

**INTERNATIONAL JOURNAL OF UNIVERSAL  
PHARMACY AND BIO SCIENCES****IMPACT FACTOR 4.018\*\*\*****ICV 6.16\*\*\*****Pharmaceutical Sciences****Research Article.....!!!****SIMULTANEOUS ESTIMATION OF DOXYCYCLINE MONOHYDRATE AND  
ORNIDAZOLE IN BULK AND COMBINED TABLET DOSAGE FORM BY UV-  
SPECTROPHOTOMETRIC METHODS****Poornima A.N\*, Rashmi D.R, Surya K.S, Pavan Kumar H.K**

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**ABSTRACT****KEYWORDS:**

Doxycycline monohydrate,  
Ornidazole, Simultaneous  
equation method, Q – analysis  
or absorbance ratio\_method.

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Two simple, precise and accurate spectrophotometric methods have been developed and validated for the simultaneous estimation of Doxycycline monohydrate (DOXI) and Ornidazole (ORNI) in bulk and pharmaceutical formulation. Method A is Simultaneous equation method, which involved measuring the absorbance values at 271 nm and 319 nm of overlay spectrum of DOXI and ORNI respectively. Method B is Q – analysis or absorbance ratio\_method, which involved the measurement of isoabsorptive point between 294 to 319 nm for the estimation of DOXI and ORNI respectively, without mutual interference with a linearity range of 1-5µg/ml and 5-25µg/ml for the estimation of DOXI and ORNI respectively for both the methods. Results of analysis were statistically reported and were found to be satisfactory.

**INTRODUCTION:**

Doxycycline is a tetracycline antibiotic<sup>1-5</sup>, and is commonly used to treat a variety of infections. It is used in prophylaxis against malaria. Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl-tRNA to the mRNA and inhibiting bacterial protein synthesis. Doxycycline prevents the normal function of the apicoplast of *Plasmodium falciparum*, a malaria causing organism.

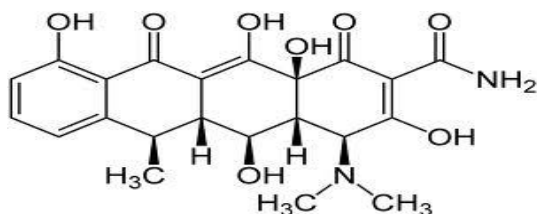


Fig.1 Chemical structure of Doxycycline monohydrate

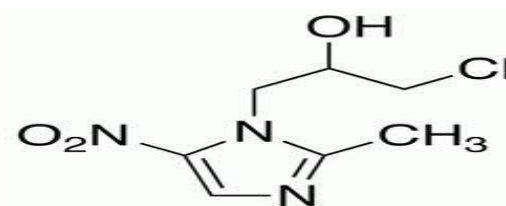


Fig. 2 Chemical structure of Ornidazole

Ornidazole is antiprotozoal medication<sup>2, 5</sup>. The nitroimidazoles, which include ornidazole, were initially introduced for the treatment of trichomonal vaginitis. They were subsequently recognized to be active against other protozoa as well as facultative anaerobes (*Helicobacter pylori* and *Gardnerella vaginalis*) and anaerobic bacteria, and are used on a worldwide basis for the treatment of infections caused by these organisms.

On literature survey, it was found that no method could be found for the simultaneous estimation of Doxycycline monohydrate and Ornidazole in combined dosage forms and no method is available in the pharmacopoeias. Few analytical methods have been developed for the determination of DOXI and ORNI individually<sup>6,7</sup>, and in combination with other drugs<sup>8,9</sup>. Hence an attempt has been made to develop a simple, accurate, precise and reproducible simultaneous equation method and Q-Absorbance Ratio methods for simultaneous estimation of DOXI and ORNI in combined dosage form with validation as per recommendation of ICH guidelines.

