Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):580-585

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

UV spectrophotometric estimation of ornidazole by first order derivative and area under curve methods in bulk drug and pharmaceutical dosage form

Rajan V. Rele

Central Research Laboratory, D.G. Ruparel College, Matunga, Mumbai

---

ABSTRACT

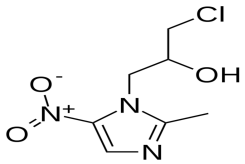
Simple and precise UV spectrophotometric methods by first order derivative and area under curve [AUC] - have been developed and validated for the estimation of ornidazole in bulk and its tablet formulation. The standard and sample solutions of ornidazole were prepared in 0.1 N hydrochloric acid. Ornidazole was estimated at 261 nm for the first order derivative UV-spectrophotometric method (A), while in area under curve (AUC) method (B) the zero order spectrum of ornidazole was measured in between 272 nm to 282 nm. Beer's law was obeyed in the concentration range of 1 to 10 µg / ml with coefficient of correlation value 0.9996 for first order derivative method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 1 to 10 µg / ml with coefficient of correlation value 0.9997. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation were of 1.276 % and 0.1493 % for the above two methods respectively. The proposed methods were successfully applied for the determination of ornidazole in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Ornidazole, UV spectroscopy, Derivative spectroscopy, Area under curve method.

INTRODUCTION

Ornidazole [1] is a 5-nitro-imidazole derivative used as anti-infective agent. It is not official in any Pharmacopoeia. Literature survey reveals Spectrophotometric [1,2] and HPLC [3-5] methods for estimation of ornidazole in dosage form. Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of ornidazole. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.

Structure of ornidazole



EXPERIMENTAL SECTION

Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.

A Shimadzu analytical balance with 0.01 mg was used.

CHEMICAL AND REAGENTS

Reference standard of ornidazole was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

PREPARATION OF STANDARD SOLUTION

About 10 mg of standard ornidazole was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes. The volume was adjusted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml.

Estimation from tablets

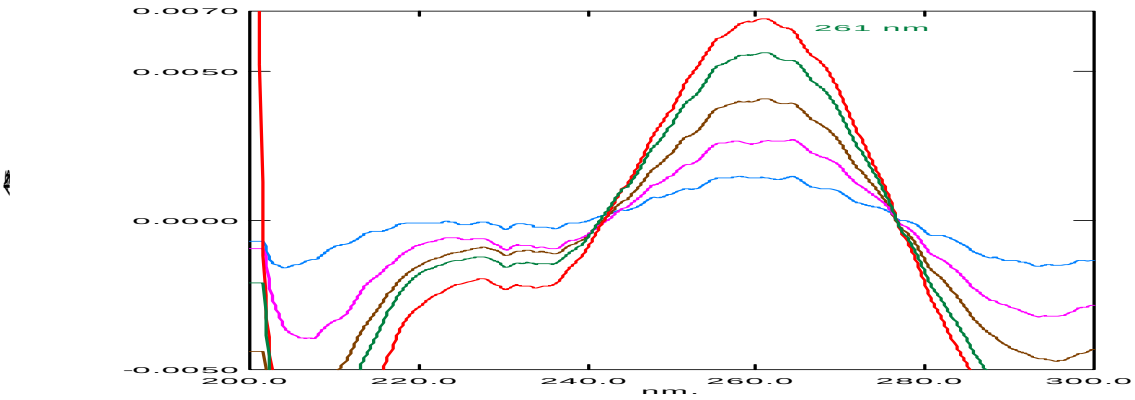
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of ornidazole was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method A: First order derivative method

