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Research Paper

Validation and Analytical Method Development for Determination of Ornidazole in Ointment Formulation by U.V Spectrophotometric Method

Shivani[1*], Ajay Kumar Kpilesh[2], Megha Sharma[1]

[1]School of Pharmaceutical Ssiences, Shoolini University, Solan-173229, Himachal Pradesh, India
[2]Department Regulatory Affairs, Cosmas Research Lab Limited, Ludhiana, Punjab, India

ABSTRACT

A new, simple and accurate UV method has been developed for the estimation of Ornidazole in ointment formulation using Systronic UV Visible Double beam spectrophotometer in presence of Povidone iodine using distilled water as a solvent system. The absorbance maxima of Ornidazole in presence of Povidone iodine was found to be 320 nm, while the λ_{max} of pure Ornidazole was found to be 315.2 nm in distilled water. The calibration curve was linear over the concentration range 5-25µg/ml for Ornidazole. The method has been validated by critical parameters like accuracy, precision, robustness, LOD and LOQ etc. This method was found to be accurate and precise for determination of ornidazole in bulk and semisolid (ointment) dosage form.

Keywords: - Method validation, Ornidazole, Povidone iodine, Ointment

INTRODUCTION

Ornidazole is tissue amoebicide. It belongs to the category of nitroimidazoles. This is for both intestinal and extra intestinal amoebiasis. It has broad spectrum cidal activity against protozoa including Giardia lamblia. Ornidazole chemically is known as 1-(3-chloro-2hydroxypropyl)-2-methyl-5 nitroimidazole ($C_7H_{10}CLN_3O_3$), having molecular weight 219.625.

***Corresponding Author:**
Shivani
School of Pharmaceutical Ssiences, Shoolini University, Solan-173229, Himachal Pradesh, India
E.Mail: shivanikashyap28@gmail.com
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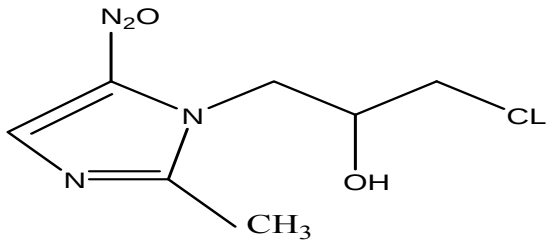


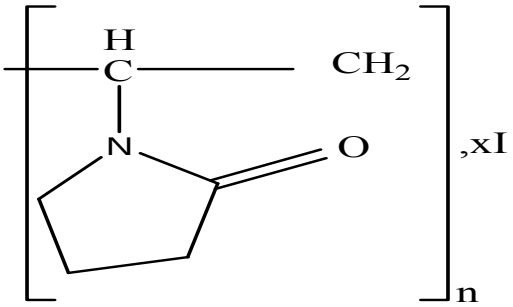
Structure of Ornidazole

Ornidazole is a white to yellowish white,crystalline powder. Ornidazole contains not less than 98.0% and not more than 102.0% of ornidazole($C_7H_{10}CLN_3O_3$),calculated on anhydrous basis. Ornidazole is soluble in chloroform, methanol, water, ether, and ethanol and having melting point 85-90^{0}c.

Mechanism of action: Nitroimidazole drugs require reductive activation to exert

antimicrobial antimicrobial activity.This activity has been proposed to be due to the reduction of nitro group to a more reactive amine that attack microbial DNA,inhibiting further synthesis and causing degradation of existing DNA.

Povidone Iodine is a complex produced by interaction between iodine and poly (2oxypyrolidine 1-ethylene).



Structure of Povidone Iodine

Povidone iodine is a yellowish brown amorphous powder, slight and characteristic odour of I_2.It contains not less than 9.0% and not more than12.0% of available iodine I, calculated on the dried basis. Povidone Iodine is easily soluble in cold water, ethyl alcohol, isopropyl alcohol, glycol, glycerin, acetone and polyethylene glycol.