**2. EXPERIMENTAL**

**2.1 Instrument and materials:-** For both the methods Shimadzu-1800 UV/Vis Spectrophotometer was used with 1 cm quartz cell of 10 mm optical path length, spectral band width of  $1 \pm 0.2$ nm,

and wavelength accuracy of  $\pm 0.3$  nm . The tablet formulation of DOXI and ORNI (Label claim: Doxycycline monohydrate 100 mg and Ornidazole 500 mg), DOX M-OZ (Moraceae pharmaceuticals) was purchased from the UP market. Standard Doxycycline monohydrate and Ornidazole were obtained as gift samples from Micro labs Ltd, Bangalore, and FDC Ltd, Goa respectively. Distilled water was used as solvent throughout the experiment.

**2.2 Preparation of standard stock solution:-**100 mg each of Doxycycline monohydrate and Ornidazole were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of Distilled water by ultrasonication and then volume was made up to the mark with Distilled water to obtain the concentration of 1000  $\mu\text{g}/\text{ml}$  of each component (stock A and A' solution). From the above stock A and A' solution 10 ml of aliquot was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with Distilled water to obtain the final concentration of 100 $\mu\text{g}/\text{ml}$  of each component (stock B and B' solution).

**2.3 Preparation of sample stock solution using formulation:-**Twenty tablets of Doxycycline monohydrate and Ornidazole (DOX M-OZ) in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 100 mg of Doxycycline monohydrate was weighed which also contains 500 mg of Ornidazole and transferred to 100 ml volumetric flask and dissolved in 50 ml solvent and the content was kept in ultrasonicator for 15 min. The solution was filtered through Whatmann filter paper No.41, finally the volume was made up to the mark with solvent, which gave a concentration of 1000 $\mu\text{g}/\text{ml}$  of Doxycycline monohydrate and 5000 $\mu\text{g}/\text{ml}$  of Ornidazole and this solution was used as stock 'A' solution. From the above stock 'A' solution, 5 ml of the aliquot was pipetted out and was transferred to a 50 ml volumetric flask. The volume was made up to 50 ml with solvent to obtain a solution with final concentration of 100 $\mu\text{g}/\text{ml}$  Doxycycline monohydrate and 500 $\mu\text{g}/\text{ml}$  of Ornidazole (stock B).

2.4 Methods:

**METHOD A: SIMULTANEOUS EQUATION METHOD:-**From the standard stock solutions, dilutions ranging between 1-50 $\mu\text{g}/\text{ml}$  for both were prepared and scanned in the wavelength range of 400-200nm using UV- visible spectrophotometer. At271 nm Doxycycline showed maximum absorbance and at 319nm Ornidazole shows maximum absorbance. Both the drugs did not show any interference at either of the wavelengths. Hence 271 nm and 319 nm for Doxycycline and Ornidazole were selected as the working analytical wavelength respectively.

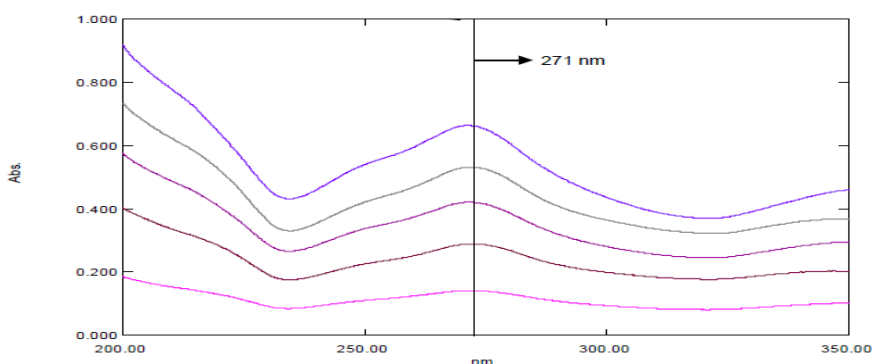
**METHOD B: Q – ANALYSIS OR ABSORBANCE RATIO METHOD:-** Appropriate aliquots were pipetted out from the standard stock B and B' solution in to a series of 10 mL volumetric flasks. The volume was made up to the mark with solvent to obtain a set of solutions having the concentration