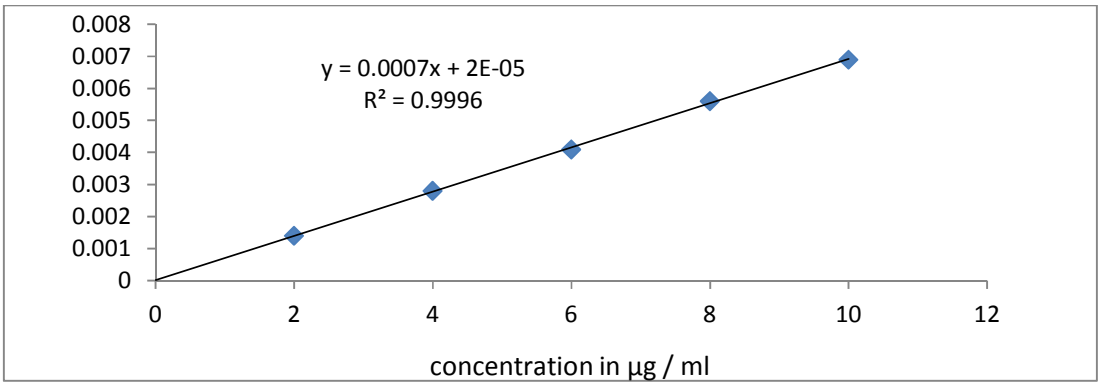
For the selection of analytical wavelength, 10 µg /ml solution of ornidazole was scanned in the spectrum mode from 300 nm to 200 nm by using absolute alcohol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 261 nm (Fig. 2).

Fig. 2. Overlay spectra of first order derivative spectrum of ornidazole (2-10 µg/ml) showing absorbance at 261 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of ornidazole was pipette out and volume was adjusted with absolute alcohol as solvent. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 261 nm by using 0.1 N hydrochloric acid as blank. The calibration curve was prepared in the concentration range of 1 to 10 µg/ml. (Fig. 3)

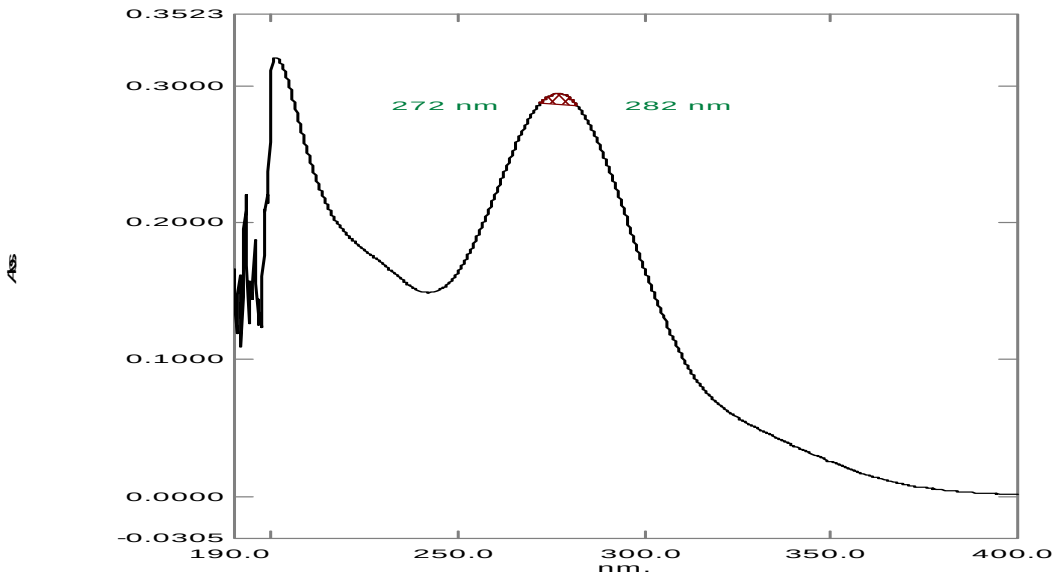
Fig. 3. Calibration curve for ornidazole at 261 nm by first order derivative Spectroscopy



Method B: Area under curve (AUC) method

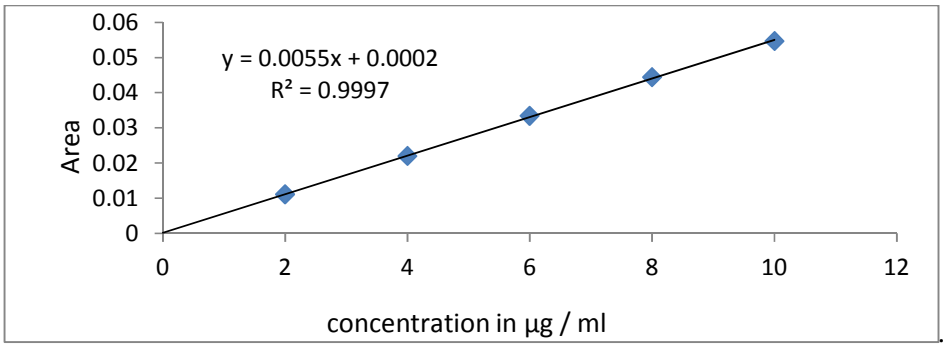
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of ornidazole was scanned in the spectrum mode from 300 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 272 nm to 282 nm (Fig. 4).