Mechanism of action: Iodophores are most soluble complexes of iodine with large molecular organic compounds that serve as carriers-releases free I_2 slowly. The most popular povidone iodine is non- irritating, nontoxic, nonstaining and exerts prolonged germicidal action [18-19].

Ornidazole is a non official drug, as it is not described in any pharmacopeia. The combination of two drugs is not official in any pharmacopoeia, hence no official method is available for estimation of ornidazole in presence of povidone iodine in combined dosage form (ointment).Literature review reveal that there are method of determining ornidazole by Rp-HPLC[5, 6, 9, 10, 12, 17 & 20], UV[1, 3, 4, 7, 8, 13, 14 & 15], HPTLC[11], Electroanalytical [16], Visible [2]. Not a single method was found to determine the ornidazole in ointment formulation by UV.

Ointment formulation used are Metrogyl P (A) and Alphadinoz(B)

Ornidazole IP 1%w/w + Povidone Iodine IP(0.5%w/w available iodine)5%w/w ointment has been approved on 2012.Indicated for the prevention and treatment of surgical wound infection in adult patients undergoing surgery.

MATERIAL AND METHOD

Materials

Ornidazole and Povidone iodine were received as a gift sample from Meridian Medicare Private Ltd, Solan, Himachal Pradesh, India. The ointments of said combination were purchased from a local pharmacy (the label claims Ornidazole1% and Povidone Iodine5%).

Instrumentation

FT-IR (Cary 630 Agilent Technologies) studies were carried out on the samples as soon as received for their actual structure determination. A Systronic UV Visible Double Beam Spectrophotometer 2202 series with quartz cell was used for all spectral measurements. An ultrasonic cleaner was used for appropriate dissolution of contents in the solvent.

Analytical Method Development

a. Selection of common solvent

After assessing the solubility of both drugs in different solvents, distilled water was selected as a common solvent for developing spectral characteristics.

b. Preparation of Standard Stock solutions

i. Ornidazole Standard solution

A standard stock solution of ornidazole was prepared by dissolving 10mg of drug in 10 ml of distilled water. Above solution was further diluted with same solvent to get the final concentration of 100µg /ml and used as a standard solution.

ii. Mixed Standard Stock Solutions

Mixed solution was prepared in the ratio as claimed in the marketed formulation.10mg of ornidazole drug was dissolved in 10 ml of distilled water to form 1000µg/ml.10mg of povidone iodine was dissolved in 10 ml of distilled water to form 1000µg/ml solution.2 ml from 1000µg/ml ornidazole, 10 ml of povidone iodine solution were mixed and volume was made up to 20 ml in a volumetric flask with the solvent to obtain a required concentration as label claims.

c. Preparation of Sample Stock Solution

Accurately weighed and transferred 1gm of samples (both A&B) in beaker and mixed thoroughly, made the volume upto 10 ml to get the required concentration. From the above solution 2 ml was taken out in volumetric flask and diluted up to 20 ml for desired concentration.

d. Calibration Plots and determination of λ max

i. Calibration plot of Ornidazole in distilled water

Ornidazole Standard solution was further diluted to obtain the concentration in the range of 5µg/ml to 25µgm/ml. The scanning of every aliquot was performed in the UV Visible spectrophotometer ranging from 200 to 800 nm and lambda maximum was selected at 315.2 nm. Fig.1-A

ii. Calibration plot of Mixed Solution in distilled water

Mixed Standard Solution was further diluted to obtain the concentration in the range of 5µg/ml to 25µg/ml. The scanning of every aliquot was performed in the UV Visible spectrophotometer ranging from 200 to 800 nm and λ maximum was selected at 320 nm. Fig.1-B

Assay procedure for Ornidazole in marketed formulations

1 gm of ointment (A & B) accurately weighed and dissolved in distilled water, volume was made up to 10 ml. 20 µg/ml solution from above was prepared and absorbance was noted down at 320 nm wavelength and value of the absorbance was substituted in the regression equation of the standard solution of the ornidazole. The values were in the range for A, for B it was somewhat not in the range. (Table 1)