range of 1, 2, 3, 4 and 5  $\mu\text{g/mL}$  of DOXI and 5, 10, 15, 20 and 25  $\mu\text{g/mL}$  of ORNI. All the above solutions were scanned separately in the “Spectrum Mode” from 400 nm to 200 nm. With the help of a overlay spectrum absorbance of the above solutions was measured at 319 nm ( $\lambda$  max of ORNI) and 294 nm (Isoabsorptive point). A calibration curve of absorbance against concentration was plotted. Both the drugs obeyed the Beer’s law in the concentration range of 1-5  $\mu\text{g/mL}$  and 5-25  $\mu\text{g/mL}$  for DOXI and ORNI respectively.

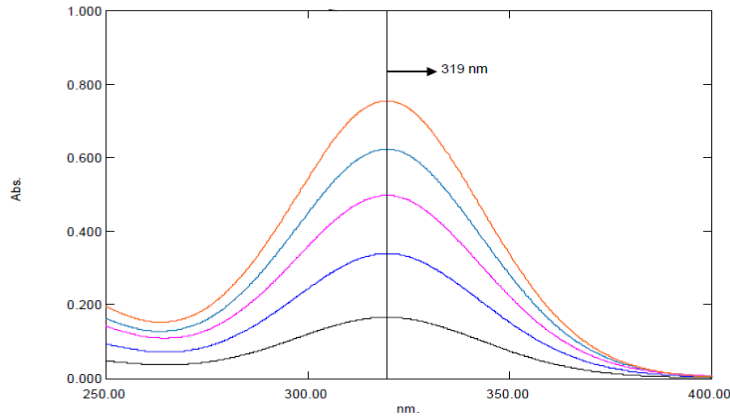
**2.5 Validation of the methods:-** All the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e., 80%, 100% and 120% by adding the pure drug to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated.

**3 Results and Discussion:-** The estimation of Doxycycline monohydrate and Ornidazole in tablet formulation was found to be accurate and reproducible with a linearity of 1-5 $\mu\text{g/ml}$  and 5-25 $\mu\text{g/ml}$  respectively for both the methods and the correlation coefficient 0.998 and 0.999 for method A and 0.998 and 0.998 for method B. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1 and Table 2 for method A and method B respectively. Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r) were calculated and are presented in Table 1 and Table 2 for method A and method B respectively. The accuracy was found by recovery studies at three different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods.

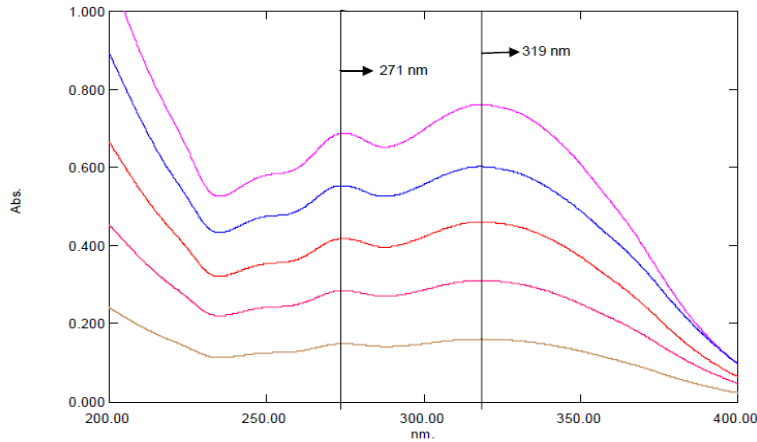
The results of Formulation were reported in Table-3. The spectra of Doxycycline monohydrate, Ornidazole and formulation are reported by Simultaneous Estimation method (Fig. 3, 4 and 5) and calibration curve was plotted (Fig. 6, 7, 8 and 9).