Fig. 4. Area under curve spectrum of ornidazole(10 µg/ml) showing area from 272 nm to 282 nm.



Into series of 10 ml graduated flask, varying amount of standard solutions of ornidazole was pipette out and volume was adjusted with 0.1 N hydrochloric acid. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for ornidazolewas plotted in the concentration range of 1 to 10 µg/ml (Fig. 5).

Fig. 5. Calibration curve for ornidazole by area under curve spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve (AUC) method
Detection Wavelength (nm)	261	272-282
Beer Law Limits (µg/ml)	1-10	1-10
Correlation coefficient(r^2)	0.9996	0.9997
Regression equation (y=b+ac)		
Slope (a)	0.0007	0.0055
Intercept (b)	0.00002	0.0002

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of ornidazole for first order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.020408	101.0204	0.128534	6.361766
2	2	3.959184	98.97959	0.10799	2.727579
2	4	5.959184	99.31973	0.10799	1.812158
2	6	7.979592	99.7449	0.128534	1.61078
				Mean = 0.11826	Mean = 3.128071

Table 3: Results of recovery of ornidazole for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.984556	99.2278	0.073873	3.722386
2	2	3.997426	99.93565	0.028353	0.709281
2	4	5.997426	99.9571	0.026376	0.439783
2	6	8.003861	100.0483	0.01791	0.223773
				Mean =0.036628	Mean =1.273806

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard

deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	Weight of ornidazole taken in mg	Content in mg. of ornidazole	
		First order derivative method	Area under curve method
1	10	10.000	9.8558
2	10	10.142	9.8378
3	10	10.000	9.8738
4	10	9.857	9.8558
5	10	9.857	9.8378
6	10	10.142	9.8378
	Standard deviation	0.1276	0.0147
	%RSD	1.2760	0.1493

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of ornidazole was transferred to 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

For first order derivative method

Solution was scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 261 nm by using 0.1 N hydrochloric acid as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell 261 nm for first order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of ornidazole was estimated by comparison with standard at 261 nm for first order derivative, table 5.

For area under curve method

Solution was scanned between 300 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 272 nm to 282 nm by using 0.1 N hydrochloric acid as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 272 nm to 282 nm (method B). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of ornidazole was estimated by comparison with standard at 272 nm to 282 nm, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	First order derivative method	Area under curve (AUC) method
(A)	Intra-day precision (n=3) Amount found ± % RSD	99.87 % 0.1685	99.45% 0.02446
(B)	Inter-day precision (n=3) Amount found ± % RSD	98.89% 0.1766	98.56% 0.03765
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.1678	0.023

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	First order derivative method	Area under curve (AUC) method
Limit of Detection (µg/ml)	0.4241	0.2459
Limit of Quantification (µg/ml)	1.2842	0.7452

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of ornidazole sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of ornidazole was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The first order derivative and area under curve UV-spectroscopic methods are useful for routine analysis of ornidazole in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Ornidazole has the absorbance maxima at 261 nm (method A) and in the AUC spectrum method areas were measured between 272 nm to 282 nm (method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 10 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of ornidazole in pharmaceutical formulation.

Acknowledgment

Authors express sincere thanks to the Principal, Dr. Tushar M. Desai of D. G. Ruparel college.

REFERENCES

[1] G. Mubeen, Vineeta Prakash, P.L Somashekar, Kadri Uvesh, *International Journal of ChemTech Research*, **2009**,1(2), 318-321.

[2] P.Tulasamma, V.Govind and P. Venkateswarlu**,** *International Journal of Pharma Sciences and Research*, **2011**,2(1), 44-48.

[3] Senem Şanlı**,** Tuğrul Yıldırım, Bediha Akmeşe, Nurullah Şanlı**,** *Hacettepe J. Biol. and Chem.*, **2012**, 40 (4), 401-407.

[4] Fatma I Khattab, Nesrin K Ramadan, Maha A Hegazy and Nermine S Ghoniem, *Pharmaceut Anal Acta*, **2012**, 3,8.

[5] Mônica Felts de La Roca Soares e José Lamartine Soares Sobrinho, *Quim. Nova,* **2010,** 33(2), 478-481,.