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Original Research Article

Analytical method development and validation of ornidazole by using uv spectroscopy

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ABSTRACT

The present research work discusses the development and validation of a UV spectrophotometric method for ornidazole. Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of ornidazole in pure drug form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 430 nm. Beers law was obeyed in the concentration range of 1μg/mL. Calibration curves shows a linear relationship between the absorbance and concentration. Validation was performed according to ICH guidelines for Linearity, accuracy, precision, LOD (limit of detection) and LOQ (limit of quantification). The sample solution was stable up to 36 hours. The proposed method may be suitable for the analysis of ornidazole in drug formulation for quality control purposes.

1. Introduction

Protozoal infections are a major cause of disease in countries where hygiene conditions are lesser. These unicellular protozoan cells are more or less resembling eukaryotic and hence have metabolic functions closer to human host cells. They include amoebiasis, giardiasis, trichomoniasis, trypanosomiasis, toxoplasmosis and leishmaniasis. The amoebic infection caused by Entamoeba histolytica could be Asymptomatic or may present as mild to moderate colitis or as dysentery or as liver abscess. This Entamoeba histolytica exist in two forms 1. Cyst form (survives outside the host body), 2.Trophozoites (Survives within the host body).

This Ornidazole, chemically (2S)-1-chloro-3-(2-methyl-5- nitro-1H- imidazol-1-yl) propan-2-ol, with molecular formula C7H10N3O3Cl. Ornidazole is a 5-nitroimidazole derivative. It is converted to reduction products that interact with DNA of the microorganism, to cause destruction of helical DNA structure and strand, leading to a protein synthesis inhibition and cell death in susceptible organisms. It is an official drug in Indian pharmacopoeia.

2. Aim and Objective

The aim of the present work is to develop a simple, precise, accurate and sensitive method using visible spectrophotometry. The method is a derivatization technique which involves reduction of nitro group of ornidazole by reacting with Zn/HCl (Clemmenson reduction) to form amino compound followed by formation of diazonium salt by reacting with Sodium Nitrite in Conc HCl. This Diazonium salt reacts with aromatic compound having ortho para directing -OH group to yield yellow coloured complex which is measured by Double beam UV spectrophotometry of Systronics.

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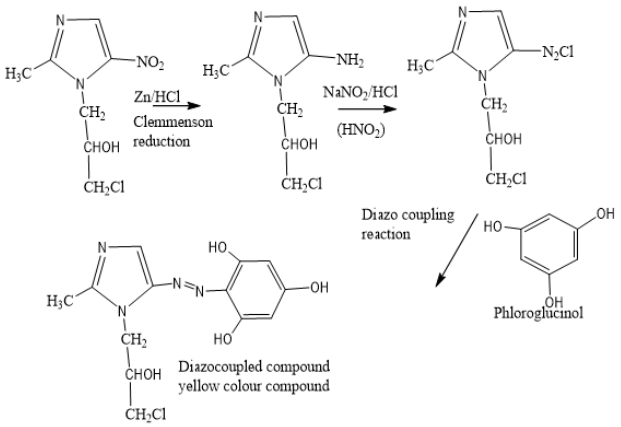
In this method development technique the Nitro group of ornidazole is reduced by Clemmenson reduction to form an amino derivative. This amino group is reacted with $NaNO_2$ /Conc HCl to form diazonium salt. This diazonium salt reacts with Phloroglucinol to form a diazo-coupling compounds, which gives yellow colored Hydroxy azo derivative. The UV absorption of this colored derivative is measured at 430 nm. A standard linear graph is obtained. The ornidazole tablet sold in the market is compared with the Standard linear graph. The results are tabulated, compared and reported.

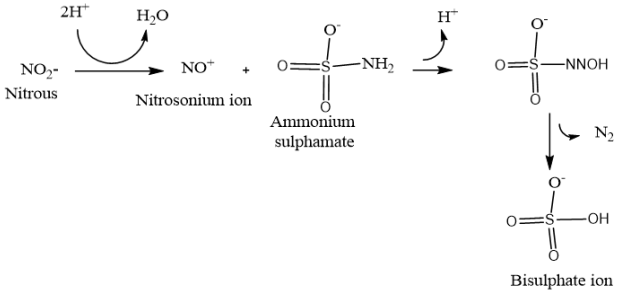
3. Method development

The reaction of Diazonium salt with aromatic compounds having Ortho para directing groups like -OH, - NH_2 to yield Diazo coupling product is called Diazo coupling reaction. This p^H should be carefully monitored at acidic condition to enhance the reaction



Ornidazole has a nitro group (-NO2) at its 5 th position of the imidazole nucleus. This nitro group on treatment with zinc dust in HCl (Clemmenson reduction), gets reduced to Primary amino group. This primary amino group on reacting with sodium nitrite in conc HCl (Nitrous acid is formed insitu), forms diazonium salt. This diazonium salt is reacted with phloroglucinol to yield diazo coupling product (yellow colored product). The UV absorption at 430 nm of this colored diazo coupling product forms the basis for this method development.