**Fig. 3: Overlay Spectra of Doxycycline monohydrate at 271.0 nm.**



**Fig. 4: Overlay Spectra of Ornidazole at 319.0 nm.**



**Fig. 5: Overlay Spectra of Formulation at 271.0 nm and 319.0 n**

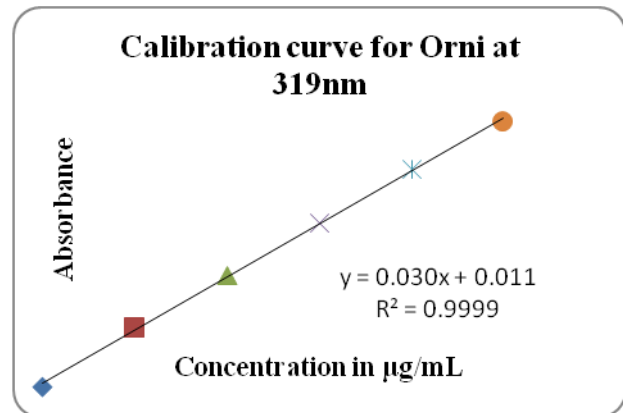
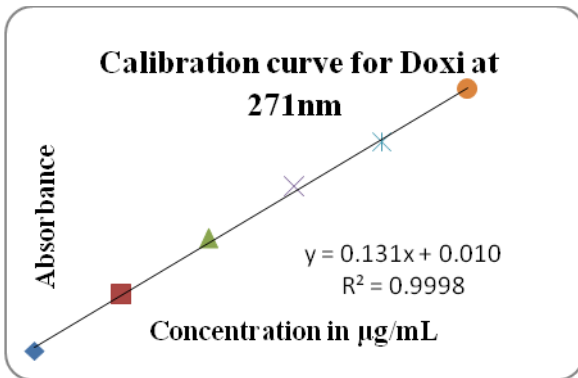


Fig. 6 & 7: Calibration curve for Doxycycline monohydrate at 271.0nm and Ornidazole at 319nm by Simultaneous Equation Method.

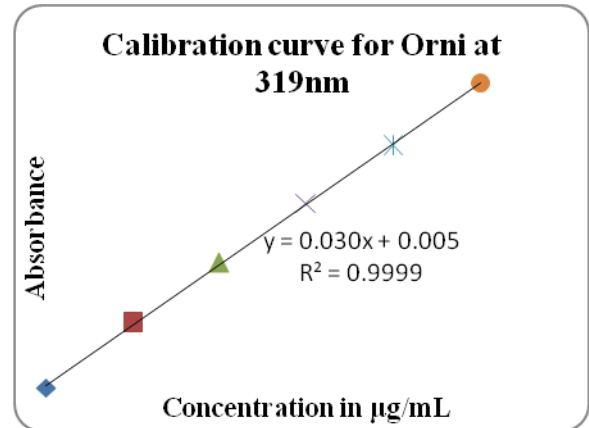
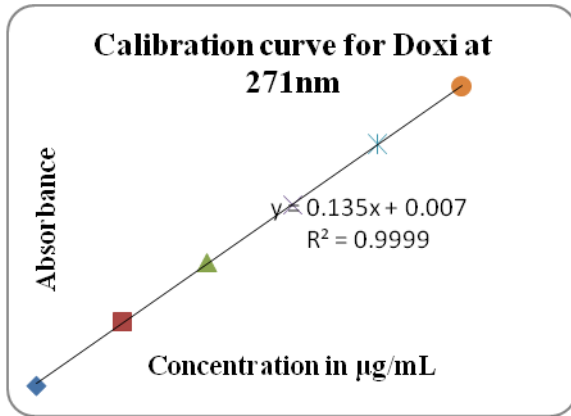


Fig.8 & 9: Calibration curve for Doxycycline monohydrate & Ornidazole in Formulation at 271.0nm & 319.0 nm respectively by Simultaneous Equation Method.

