Ornidazole in the bulk and pharmaceutical dosage forms.
Keywords: Visible spectrophotometry, colorimetry, estimation, ornidazole.

INTRODUCTION

Ornidazole, chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol, with molecular formula $C_7H_{10}N_3O_3Cl$ is an antimicrobial agent[1]. Ornidazole is used in the treatment of amoebiasis and other protozoal diseases. It is used in treatment of amoebiasis, giardiasis, trichomoniasis etc. This drug is under the category anthelmentics[2]. Ornidazole is a 5-nitroimidazole derivative. It is converted to reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to a protein synthesis inhibition and cell death in susceptible organisms[3].

It is an official drug in Indian pharmacopoeia. Literature review revealed that few methods are available for the determination of ornidazole in bulk and solid dosage form based on spectrophotometry[4-13] and RP-HPLC[14]. The aim of present study work is to develop a simple, precise, accurate and sensitive method based visible spectrophotometry using orcinol. The method involves reduction of ornidazole by reaction with Zn/HCl followed by diazotisation and coupling.



Structure of Ornidazole

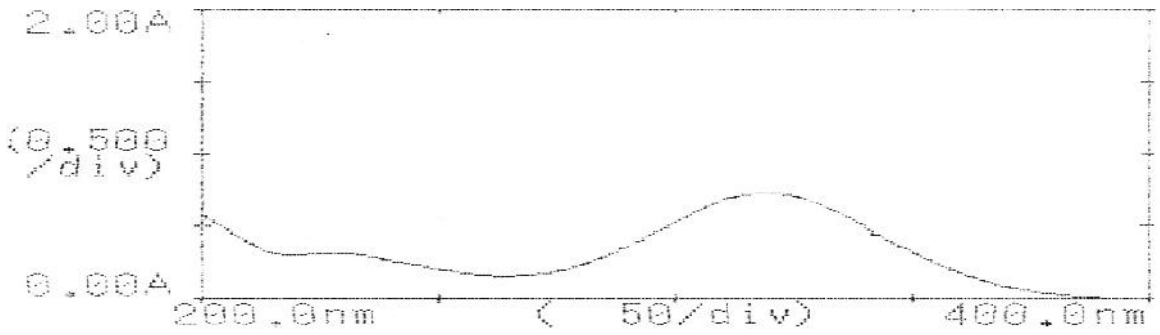
EXPERIMENTAL

Instrument: A Shimadzu UV-Vis Double beam spectrophotometer (Pharmaspec-1700) with 1 cm matched quartz cells was used for all spectral measurements.

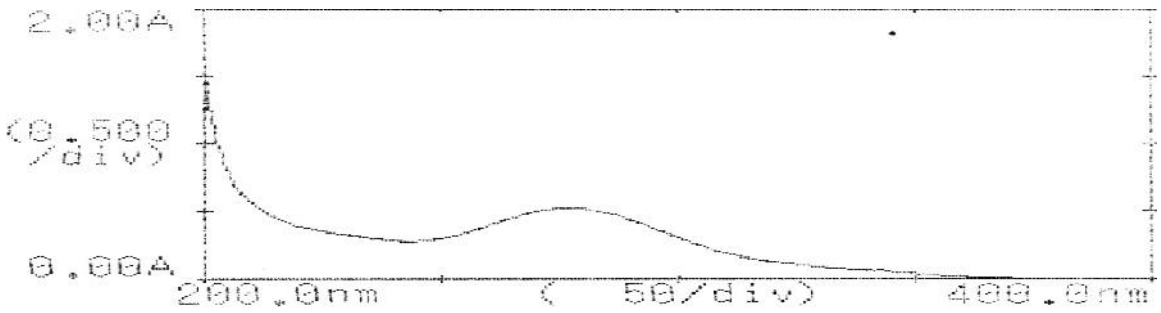
Materials: The tablets (referred as Dazolic®) were purchased from a local pharmacy (The label claim contained 500 mg of Ornidazole). All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Various chemicals used were Sodium nitrite, Hydrochloric acid, Zinc granules, Sulphamic acid, Orcinol and Ice-bath.