RESULT AND DISCUSSION

The Maximum absorption for pure ornidazole in distilled water using U.V.Spectrophotometer was recorded at 315.2 nm. The method was validated according to I.C.H. Guidelines. The λmax for ornidazole in sample solution against distilled water was recorded 320 nm.The correlation coefficient (r^2) for pure ornidazole was found to be 0.9994 indicates a good linearity between absorbance and concentration range of 5-25 µg/ml.The correlation coefficient(r^2) for mixed standard solution was

found to be 0.99.The % Recovery for Formulation A and Formulation B were found to be in the range of about 93.85%-108.03% and 84.24%-104.26%.Robustness was done at different conditions like concentration, wavelength. The result shows mean %RSD as 0.236 and acceptance criteria is 2%. The developed UV Spectrophotometric method for the Ornidazole in presence of povidone iodine is simple, accurate and economical.Ornidazole as pure drug shows λmax at 315.2nm but with the addition of povidone iodine there is an bathocromic shift appears at 320nm,means the mixed sample solution shows λmax at 320nm.The %Recovery for A formulation in the limit,but for B formulation it is somewhat outside the limit.Hence this developed method could be used for routine estimation of ornidazole in presence of povidone iodine in the ointment formulation.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific method will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

As per I.C.H. guidelines, validation of analytical procedure, methodology Q2B was followed. The following parameters were determined: Linearity, Range, Accuracy, Precision, Detection limit, Quantitation limit and Robustness.

Linearity and Range

Various aliquots were prepared from Mixed Standard Solution (100µg/ml) and Ornidazole standard solution (100µg/ml) ranging from 5-25µg/ml. The samples were scanned in UV Spectrophotometer against distilled water as blank. It was found that selected drug (ornidazole) shows linearity between the ranges of 5-25µg/ml at 320 nm and Ornidazole alone at 315.2 nm. (Table 1)

The acceptable limitis should be linear in the specified range and regression coefficient should not be less than 0.99.

Accuracy (Recovery Study)

This parameter was evaluated by the percent recovery studies at concentration level of 80%, 100%, and 120% which consisted of adding known amount of ORNIDAZOLE pure drug to prequantified solution and taken absorbance of each solution in triplicate as shown in Table 2, 3.

Discussion-The recoveries were verified by estimation of drug in triplicate preparations at each specified concentration levels. The results are reported in terms of %recovery, RSD.

Precision

Precision of the method was demonstrated by Repeatibility,Intraday and Interday variation studies.For repeatability study nine samples of same concentration were taken their absorbances noted down and %RSD was calculated.(Table 4)

Discussion-The results of nine determinations having the same concentration are well and within the limits. The %RSD was found to be 1.16 and acceptance criterion is 2%.

In Intraday variation study nine different solution of same concentration were analysed 2 times a day and the absorbance noted. From the absorbance result mean, standard deviation and %RSD was calculated.(Table 5)

Discussion-Intraday precision of nine determinations having the same concentration

shows within the limits. The acceptance criterion is 2%.

In the Interday variation study, solution of same concentration were analysed for three consecutive days and the absorbance result mean, standard deviation and %RSD was calculated.(Table 6)

Discussion-The interday precision shows the %RSD results within the limits and average %RSD is 0.899.The acceptance criterion is 2%.

Limit of Detection and Limit Of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines. (Table 7)

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Robustness

Robustness of the method was carried out taking reading under different concentration and wavelength conditions. The absorbance were noted down and their %RSD was calculated. (Table 8)

Discussion-Robustness was done at different conditions like concentration and wavelength. The result shows mean %RSD as 0.236 and acceptance criteria is 2%.