The isoabsorptive point of Doxycycline monohydrate and Ornidazole formulation by Q – analysis or absorbance ratio method are reported (Fig.10) and calibration curve was plotted (Fig.11,12, 13,14)

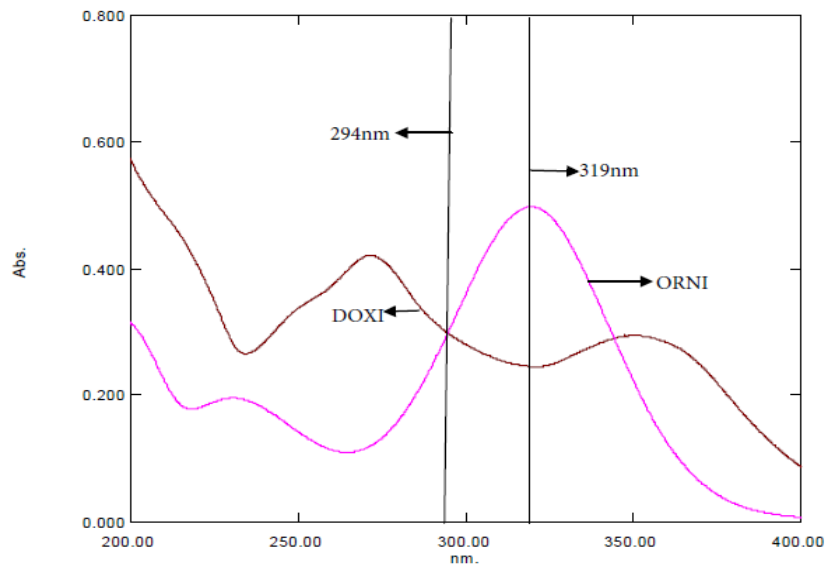


Fig. 10: Isoabsorptive point of Doxycycline monohydrate and Ornidazole.

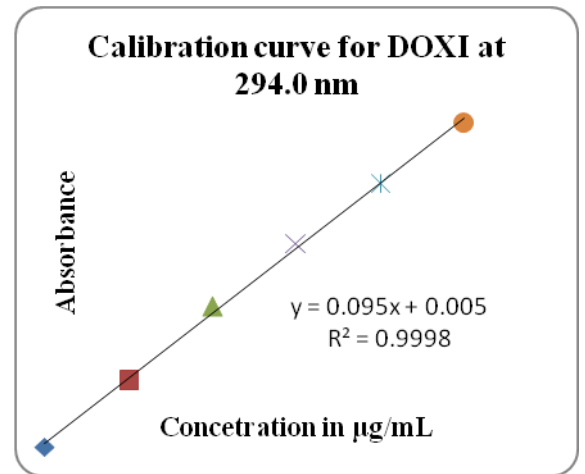
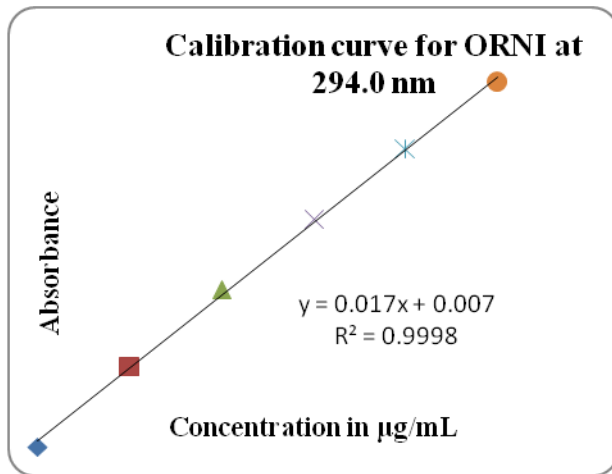


Fig. 11 & 12: Calibration curve of ORNI & DOXI at 294.0 nm by Q-Ratio method.

