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Development and Validation of First Order Derivative Method for Simultaneous Estimation of Doxycycline Monohydrate and Ornidazole in Synthetic Mixture.

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Abstract

The present manuscript describe simple, novel, rapid, precise, accurate, specific and cost effective first order derivative spectrophotometric method for the determination of Doxycycline monohydrateand Ornidazole in combined dosage forms. First order derivative spectrophotometric method involves measurement of absorbance at ZCP of Doxycycline monohydrate for estimation of Ornidazole and at ZCP of Ornidazole for estimation of Doxycycline monohydrate. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines and all validation characteristics were found within the acceptance limits. Thus, the proposed method could be successfully applied for simultaneous determination of Doxycycline monohydrate and Ornidazole in combined dosage forms.

Keywords:- Doxycycline monohydrate, Ornidazole, First order derivative spectrophotometric method.

1. Introduction

Doxycycline is a broad spectrum antibiotic which acts against both gram positive and gram negative organisms. It inhibits bacterial protein synthesis by attaching to 30 S subunit of bacterial ribosome (which are absent in mammals). Doxycycline interfering the attachment of aminoacyl-tRNA to the mRNA-ribosome complex and peptide chain fails to grow.

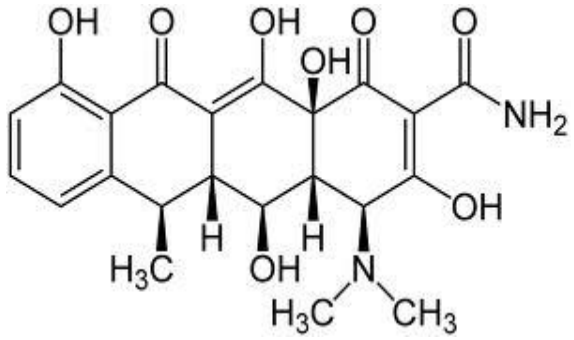

Figure 1: Structure of Doxycycline Monohydrate

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.

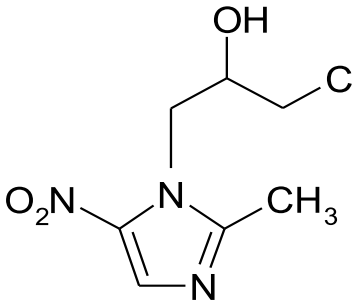

Figure 2: Structure of Ornidazole

Doxycycline monohydrate and Ornidazole in their combined dosage form mainly used as broad spectrum antibiotics because doxycycline act as antibacterial activity, whereas, ornidazole act as antiprotozoal activity. In India, Avidox-OZ tablet and DOX-M-OZ tablet are marketed as combined dosage form of Doxycycline monohydrate and Ornidazole.

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2. Material And Methods

2.1 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, Goettingen, Germany)
- ✓ Ultrasonic cleaner (Frontline FS 4, Mumbai, India)
- ✓ Corning volumetric flasks, beakers and pipettes of borosilicate glass were used in the study.

2.2 Materials and Reagents

- ✓ Doxycycline monohydrate and Ornidazole standard powder. (Acme Pharmaceutical Ltd., Ahmedabad, India)
- ✓ Methanol AR grade as solvent (S.D. Fine Chemical Ltd., Mumbai, India.)
- ✓ Whatman filter paper no. 41 (Whatman International Ltd., Maidstone, Kent,, England)

2.3 Preparation of Solutions

2.3.1 Preparation of standard stock solution

An accurately weighed DOX and ORN powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution having concentration 100 µg/ml for both of drugs.

2.3.2 Preparation of working standard solution

The working standard solutions of DOX and ORN were prepared be transferrin aliquots' of standard stock solution of DOX (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 & 3.6 ml) and ORN (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 & 3.6 ml) was transferred in a series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and mixed.

2.3.3 Preparation of synthetic mixture

Accurately weigh 700 mg of synthetic mixture was prepared by using DOX (100 mg) and ORN (500 mg) and excipients (100 mg) like MCC (Microcrystalline cellulose), Starch, Magnesium stearate and Talc.

2.3.4 Preparation of sample solution

Quantity of the powder equivalent to 10 mg DOX & 50 mg ORN was transferred in 100 ml volumetric flask 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. The solution 0.6 ml was transferred to 10 ml volumetric flask, the volume was diluted up to mark with methanol.

3. Method Development

3.1 Determination of the zero crossing points

The standard solutions of DOX (10 µg/ml) and ORN (10µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. At zerocrossing point of first drug second drug showed reasonable absorbance, while at zerocrossing point ofsecond drug first drug showed reasonable absorbance so these two wavelengths were selected for further measurement.