Preparation of standard stock solution: The standard stock solution of Ornidazole was prepared, by dissolving 100 mg of pure ornidazole in 10 ml of methanol and treated with 20 ml of 4 N HCl and 1.2 gm of zinc granules was added in proportions. After standing for 2 hours at room temperature, total volume of the reaction mixture was made upto 100 ml with distilled water, to get the final concentration of 1 mg/ml.

From this stock solution, appropriate dilutions were made with distilled water to obtain 20 µg/ml of Ornidazole and the sample was scanned between 200 nm to 400 nm on a double beam spectrophotometer. The UV spectrum of ornidazole is given in figure below, λ_{max} of ornidazole was found to be 320 nm.



The UV spectrum of reduced ornidazole is given in the figure below, λ_{max} of reduced ornidazole was found to be 278 nm.



Construction of calibration curve: Appropriate aliquot of standard stock solution 1, 2, 3, 4, 5 and 6 ml was transferred into six different 10 ml volumetric flasks to obtain the concentration of the drug given in the Table 1. 1 ml of 5 N HCl and 1 ml of 0.1 N $NaNO_2$ were transferred into each volumetric flask and then kept in an ice bath for 10 minutes. 1 ml of 1% Sulphamic acid solution and 1 ml of 1.5% Orcinol solution were added into each flask and then they were kept in an ice bath for another 5 minutes to develop the Yellow colour chromogen having λ_{max} 420 nm. The absorbances were plotted against the respective concentrations to obtain the calibration curve. The blank reagent was prepared by using all the reagents except the drug solution. The linearity data is given in Table 1**.**

Stability study: The reduced primary amino group forms a diazonium salt and forms coupling complex with Orcinol. Stability study of the developed yellow chromogen was carried out, by measuring the absorbance values at time intervals of 20 minutes for 5 hours, and it was found to be stable for more than 4 hours at room temperature.

Analysis of pharmaceutical formulations: Twenty tablets were weighed and ground to fine powder. An accurately weighed powder sample equivalent to 100 mg of Ornidazole was transferred to a 100 ml volumetric flask. The powder was dissolved in 10mL of methanol and the volume was made up with water. The solution was then filtered through Whatmann Filter paper no 40. Ten ml of this filtrate and 20ml of 4N HCl and 1.2 gm of zinc granules was added in proportions. After standing for 2 hours at room temperature, total volume of the reaction mixture was brought to 100 ml with distilled water to get stock solution of 100 µg/ml. Aliquot 1 ml of working stock solution was transferred into four different 10 ml volumetric flasks and the volume was made up to mark with water. Analysis of the tablet was done as per the proposed colorimetric method and the concentration was determined by utilizing the linear regression equation. The results of tablet analysis are given in the Table 2.

Validation: As per I.C.H. guide lines.

Linearity: The linearity range was found in between 10 - 60 µg/ml.

Precision: Precision study was performed to find out intra-day (within a day) variations in the estimation of Ornidazole of different concentrations with the proposed method. Percentage relative standard deviation (% RSD) was found to be less than 1% for within 5 hours, which proves that method is precise.

Accuracy: It was found out by recovery study using standard addition method. Known amounts of standard Ornidazole was added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed colorimetric method. Results of recovery studies are shown in Table 3.

