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Analytical Method Development and Validation of Ornidazole in Bulk and Dosage Form

Basant Kumar[1], Dr. Meenu Chaudhary[2]
[1]*School of Pharmaceutical Sciences, SGRR University, Dehradun, Uttarakhand, India*
[2]*Associate Professor, School of Pharmaceutical Sciences, SGRR University, Dehradun, Uttarakhand, India*

***Abstract:** In the given study, of an For the purpose of estimating ornidazole in bulk and dose form, an correct, exact, and specific approach has been established. by ultraviolet spectroscopy. Ornidazole belongs to the categories of antibacterial medications (nitroimidazoles) also used to treat infections caused by anaerobic bacteria and protozoa. The procedure includes calculation of the absorbance of Ornidazole blend in Methanol water 1:9. The calibration curve method shows wavelength maxima for Ornidazole at 315 nm with Methanol water 1:9 in the wavelength having a correlation value of 0.999, between 2-28 g/ml. The mean recovery of Ornidazole was discovered to be in pharmaceutical dosage form 95.5%. Results of experiments were authenticated for Accuracy: correctness, exactness, precision, truthfulness, reliability, validity established adequately. The submission process is easy, quick, and appropriate for standard analysis. Statistics were used to validate the analysis' findings, and recovery studies were administered as per the guidelines of ICH.*

***Keywords:** Ornidazole, Methanol, UVspectroscopy, absorption.*

I. INTRODUCTION

The aim of this research is developing and validating a Novel UV spectrophotometric technique for estimation of Ornidazole in large quantities and as a pill. The molecular formula of Ornidazole is C7H10ClN3O3. It has a molecular weight of and is a white powder of 219.625g/mol. (Fig.1). It is soluble in organic solvents, it has highest solubility in methanol, ethanol.[(1)] It comes under the categories of anti bacterial and used for treatment of particular vaginal, urinary tract, and intestinal diseases, as well as various bodily infections in general where the mechanism of action of Ornidazole works by preactivating by reducing the nitro group and producing toxic derivatives and radicals to cure infections caused by protozoa and anaerobic bacteria.[(2)] The adverse affects of this drug include Vomiting, Nausea, Headache, Dizziness, Increased sweatingA review of the literature revealed that numerous techniques for characterising ornidazole, as well as its preparation techniques, analytical method development, and validation in various media, have been documented.[(3,4)]

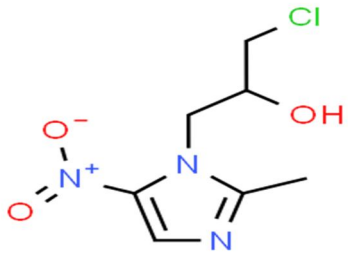


Fig.1 Molecular structure of Ornidazole.

II. MATERIALS AND METHODS

Table 1: Instrumentation:

S.No.	Instrument	Model	Manufacturer
1.	UV Spectrophotometer	Cary 60 Spectrophotometer	Agilent Technologies
2.	Digital weighing balance	TX 323L	Shimadzu Corporation
3.	Ultrasonicator	SONAR	Sonar

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Table 2: Reagents and solvents:

S.No.	Reagent/Solvent	Grade	Supplier
1.	Methanol	96.6%	SGRRU Pharmaceutical division

A. Standard stock solution Preparation:

A 50 ml volumetric flask was used to transfer 50 mg of the medication, which was then dissolved in 50 ml of solvent (1:9 methanol to distilled water). A further 10 ml volumetric flask was filled with a 1:9 solution of methanol and water after 1 ml of this stock solution had been taken and transferred. The standard solution has a drug concentration of 10 g per ml..(5)

B. Selection of Absorption Maximum

To obtain a concentration of 10 g/ml, pipette 1 ml of stock solution into a 10 ml volumetric flask, then top off the liquid to 10 ml. Using methanol + water in a 1:9 ratio as a blank, the final solution was scanned in a UV-Spectrophotometer between 200 and 400 nm. At 315 nm, the wavelength maxima were determined.

C. Wavelength Maxima

Ornidazole

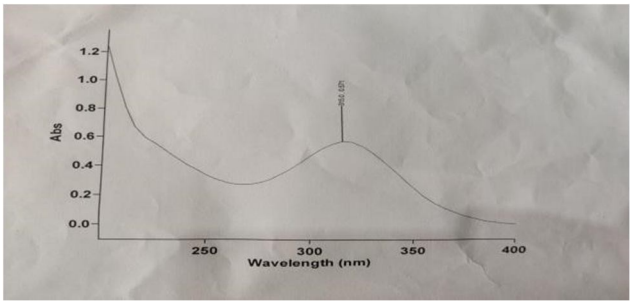


Fig.2 λmax was found to be 315 nm with methanol:water in 1:9.