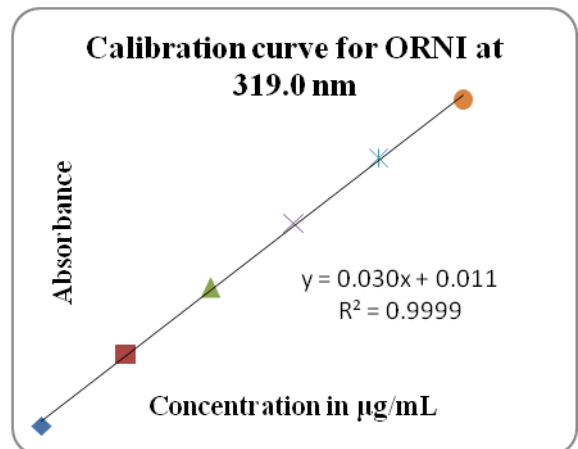
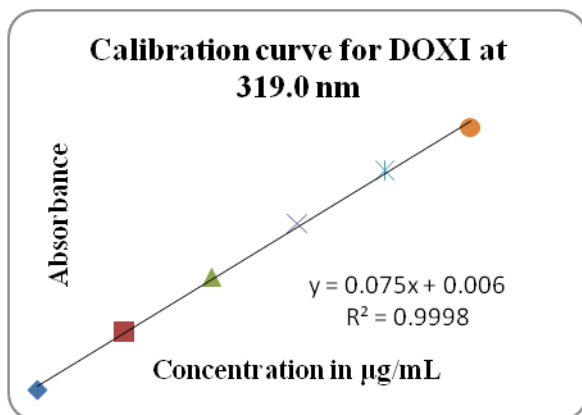


Fig. 11 & 12: Calibration curve of ORNI & DOXI at 319.0 nm by Q-Ratio method

**Table1: Optical characteristics and other parameters for Method A**

Parameters		DOXI	ORNI
Linear range (µg/ml)		01-05	05-25
$\lambda_{\max}$ /wavelengthrange(nm)		271.0nm	319.0nm
Coefficient of correlation		0.9998	0.9999
Slope*(m)		0.131	0.030
Intercept*(c)		0.010	0.011
Accuracy (%RSD)	80%	0.6993	0.4499
	100%	0.25	0.3617
	120%	1.3326	0.2858
Precision (%RSD)	Intra-day	0.9439	0.3590
	Inter-day	0.2409	0.1385
Limit of Detection (µg/ml)		0.0292	0.1155
Limit of Quantification (µg/ml)		0.0887	0.350

\* $y=mx+c$ ; when x is the concentration in µg/ml and y is absorbance unit.

**Table2: Optical characteristics and other parameters for Method B**

Parameters		DOXI	ORNI
Linear range (µg/ml)		01-05	05-25
$\lambda_{\max}$ /wavelengthrange(nm)		294.0nm	319.0nm
Coefficient of correlation		0.9998	0.9999
Slope*(m)		0.095	0.030
Intercept*(c)		0.005	0.011
Accuracy (%RSD)	80%	0.2715	0.2967
	100%	0.2081	0.2081
	120%	0.2804	0.2950
Precision (%RSD)	Intra-day	0.77153	0.67414
	Inter-day	0.09814	0.14433
Limit of Detection (µg/ml)		0.03654	0.1155
Limit of Quantification (µg/ml)		0.1107	0.350

\* $y=mx+c$ ;when x is the concentration in µg/ml and y is absorbance unit.



**Table3: Results of formulation:**

method	Brand name	Label claim of DOXI (mg)	Label claim of ORNI (mg)	Amount found for DOXI (mg)	Amount found for ORNI (mg)	%Recovery $\pm$ SD** for DOXI	%Recovery $\pm$ SD** for ORNI
A	DOX	100	500	99.98	499.98	99.98 $\pm$ 0.02250	99.99 $\pm$ 0.03633
B	M-OZ	100	500	99.99	500.12	99.99 $\pm$ 0.02250	100.01 $\pm$ 0.03633

\*\* Average of six determinations.

### 3. CONCLUSION:-

The proposed two spectrophotometric methods were found to be simple, accurate and precise and in expensive and can be used for routine analysis of Doxycycline monohydrate and Ornidazole in bulk and its formulation.

### 4. ACKNOWLEDGEMENT:-

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