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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF ORNIDAZOLE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

The present manuscript describe simple, novel, rapid, precise, accurate, specific and cost effective Differential Spectrophotometric method for the determination of Ornidazole in Pharmaceutical formulation. Methanol was used as solvent. Differential Spectrophotometric method involves measurement of absorbance at 268 nm Peak minima and 313 nm Peak maxima. The amplitude, which is sum of magnitude of absorbances at above two wavelengths, was selected for the measurement. The drug comply with Beer Lambert's law over the linearity range 5-30 µg/ml. The method was validated as per ICH guideline rules in terms of Linearity, accuracy (recovery study), Precision (repeatability, intraday, interday validation), limit of detection, limit of quantification. All the validation parameters were found to be within acceptable limits. The method was found to be simple, novel, rapid, cost effective, accurate, and precise therefore is utilized for the routine analysis of drug in Pharmaceutical formulation.

Keywords: Ornidazole, Differential Spectroscopy, UV Spectrophotometric method, Validation.

INTRODUCTION

Ornidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. Nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which excerts cytotoxic action by damaging DNA and other critical biomolecules[1].

Ornidazole in their dosage form mainly used as broad spectrum antibiotics because ornidazole act as antiprotozoal activity[1, 2].

MATERIAL AND REAGENTS

INSTRUMENTS

- A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.
- Analytical balance (CP224S, Sartorius, Germany)
- Ultrasonic cleaner (Frontline FS 4, Mumbai, India)
- Corning volumetric flasks, beakers and pipettes of borosilicate glass were used in the study.

REAGENTS AND MATERIALS

- Ornidazole standard powder. (Acme pharmaceutical Ltd.)
- ORNI 500 (Zydus Cadila Healthcare Ltd.)
- Methanol AR grade as solvent (S.D. Fine Chemical Ltd., Mumbai, India.)
- AR grade Hydrochloric acid and Sodium hydroxide
- Whatman filter paper no. 41 (Whatman International Ltd., England)

Preparation of Reagents and Solutions

Preparation of 0.1N Hydrochloric acid

0.1N Hydrochloric acid can be prepared by adding concentrated hydrochloric acid (8.5 ml) in 100 ml volumetric flask and diluting up to the mark with methanol (0.1N HCl)[2].

Preparation of 0.1N Sodium hydroxide

0.1 M Sodium hydroxide can be prepared by adding sodium hydroxide (0.4 gm) in 100 ml volumetric flask and diluting up to the mark with methanol (0.1N NaOH)[2].

Preparation of Standard Stock Solution

An accurately weighed ORN powder (10 mg) was weighed and transferred to 100 ml separate volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol to give a solution having concentration 100 µg/ml [3].

Preparation of working standard solution

The working standard solutions of ORN was prepared be transfer in aliquots' of standard stock solution of ORN (0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 ml) was transferred in a series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and mixed[3].

Preparation of Sample Solution

Quantity of the powder equivalent to 10 mg ORN was transferred in 100 ml volumetric flask separately and powder was dissolved in 50 ml of methanol with sonication having

slight warming temperature to dissolve drug as completely as possible. Then the volume was adjusted up to mark with methanol. Transfer 0.1 ml of above solution to 10 ml volumetric flask to get final concentration around 10 µg/ml of ORN. Then the volume was adjusted up to mark with methanol[4].

METHOD DEVELOPMENT
DETERMINATION OF WAVELENGTH

In Difference spectroscopic method the absorption spectra of equimolar solutions of Drug in two different pH (acidic or basic) were taken (Figure 2). The difference absorption spectrum is a plot of the difference in absorbance between the solutions against wavelength. It may be generated automatically using a double beam recording spectrophotometer with the solution 1 in the sample cell and the solution 2 in the reference cell[5]. The absorbance was measured at two wavelengths, one being the peak maxima and other being peak minima. For this measurement, equimolar solution of Ornidazole was prepared separately in 0.1 N HCl as well as in 0.1 N NaOH at a concentration of 10 µg /ml. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. From the difference spectrum of drug in two different form, absorbances were measured at selected wavelength i.e. 268 nm Peak minima and 313 nm Peak maxima[6]. The amplitude, which is sum of magnitude of absorbances at above two wavelengths, was selected for the measurement. It was calculated and used to obtain the concentration. The isobestic points (points representing zero absorbance corresponding to cutting points of acidic and alkaline spectra) was recorded at 287 nm, which were identical irrespective of the pH of solution in reference cell. There was no change in isobestic points[7], which reveals that there was no interference by additives.