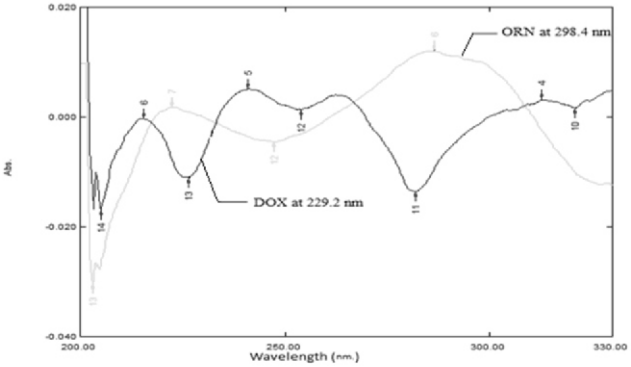

Figure 3: Derivatised spectra of DOX (12µg/ml) and ORN (12 µg/ml)

3.2 Preparation of Calibration Curves

From standard stock solutions, aliquots of DOX (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 & 3.6 ml) and ORN (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 & 3.6 ml) were transferred in a series of 10 ml volumetric flasks. The volume was adjusted to the mark with methanol and mixed.The Absorbances of derivatised spectra were measured at 229.2 nm (zero crossing point of ORN) and 298.4 nm (zero crossing point of DOX) against methanol as blank. The calibration curves were prepared by plotting the graph of absorbance Vs concentration.

4. Results And Discussion

The working standard solutions of DOX and ORN were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. From the overlay derivatised spectra of two drugs, it is evident that DOX and ORN show a zero crossing point at 229.2 nm and 298.4 nm. These two wavelengths were employed for the determination of DOX and ORN. Overlain and derivatised spectra of both the drugs are shown in Figure 3.An attempt has been made to develop a rapid, sensitive, economic, precise and accurate an alytical methods for simultaneous estimation of DOX and ORN in bulk and in pharmaceutical dosage form. The proposed methods are based on spectrophotometric absorption for the simultaneous estimation of DOX and ORN in UV region derivative spectra using methanol assolvent. Beer's law

obeyed in the concentration range of zero crossing point at 229.2 nm and 298.4 nm, considering wavelengths selected for method. The correlation coefficient values were found0.9991 and 0.995, which shows that absorbance of all the drugs was linear with concentration.

5. Method Validation

The developed method was validated according to the International Conference on Harmonization (ICH) guidelines.

5.1 Validation of the First order derivative Method

5.1.1 Linearity

Calibration range was observed in the concentration range of 4-36 µg/ml for both DOX and ORN.

PARAMETERS	DOX	ORN
Wavelength (nm)	229.2	298.4
Beer's law limit (µg/ml)	4-36	4-36
Regression Equation Y=mX+C	Y=0.001X-0.0026	Y=0.0008X-0.0001
Slop (m)	0.001	0.0008
Intercept (C)	0.0026	0.0001
Correlation coefficient (r^2)	0.9991	0.9995

Table 1: Regression parameters of DOX and ORN by First derivative method

5.1.2 Precision

5.1.2.1 Method Precision (Repeatability)

The RSD values for repetability data of DOX and ORN are given in table 2.

Sr. No	Absorbance of DOX (20 µg/ml)	Absorbance of ORN (20 µg/ml)
1	0.0171	0.0167
2	0.0173	0.0172
3	0.0169	0.0167
4	0.0168	0.0170
5	0.0171	0.0168
6	0.0169	0.0171
MEAN	0.0170	0.0169
SD	0.00018	0.00021
RSD	1.0782	1.2632

Table 2:Repeatability data for the simultaneous equation method

5.1.2.2 Intermediate Precision (Reproducibility)

The RSD values for reproducibility data of DOX and ORN are given in table 3.

DRUG	% RSD	
	Intraday Precision	Interday Precision
DOX	1.11-1.58	1.10-1.29
ORN	0.82-1.87	0.82-1.49

Table 3: Precision (Reproducibility) data for first derivative method

5.1.3 LOD and LOQ

LOD and LOQ values for DOX were found to be 0.76 and 2.30 µg/ml at 229.2 nm respectively. While the, LOD and LOQ values for ORN were found to be 0.41 and 1.25 µg/ml at 298.4 nm respectively. Low value of LOD & LOQ indicates that the method is sensitive.

5.1.4 Accuracy

The mean recoveries were found to be 99.94 ± 0.44 and 99.97 ± 0.89 for DOX and ORN, respectively. The recoveries results indicate that the proposed method is accurate. Results of recovery studies are shown in Table 4.

DRUG	LEVEL	Amt. Present (µg/ml)	Amt. added (µg/ml)	% Mean Recovery ± SD
DOX	I	6	3	99.44±1.38
	II	6	6	100.27±1.43
	III	6	9	100.11±0.77
ORN	I	30	15	100.25±1.90
	II	30	30	100.61±1.93
	III	30	45	99.06±1.11

Table 4- Recovery data for first derivative method

5.1.5 Assay

The proposed validated method was successfully applied to determine DOX and ORN in combined synthetic mixture. Results are given in Table 5.5. No interference of the excipient with the absorbance of analyte of interest has been seen hence the proposed

Sample No.	Label claim (mg/Tablet)		Amt. found (mg/Tablet)		% Label claim (%)	
	DOX	ORN	DOX	ORN	DOX	ORN
1	100	500	98.50	496.25	98.50	99.25
2	100	500	98.83	504.37	98.83	100.87
3	100	500	100.83	507.50	100.83	101.50
4	100	500	99.00	491.04	99.00	98.20
5	100	500	100.33	497.70	100.33	99.54
6	100	500	98.16	508.75	98.16	101.75
				MEAN	99.28	100.18
				SD	1.06	1.40

Table 5:Analysis of synthetic mixture

6. Conclusion

The method described for the simultaneous estimation of DOX and ORN was found to be sensitive, accurate and precise for routine simultaneous estimation of two drugs. 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 drugs. 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 drugs in combined dosage form.

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