D. Calibration Curve

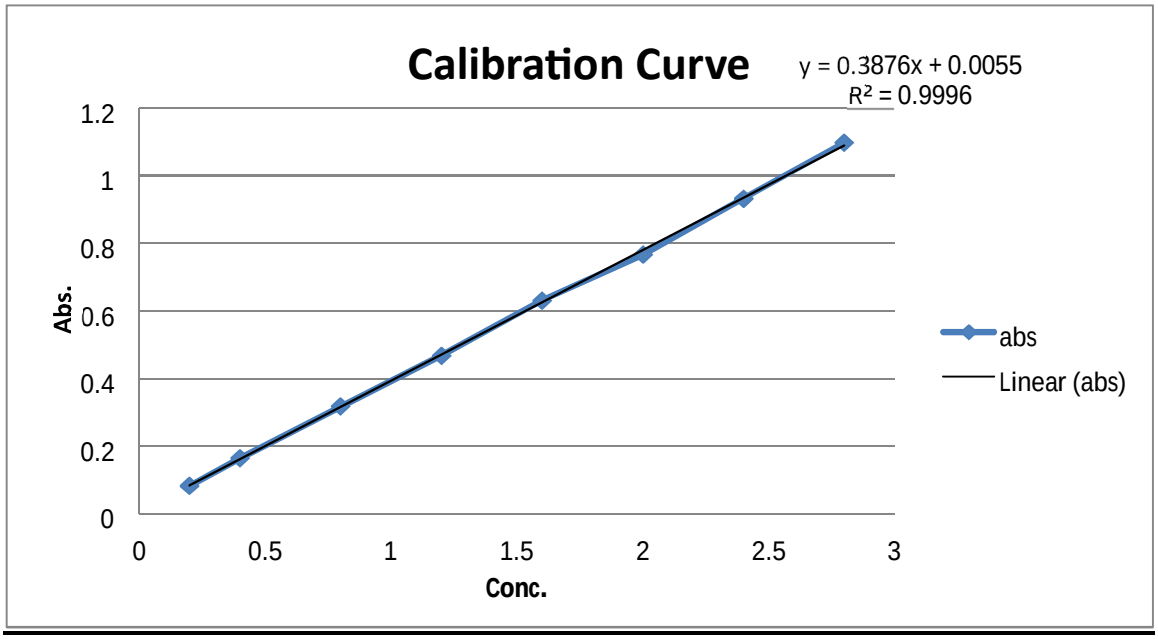


Fig.3 Calibration curve of Ornidazole.

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1) Range

The range of Ornidazole was found to be of range 2- 28 µg/ml.

2) Linearity

Accurately measured aliquot portions of working standard solutions of Ornidazole were transferred to a volumetric flask measuring 10 ml. The volume was adjusted using a 1:9 methanol to water solution to get the following concentrations: 2, 4, 8, 10, 12, 16, 20, 24, and 28 g/ml. The study of regression of Ornidazole was carried out in which regression coefficient of Ornidazole is 0.999.

E. Preparation of Calibration Curve

With the help of pipette deliver 0.2,0.4,0.6,0.8,1.2,1.6,2.0,2.4 and 2.8 ml of working solution and transfer into six different volumetric flask and make the volume up to the level with ethanol to get the desired concentration levels of 2 µg/ml, 4 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 16 µg/ml, 20 µg/ml, 24 µg/ml, 28 µg/ml respectively. Using methanol as a blank, the aforementioned produced solutions were measured at 315 nm. The concentration data and the associated absorbance values were plotted. The drug's graphic reaction was linear across its experimental 2-28 g/ml range. Ornidazole's calibration equation was discovered to be y = 0.3876x + 0.0055 with a correlation value of 0.999 [6]. Estimation of Ornidazole in pharmaceutical dosage form (Orni 500mg):
10 capsules were weighed for the assay, and the weight was calculated. The capsules were then accurately ground into a powder that contained 10 mg of ornidazole, and the powder was poured into a 100 ml volumetric flask with 50 ml of methanol, sonicated for 30 minutes, and then thoroughly shaken and filtered. Put 1 ml of the filtrate in a 10 ml volumetric flask, and add methanol to bring the volume up to 10. Using methanol water in a 1:9 ratio as a blank, the absorbance of the produced solution was measured at 315 nm(7,8). Three times this surgery was carried out. The table 3 provides a summary of the results.