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ANNEXURE

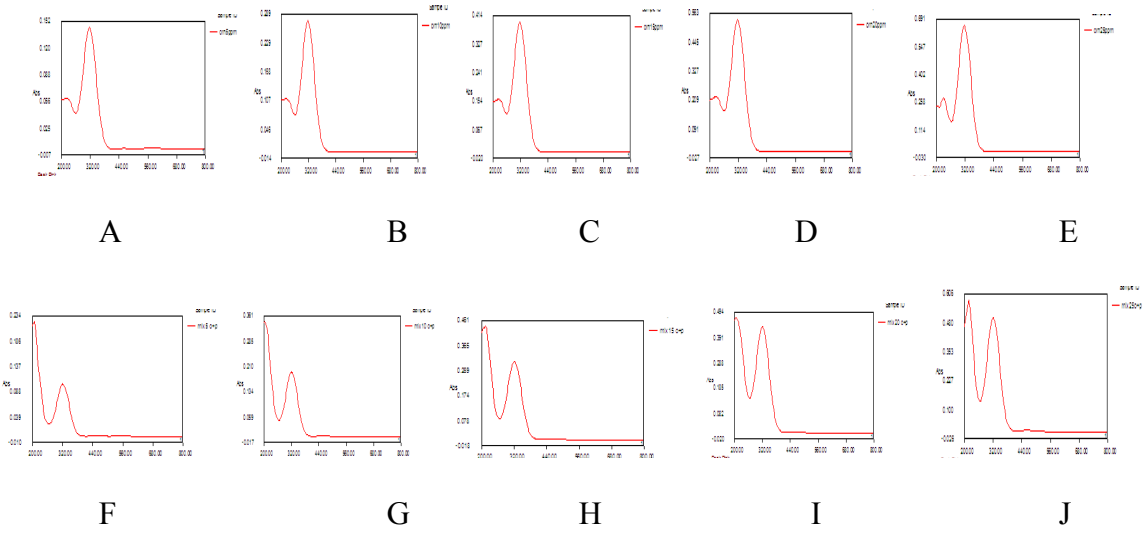

Figure1: Graph of Ornidazole bulk A. 5µg/ml B.10µg/ml C. 15µg/ml D. 20µg/ml E. 25µg/ml and in presence of Povidone Iodine F. 5µg/ml G. 10µg/ml H. 15µg/ml I. 20µg/ml J.25µg/ml