METHOD VALIDATION[15]

The developed method was validated according to the International Conference on Harmonization (ICH) guidelines[15]. The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ). The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the Pharmaceutical formulation[14]. The precision (% RSD) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations.

Linearity & Range

Calibration curve was plotted over a concentration range of 5-30 µg/ml for ORN. Accurately measured standard working solution ORN (0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 ml) was pipette out in to a separate series of 10 ml volumetric flask[8]. The volume was adjusted to the mark with methanol and the amplitude of absorbance of the solutions was measured at 268.0 nm (Peak minima) and 313 nm (Peak maxima) against methanol as a blank. The calibration curve was constructed by plotting absorbance Vs concentration[11].

Precision
Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n = 6) of ORN (10 µg/ml) without changing the parameters of the proposed Method[9]. The results are reported in terms of relative standard deviation (% RSD) [13, 15].

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was evaluated by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of sample solutions of ORN (5, 10, and 15 µg/ml)[9]. The results are reported in terms of relative standard deviation (% RSD)[13, 15].

Limit of detection (LOD) & Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated by using the following equations[15].
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where, σ = the standard deviation of the response, S = slope of the calibration curve

Accuracy (% Recovery study)

The accuracy of the method was determined by calculating recoveries of ORN by the standard addition method. Known amounts of standard solution of ORN were added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of ORN[10].

ANALYSIS OF ORNIDAZOLE IN PHARMACEUTICAL FORMULATION

Pharmaceutical formulation of Ornidazole was supplied by Zydus Cadila Healthcare Ltd. Sample solutions were prepared as described earlier. These solutions were then analyzed to obtain the spectra, and absorbance values at 268 nm and at 313 nm or amplitude absorbance was noted. Spectra of sample solution shown in Figure 3. These values was then equated in regression equation and the concentration of drug was calculated[12].

RESULTS AND DISCUSSION

Owing to the solubility of Ornidazole in the methanol it was selected as solvent. Calibration range was observed in the concentration range of 5-30µg/ml for ORN. The calibration curves at different wavelengths are shown in Figure 4. The RSD values of repetability for ORN respectively at amplitude absorbance of ORN shown in Table 2. Low value of RSD indicates that proposed method is repeatable. The RSD values of ORN for inter-day and intra-day was 1.24-1.57 and 0.80-1.35 at amplitude of absorbance respectively. The RSD values of intermediate precision less than 2 indicates the propose method is reproducible. LOD values for ORN was found to be 0.22 µg/ml at amplitude of absorbance. While the, LOQ values for ORN was found to be 0.67 µg/ml at amplitude of absorbance. Low value of LOD & LOQ indicates that the method is sensitive. The mean recoveries was found to be 98.90 ± 1.94 for ORN. The recoveries results indicate that the proposed method is accurate. Results of recovery studies are given in Table 3. The proposed validated method was successfully applied to determine ORN in Marketed formulation. Results are given in Table 4. The proposed Spectrophotometric method was successfully applied to Ornidazole in pharmaceutical dosage forms.

Table 1. Regression parameters of ORN

PARAMETERS	ORN
Wavelength (nm)	268-313 nm
Beer's law limit (µg/ml)	5-30
Regression Equation Y=mX+C Slop (m) Intercept(C)	 Y=0.0555X+0.0058 0.0555 0.0058
Correlation coefficient (r^2)	0.9997

Table 2. Repetability data of ORN

Sr. NO	Absorbance difference at 313-268 nm
1	0.563
2	0.559
3	0.568
4	0.572
5	0.581
6	0.575
MEAN	0.569
SD	0.008
RSD	1.41

Table 3. Recovery data for ORN in Pharmaceutical formulation

DRUG	LEVEL	Amt. Present (µg/ml)	Amt. added (µg/ml)	% Mean Recovery ± SD
ORN	I	10	5	98.61±1.50
	II	10	10	97.11±0.54
	III	10	15	100.98±0.30

Table 4. Analysis of Pharmaceutical formulation

Sample No.	Label claim (mg/Tablet)	Amt. found (mg/Tablet)	% Label claim (%)
1	500	502.88	100.57
2	500	488.46	97.69
3	500	501.98	100.39
4	500	492.07	98.41
5	500	499.27	99.85
6	500	483.96	96.79
MEAN		494.77	98.95
SD		7.77	1.55