Table 3 : % Assay of Ornidazole in pharmaceutical dosage form.

Drug Sample	Conc. (mg)	Abs.	Conc. found (mg)	Difference (mg)	%Drug Content	Standard deviation	Relative Standard deviation
1	500	0.4735	499.5	0.5	99.9		
2	500	0.4709	499.7	0.3	99.9		
3	500	0.4713	499.2	0.2	99.8	0.10	0.10
			Mean		99.9		

Limits of Quantification (LOQ) and of Detection (LOD) LOD, or Limit of Detection
The detection limit was established using
DL = 3.3 σ/s Where, σ = Standard deviation of the response. s = Slope of the linearity curve.
 Limit of Quantification (LOQ) The Quantification Limit is determined by

$$QL = 10\frac{\sigma}{s}$$

σ = Standard deviation of the response.
s = Slope of the linearity curve.

Table 4 : Limit of Detection and Limit of Quantification

S. No.	Conc. (µg/ml)	Abs.	LOD	LOQ
1	2	0.082		
2	4	0.1642		
3	8	0.317		
4	12	0.4679	3.10	9.40
5	16	0.6321		
6	20	0.7667		
7	24	0.9337		
8	28	1.0993		
	MEAN	0.5578		

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F. Method Validation[9]

The following UV Spectrophotometric method has been validated as per ICH guidelines.

1) Linearity

The method developed was studied using preparation of std. sol. at varying concentration levels. Linearity range was found to be 2-28µg/ml as shown in table 5. y = 0.3876x + 0.0055 was found to be the equation of regression with 0.999 as the value correlation coefficient.

TABLE 5. Linearity, $E^{1\%}$1cm, Absorptivity (litre/gm cm), Molar Absorptivity (litre/mol cm) of Ornidazole.

S. No.	Concentratio n µg/mL	Absorbance	Abs/Conc	$E^{1\%}$1cm	Absorptivity	Molar Absorptivit y
1	2	0.082	0.041	41	4.1	900.46
2	4	0.1642	0.0410	41.05	4.105	901.56
3	8	0.317	0.0396	39.62	3.962	870.15
4	12	0.4679	0.0389	38.9	3.89	854.34
5	16	0.6321	0.0395	39.5	3.95	867.51
6	20	0.7667	0.0383	38.3	3.83	841.16
7	24	0.9337	0.0389	38.9	3.89	854.34
8	28	1.0993	0.0392	39.2	3.92	860.93
Mean				39.55	3.16	868.80

2) Precision

The precision of the developed method was studied in the form of repeatability, intraday and interday.

Repeatability - With the help of pipette transfer 0.8,1.6 and 2.4 ml standard solutions and pour into nine different volumetric flasks. After transferring, dilutions accompanied by methanol-Distilled water were made to get 8,16 and 24 µg/ml sol. The absorbance of the above prepared solutions was examined at 315nm using methanol-Distilled water as blank. The result obtained was summarized in table.

3) Intraday

With the help of pipette transfer 0.8,1.6 and 2.4 ml of working solution into 9 distinct vol. flasks and the vol. was made up to 10 ml using methyl acohol-Distilled water to get the concentration of 8, 16 and 24 µg/ml respectively. The abs. of the made sol. was examined at 315 nm using methanol-Distilled water as a blank sol. These progression studies were performed thrice a day at interval of 3hrs. The result obtained were summarized in the table .

Transfer 0.8, 1.6, and 2.4 ml of the working solution with a pipette into nine separate volumetric flasks, then add 10 ml of methanol-distilled water to the volume to achieve concentrations of 8, 16, and 24 g/ml, respectively. At 315 nm, the absorbance of the produced solutions was measured with methanol-distilled water serving as the blank. These succession experiments were carried out three times each day, separated by a 24-hour period. The results were compiled in a table.