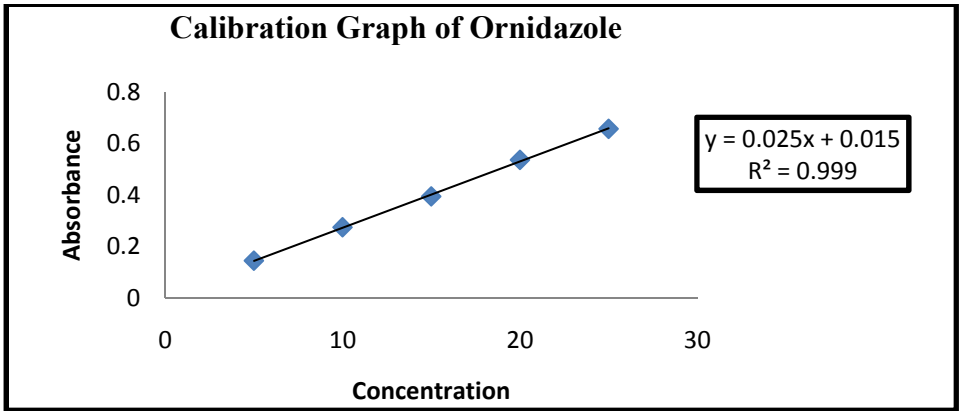

Figure 2: Calibration Graph of Ornidazole

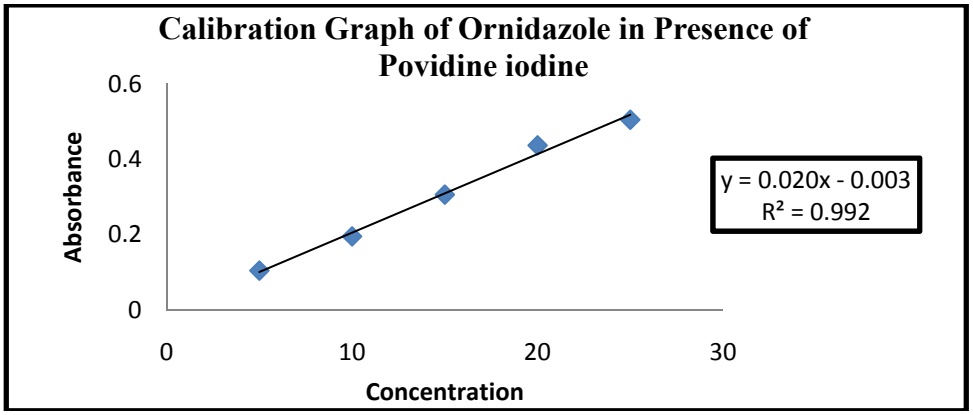

Figure 3: Calibration Graph of Ornidazole in Presence of Povidine iodine

Table 1: Linearity and Range

Concentration (ppm)	Absorbance(Ornidazole)	Absorbance(Mix)
5	0.145	0.104
10	0.275	0.195
15	0.395	0.306
20	0.537	0.437
25	0.658	0.505

Table 2: Accuracy Study [Formulation A (metrogyl p)]

No. of preparation	Amount Added(mg)	% Recovery	Mean	S.D.	%RSD
80% 80% 80%	8	108.03% 106.524% 105.77%	1.007	0.0107	1.06
100% 100% 100%	10	101.395% 99.166% 98.100%	1.043	0.0173	1.66
120% 120% 120%	12	93.85% 94.37% 94.644%	1.086	0.0045	0.42

Table 3: Accuracy Study [Formulation B (Alphadin OZ)]

No. of Preparation	Amount added(mg)	%Recovery	Mean	S.D	%RSD
80% 80% 80%	8	104.26% 102.97% 103.97%	0.976	0.0066	0.68
100% 100% 100%	10	93.835% 93.158% 93.35%	0.98	0.0036	0.36
120% 120% 120%	12	85.65% 85.39% 84.24%	0.982%	0.0085	0.86

Table 4: Repeatibility Precision

Conc(ppm)	Abs	Mean	SD	%RSD
20	0.540	0.541	0.006275	1.16
20	0.530			
20	0.540			
20	0.545			
20	0.536			
20	0.546			
20	0.551			
20	0.546			
20	0.540			

Table 5: Intraday Precision

Conc(ppm)	Absorbance		Mean	
	Afternoon	Evening	Afternoon	Evening
20	0.545	0.529	0.541	0.529
20	0.540	0.523		
20	0.530	0.533		
20	0.540	0.529		
20	0.540	0.538		
20	0.546	0.532		
20	0.532	0.518		
20	0.551	0.530		
20	0.546	0.529		
SD	0.006275	0.0057446		
%RSD	1.16	1		

Table 6: Interday Precision

Conc(ppm)	%RSD			Average
	Day 1	Day2	Day3	
20	1.16	1	0.548	0.899

Table 7: Ornidazole bulk Charateristics

Parameters	Ornidazole bulk	Ornidazole in presence of Povidone iodine
Absorption maximum(nm)	315.2	320
Beer's Law limit(µg/ml)	5-25	5-25
Regression Equation (y=mx+c)	Y=0.0258x+0.0156	Y=0.0209x+0.0038
Slope (m)	0.0258	0.0209
Intercept(c)	0.0156	0.0038
Correlation Coefficient(r^2)	0.9994	0.9924
LOD		0.76
LOQ		2.325

Table 8: Robustness

Conc(ppm)	Wavelength(nm)	Absorbance
15	318 320 322	0.439 0.441 0.440
20	318 320 322	0.707 0.708 0.705
25	318 320 322	0.873 0. 874 0.870