The nitrous acid formed in excess can be removed by adding ammonium sulphamate in acid condition, so that the nitrous acid gets converted to Nitrogen gas and sulphuric acid.



4. Materials Used

4.1. Instruments

UV Spectrophotometer

UV Visible Double beam spectrophotometer 2704-x-system with matched quartz cells.

4.2. Reagents

1. Active Pharmaceutical Ingredient “ORNIDAZOLE” (Supplied from Stedmann Pharmaceuticals, Chennai)
2. Distilled water
3. Sodium hydroxide
4. Ammonium Sulfamate
5. Phloroglucinol
6. Concentrated Hydrochloric acid
7. Sample tablet
8. Tablet Formulation Available Market: ORNI 500
9. Manufacturer: Zydus Cadilla.

4.3. Sample bablet

1. Tablet Formulation Available Market: ORNI 500
2. Manufacturer: Zydus Cadilla

4.4. Parameter estimated

1. Determination of absorption maximum (λ max)
2. Linearity (beer’s law concentration range)
3. Estimation of dosage form
4. Accuracy (recovery study)

4.5. Establishment of parameter

1. Absorption Maximum (λ max)
2. Beer’s Law concentration (linearity)
3.

5. Preparation of Standard Drug Solution: [1]

Zn /Hcl Reduction: 200mg zinc powder + 5ml water + 2.5ml of 4NHcl. keep it for reduction for 1 hour and then filter it.

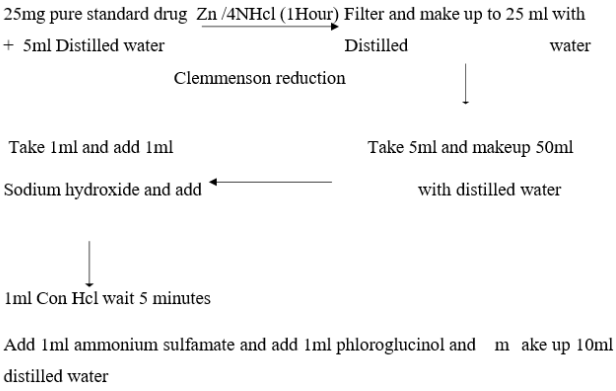


Chart 1:

5.1. *Absorption maximum*

It is wavelength of light at which a substance shows maximum absorbance. The standard stock solution is diluted to 25mg/ml and scanned over a visible region of 400-500nm. The absorption values were tabulated to find the absorption maximum. Then the absorption maximum was found at the wavelength of 430nm.

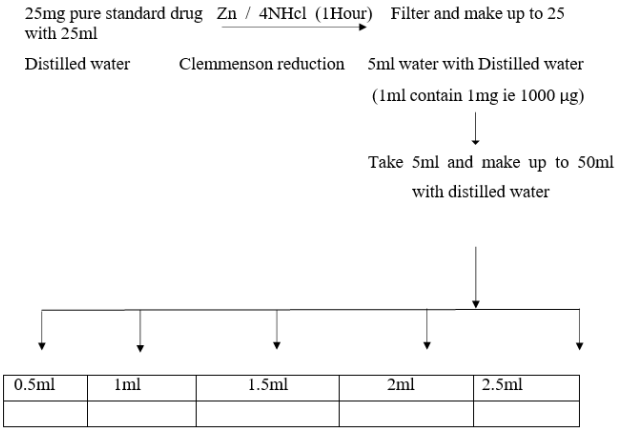


Chart 2:

Add 1 ml of sodium hydroxide and 1ml of Conc Hcl and wait for 5 minutes and add 1 ml of ammonium sulfamate and 1 ml of phloroglucinol solution and makeup to 10ml with distilled water.

5.2. *Linearity*

Beer's Law Concentraton Range

The stock solution was suitably diluted with distilled water to get various concentration of ornidazole and their absorbance was measured and was found that ornidazole obeys beer's law.

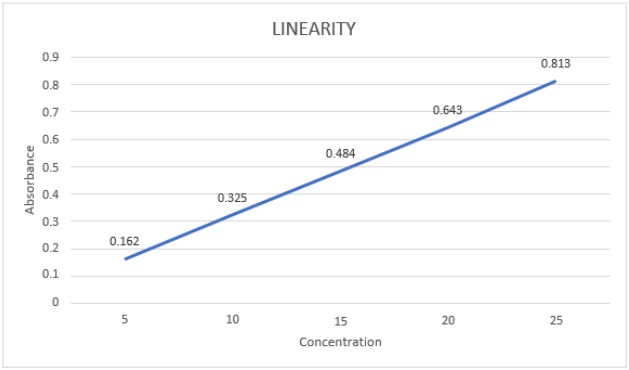


Figure 1:

Table 1:

Concentration in µg	Quantity in ml	Absorbance
5	0.5	0.162
10	1.0	0.325 std absorbance
15	1.5	0.484
20	2.0	0.643
25	2.5	0.813

5.3. *Preparation of test solution*

Twenty tablets (Brand: ORNI 500) were weighed and the label claim of tablet content is noted and the average weight of each tablet was calculated.