Table 6: Repeatability studies

Nominal Conc. (µg/ml	Absorbance			Mean±SD	%RSD
	0hr	24hr	48hr		
8	0.3739	0.3759	0.3848	0.3782± 0.007283	1.9257
16	0.6762	0.6718	0.6842	0.6774±0.01294	1.9102
24	1.0002	0.9958	1.008	1.0016± 0.006081	0.0607
				Mean	3.8966

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Table 7. Studies of Intra-day

Nominal Conc. (µg/ml	Absorbance			Mean±SD	%RSD
	0hr	3hr	6hr		
8	0.3858	0.3851	0.3817	0.3842 ± 0.001792	0.5236
16	0.6888	0.6905	0.6816	0.686967 ± 0.003858	0.5615
24	1.0013	1.0038	0.9992	1.001433 ± 0.00188	0.1877
				Mean	0.4242

Table 8. Studies for Inter-Day

Nominal Conc. (µg/ml	Absorbance			Mean±SD	%RSD
	0hr	24hr	48hr		
8	0.3739	0.3759	0.3848	0.3782± 0.007283	1.9257
16	0.6762	0.6718	0.6842	0.6774±0.01294	1.9102
24	1.0002	0.9958	1.008	1.0016± 0.006081	0.0607
				Mean	1.2988

4) Accuracy

Using methanol as a blank, the absorbance of the aforementioned solutions was measured at 315 nm. The table provides a summary of the results. 9.

Table 9 : Accuracy studies

Recovery %	Conc. Of API (µg/ml)	Conc. of addition (µg/ml)	Nominal conc. (µg/ml)	Absorbance	Observed conc. (µg/ml)	% Recovery
80	2.8	2.3	5.1	0.2496	4.9	96.0
80	2.8	2.3	5.1	0.2372	4.7	92.1
80	2.8	2.3	5.1	0.2457	5.0	98.0
100	2.8	2.8	5.6	0.2840	5.3	94.6
100	2.8	2.8	5.6	0.2708	5.4	96.4
100	2.8	2.8	5.6	0.2782	5.2	92.8
120	2.8	3.3	6.1	0.2920	5.8	95.0
120	2.8	3.3	6.1	0.2878	5.9	96.7
120	2.8	3.3	6.1	0.2915	6.0	98.3
Mean						95.54

5) Specificity

Studies on specificity are carried out to look for "any interference in drug absorbance in the presence of common additives like starch, talc, lactose, magnesium stearate, etc." At 315nm, the absorbance of a 4 µg/ml standard solution was measured both with and without additions, using methanol + water as a blank. Table 10 provides a summary of the findings..

Table 10. Specificity studies

Nominal Concentration	In presence if excipients		In absence of excipients		% Interference
(µg/ml)	Absorbance	Obtained conc.	Absorbance	Obtained conc.	
4	0.1728	3.53	0.1831	4.25	0.0103
4	0.1725	3.44	0.1844	4.35	0.0119
4	0.1708	3.37	0.1819	4.22	0.0111
				Mean	0.0111

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III. RESULTS AND DISCUSSION

The accuracy, precision, repeatability, and specificity of the discovered approach have all been confirmed. Standard solutions had a nominal concentration of roughly 2-28 g/ml. With a linear correlation coefficient of 0.999 and a linear regression equation of y=0.3876x+0.0055, the proposed technique was confirmed to be linear (fig. 3). The range of concentrations where ornidazole may be relied upon was linear, ranging from 2-28 g/ml. The method's accuracy was demonstrated by the mean recovery, which was 95.54% (Table 9). RSD was less than 2%, as were the repeatability, intraday, interday precision, specificity, and accuracy. Using the suggested method, the potency of Ornidazole dosage forms was assessed. With the new analytical technique, the brand products satisfied the requirements. The reported method's capacity to provide data on specificity for their estimation in the With the new analytical technique, the brand products satisfied the requirements. The reported method's capacity to offer data on specificity for their estimation in the presence of formulation excipients is known as specificity. The absorbance obtained with the excipient mixture did not interact in any way with the standard absorbance. The percentage of ornidazole in the marketed product was determined to be 95.54% .

IV. CONCLUSION

A simple and sensitive spectrophotometric method for Ornidazole as API and in pharmaceutical dosage form was developed. The new introduced method was found to be easy and reliable. The maximum absorbance for Ornidazole in methanol was found to be 315nm. The method was found to be progressive and behaves according to beers lamberts statue in concentration range of 2-28µg/ml for ornidazole. The correlation coefficient for Ornidazole was found to be 0.999. All the validation parameters were performed according to the I.C.H. guidelines. The method for the drug was found to be precise as %RSD for interday, intraday, repeatability was found to be less than 2.