Table 5. Summary of validation parameters

PARAMETERS	ORN
Wavelength (nm)	268-313 nm
Beer's law limit (µg/ml)	5-30
Regression Equation Y=mX+c Slop (m) Intercept (c)	Y=0.0555X-0.0058 0.0555 0.0058
Correlation coefficient (r^2)	0.9997
Method precision Repeatability (n=6, %RSD)	1.41
Interday precision (n=3, %RSD)	1.24-1.57
Intraday precision (n=3, %RSD)	0.80-1.35
LOD (µg/ml)	0.22
LOQ (µg/ml)	0.67
% Recovery ± SD (n=3)	98.90±1.94
Assay ± SD (n=3)	98.95±1.55

[a] RSD = Relative standard deviation. [b] LOD = Limit of detection. [c] LOQ = Limit of quantification [d] S. D. is standard deviation.

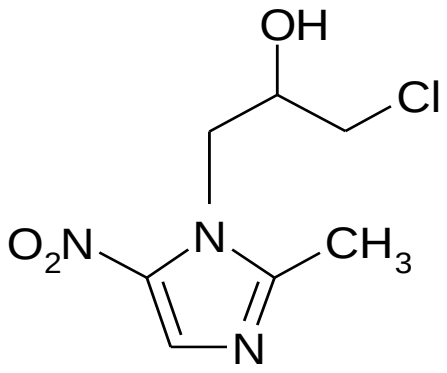

Figure 1. Structure of Ornidazole

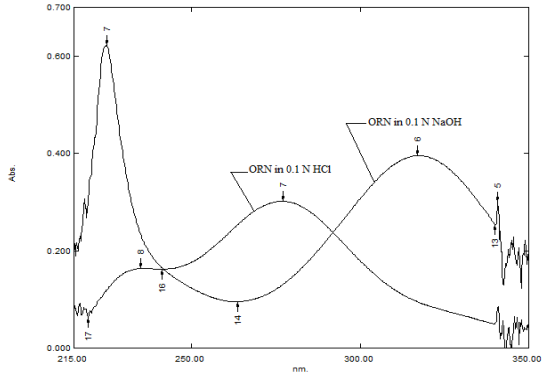

Figure 2. Overlay spectra of ORN in acidic and basic media

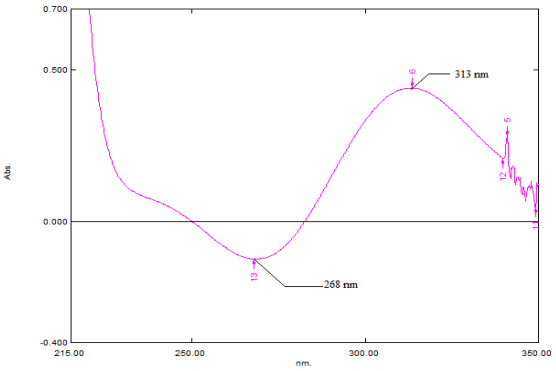

Figure 3. Difference absorption spectra of sample solution of marketed formulation

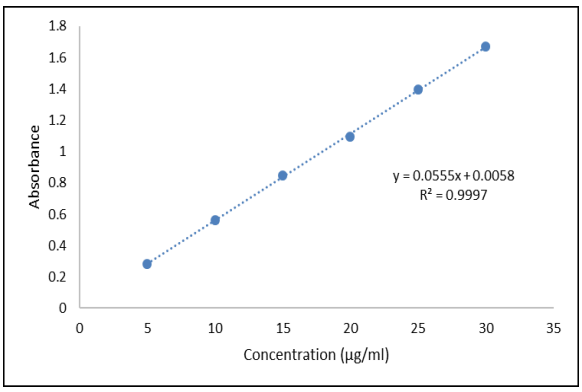

Figure 4. Calibration curve of Amplitude absorbance of ORN

CONCLUSION

The method described for the simultaneous estimation of ORN was found to be sensitive, accurate and precise for routine estimation of drug. The values of standard deviation and % RSD were satisfactorily low and recoveries studies indicate the reproducibility and accuracy of the method. The result of the analysis of the tablet dosage form by this method is reproducible and reliable and is in good agreement with label claim of the drug. The additive present in the tablet dosage form did not interfere in the analysis. So the method can be used for the routine analysis of drug in Pharmaceutical formulation.

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