Average weight of 20 tablets = 0.6576 gm Label claim or tablet content = 500 mg Test sample powder quantity to be taken

$$Equivalent\ to\ 0.025\ gm\ (25mg)\ = \frac{Avg\ weight}{Lable\ claim} X\ weight\ to\ be\ take$$

Five tablet were powdered individually and a quantity equivalent to 0.025gm of powder was taken from each tablet in separate flask and it was subjected to Clemmenson reduction using Whatman filter paper and makeup to 25ml and, from this take 5ml and make up to 50 ml, From this 1ml of solution is taken and add 1ml of 0.1% sodium Nitrite and add Conc Hcl 1ml and wait for 5 minutes. Then add 1 ml of 0.5% Ammonium sulfamate and 1ml of 0.5% phloroglucinol and makeup to 10 ml with distilled water in a standard flask.[1–3] The content of Ornidazole present in each tablet of average weight is calculated

Recovery Study (Accuracy) by Standard addition method.

5.4. *Preparation of standard drug solution*

0.025 gm of pure standard drug is made up to 25 ml using distilled water (1ml=0.001gm)

Table 2:

S.no	Weight equivalent of tablet powder taken	Absorbance (nm)	Content of Ornidazole
1	0.032	0.315	0.497
2	0.033	0.320	0.490
3	0.034	0.331	0.492
4	0.035	0.341	0.492
5	0.036	0.351	0.493

5.5. Preparation of test sample for recovery study

032gm of tablet powder equivalent to 25mg of pure drug was taken

Add 5ml (5mg) of above standard drug solution .This mixture is subjected to clemmenson reduction (Zn /Hcl), filter using filter paper make up 25 ml with distilled water and for, this dilution is done as per flow chart.[4-7][4–6] This procedure is carried out for five tablet as individual methods and the absorbance is measured

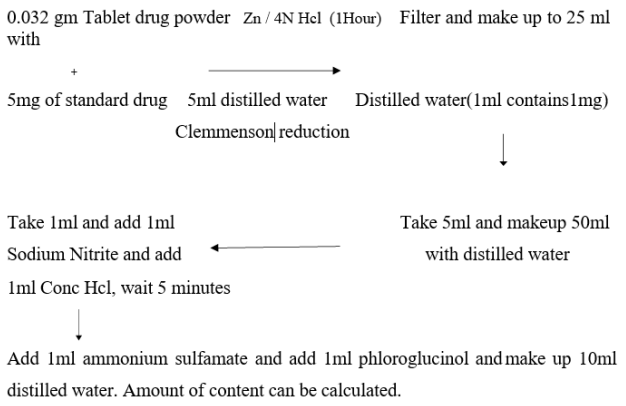


Chart 3:

6. Result and Discussion

1. The maximum absorbance of ornidazole was found to be 430nm from table no.1 five different standard solution of concentration 5,10,15,20,25, μg were prepared and the absorbance are found out and the linearity graph was plotted.
2. The quantitative estimation was carried out by taking the concentration of 100 μg/ ml. For five test sample tablets of ORNI 500 and their individual absorbance was noted in the table no.3 . More over the content of the ornidazole present in gram in all five tablet were calculated
3. The recovery study (Accuracy) is also performed by the standard addition method on adding 5mg standard drug of ornidazole to each tablet sample and their absorbance with content present in each tablet is shown in table no :4

Table 3:

Tablet . No	Absorbance	Amount present in each tablet	Percentage of content
1.	0.318	0.502	100.4 %
2.	0.324	0.496	99.2 %
3.	0.335	0.498	99.6 %
4.	0.345	0.498	99.6 %
5.	0.355	0.498	99.6 %

Average of test absorbance	Average content Present gm / tablet	Standard deviation for content present in tablet	Avg % Label claim %w/w	%Deviation
0.335	0.498	0.00195959	99.68	0.32

4. The average of test absorbance an its standard deviation were calculated.

   (a) The average content in gms present in each tablet and it standard deviation were calculated. The percentage label claim was found to be 99.68% w/w and percentage deviation from label claim was found to be

   (b) 0.32 % w/w.

7. Conclusion

1. Ornidazole was determined by a new method of formation of Diazo coupled product which produces a visible colour in acid medium. Distilled water was used as solvent. From the analytical and statistical data obtained, the method can be accurately applied for the determination of ornidazole present in formulation. The maximum absorption was found to be 430nm and the linearity range was found as 5-25 μg.
2. The recovery study shows a percentage label claim of 99.68% w/w and a percentage deviation from label claim were found to be 0.32%w/w. This recovery study by standard addition method states that this method can be successfully used for estimation of ornidazole.

8. Acknowledgment

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9. Source of Funding

None.

10. Conflict of Interest

None.

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