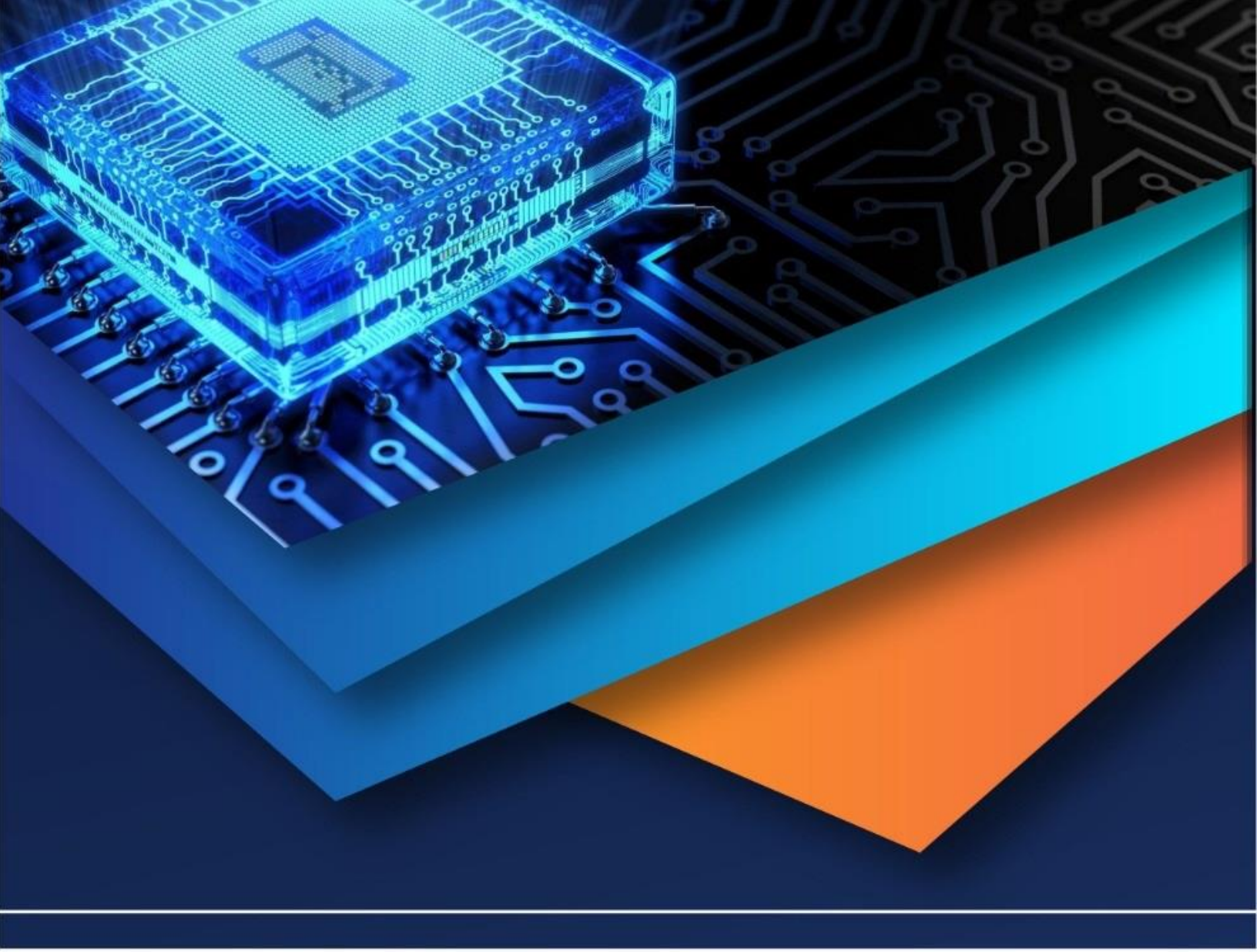
Table 11 : Summary of validation parameters

S. No.	Parameters	Observation
1	Linearity and range	2-28µg/ml
2	Correlation value	0.999
3	Precision (%RSD)	
3.1	Repeatability	1.2988
3.2	Interday	1.2988
3.3	Intraday	0.4242
3.4	% Recovery	95.5%
3.5	Specificity	No Interference found
3.6	Limit of detection	3.10
3.7	Limit of quantification	9.40
4	% Assay	99.9%

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