Table 1: Linearity data of Ornidazole

Parameters	**Value**
Linearity range (µg/ml)	10 - 60
Linear regression equation (Y = mx + c)	Y = 0.0472x - 0.1405
Regression coefficient (r^2)	0.9939

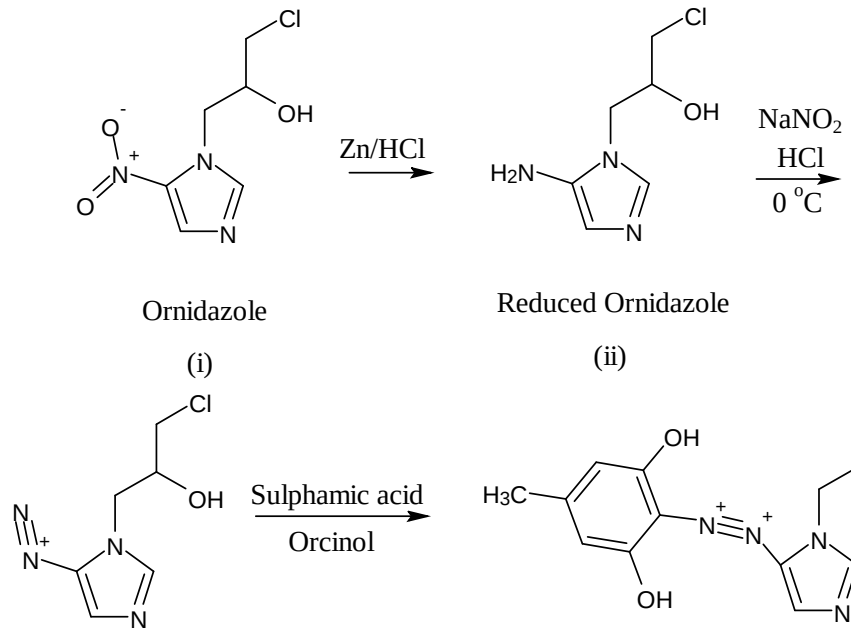
Table 2: Analysis of marketed formulation (Dazolic®)

Label claim (mg/tab)	**Found Concentration* (mg/tab)**	**C.I.**	**%RSD**
500	502.7122	100.5424 ± 2.0772	1.2982

*Average of four determinations

Table 3: Recovery data of Ornidazole

Level of recovery	**% Recovery ± SD**	**% RSD**
80 %	100.9041 ± 1.1572	1.1676
100 %	100.3774 ± 1.1044	1.1085
120 %	100.0917 ± 1.4098	1.4111



Mechanism of reaction:

Ornidazole is reduced by reaction with Zn/HCl. The reduced ornidazole is then reacted with $NaNO_2$/HCl at 0℃ to obtain the diazonium salt of reduced ornidazole. This diazonium salt of reduced Ornidazole is then reacted with orcinol in presence of sulphamic acid to form yellow coloured ornidazole-orcinol complex.

RESULTS AND DISCUSSION

In aqueous medium, Ornidazole form a yellow absorbed complex with orcinol. The analytical wavelength for measuring absorption maximum for Ornidazole-orcinol yellow complex was observed at 420 nm against the reagent blank. Absorption maximum at 401 nm observed for the reagent blank identical experimental conditions were used. The extent of formation of Ornidazole complex is governed by orcinol concentration. The absorbance of the complexes initially increased in the concentration range of (0.02-1.5%) orcinol and then attained practically a constant value in the concentration range of (1-1.5 %) orcinol. Thus it was found that 1.5% concentration of orcinol yellow in the range of 1ml for the achievement of maximum colour intensity. The effect of temperature on the product was studied at different temperatures. The colored product was stable in the temperature range of 25 to 30ºC. However, resultant product was stable for more than 4 hour at 25 -30ºC. The validity of the method for the assay of tablets was determined. The percentage recovery experiments revealed good accuracy of the data. There is no need for the separation of soluble excipients present in marketed tablets as the results were always reproducible equivalent to the labelled contents of the preparations. The recovery results of the proposed method were well agreed with the reported RP-HPLC method for Ornidazole coated tablets.

The proposed method for the determination of Ornidazole was simple, accurate, linear, precise, reproducible and free from interferences of other additives present in the formulation. Hence the method can be used for routine analysis of Ornidazole in bulk and solid dosage form.

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