WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 887-895. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH METHOD FOR THE SIMULTANEOUS ESTIMATION OF DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE IN SYNTHETIC MIXTURE

***Bhoomi H. Patel and Satish A. Patel**

Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar– 384012, Mehsana, Gujarat, India.

Article Received on
26 Sept. 2017,

Revised on 16 Oct. 2017,
Accepted on 07 Nov. 2017

DOI: 10.20959/wjpr201715-10124

***Corresponding Author**
Bhoomi H. Patel
Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar– 384012, Mehsana, Gujarat, India.

ABSTARCT

A simple, novel, rapid, precise, accurate, specific and cost effective Dual Wavelength spectrophotometric method for the determination of Doxycycline monohydrate and Ornidazole in combined dosage forms. Dual Wavelength spectrophotometric method involves measurement of absorbance at Wavelengths λ_1 and λ_2 at which absorbance difference (A270 – A252.2) of DOX was observed and there was same absorbance of ORN at this two wavelengths (270 & 252.2nm). Wavelengths λ_3 and λ_4 at which absorbance difference (A310.6–A291.4) of ORN was observed and there was same absorbance of DOX at this two wavelengths (310.6 & 291.4 nm). 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 can be successfully applied for simultaneous determination of Doxycycline monohydrate and Ornidazole in combined dosage forms.

KEYWORDS: Doxycycline monohydrate, Ornidazole, Dual Wavelength spectrophotometric method, Validation.

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.

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.

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 & DOX-M-OZ are marketed as combined dosage form of Doxycycline monohydrate and Ornidazole.

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, 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., 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 (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 & 3.5ml) and ORN (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 & 3.5ml) 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

600mg of synthetic mixture was prepared by using DOX (100mg) and OZ (500mg) 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 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 0.5 ml of above solution to 10 ml volumetric flask to get final concentration around 5 µg/ml of DOX and 25 µg/ml of ORN. Then the volume was adjusted up to mark with methanol.

2.4 Method Development

2.4.1 Selection of Wavelength

The solution of DOX and ORN were prepared separately in methanol and scanned in the UV range of 200 - 400 nm. From the overlain spectra (Figure 3) of both drugs, four specific wavelengths are selected.

(I) Wavelengths λ_1 and λ_2 at which absorbance difference (A270 – A252.2) of DOX was observed and there was same absorbance of ORN at this two wavelengths (270 & 252.2nm).

(II) Wavelengths λ_3 and λ_4 at which absorbance difference (A310.6– A291.4) of ORN was observed and there was same absorbance of DOX at this two wavelengths (310.6 & 291.4 nm).

Thus these four selected wavelengths were employed to determine the concentration of DOX and ORN.

2.5 VALIDATION OF THE DEVLOPED METHOD

The method was validated as per the rules of International Conference on Harmonization (ICH) guidelines [34].

2.5.1 Linearity & Range

Calibration curve were plotted over a concentration range of 5-35 µg/ml for DOX 5-35 µg/ml for ORN. Accurately measured standard working solution of DOX (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 & 3.5ml) and ORN (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 & 3.5ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and the absorbance of the solutions was measured at 270 nm (λ1), 310.6 nm (λ2), 252.2 nm (λ3) and 291.4 nm (λ4). The difference in absorbance between 270 nm and 252.2 nm is (due to the 0 absorbance difference of ORN) plotted against DOX concentration (µg/ml). The difference in absorbance between 310.6 nm and 291.4 nm is (due to the 0 absorbance difference of DOX) plotted against ORN concentration (µg/ml) and two different regression equations were obtained.

2.5.2 Precision

2.5.2.1 Method Precision (Repeatability)

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

2.5.2.2 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 DOX and ORN (10, 15, and 20 µg/ml). The results are reported in terms of relative standard deviation (% RSD).

2.5.3 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.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

2.5.4 Accuracy (% Recovery study)

The accuracy of the method was determined by calculating recoveries of DOX and ORN by

the standard addition method. Known amounts of standard solution of DOX and ORN were added at 80%, 100% and 120% levels to pre quantified sample solutions of DOX and ORN.

3. ANALYSIS OF DRUGS IN SYNTHETIC MIXTURE

Synthetic mixture contains DOX (100 mg) and ORN (500 mg). Transfer accurately weighed 600 mg of synthetic mixture which equivalent to 100 mg DOX and 500 mg of ORN. It was dissolved in sufficient amount of distilled water and sonicated for 30 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to 100 ml with methanol. From the resulting solution 1 ml of aliquot was further diluted up to 100 ml with distilled water. From that 0.5 ml of solution was further diluted up to 10 ml with distilled water. For elimination of the effects of an interfering component, four specific wavelengths are chosen: Wavelengths λ_1 and λ_2 at which absorbance diff of DOX was observed and there was same absorbance of ORN at this two wavelengths (270 & 252.2 nm). Wavelengths λ_3 and λ_4 at which absorbance diff of ORN was observed and there was same absorbance of DOX at this two wavelengths (310.6 & 291.4 nm). Thus these four selected wavelengths were employed to determine the concentration of DOX and ORN.

4. RESULT AND DISCUSSION

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The solution of DOX and ORN were prepared separately in methanol and scanned in the UV range of 200 - 400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. Wavelengths λ_1 and λ_2 at which absorbance difference (A270 – A252.2) of DOX was observed and there was same absorbance of ORN at this two wavelengths (270 & 252.2nm). Wavelengths λ_3 and λ_4 at which absorbance difference (A310.6– A291.4) of ORN was observed and there was same absorbance of DOX at this two wavelengths (310.6 & 291.4 nm). Thus these four selected wavelengths were employed to determine the concentration of DOX and ORN.

LIST OF FIGURES

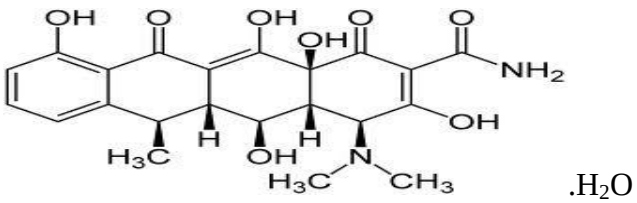

Figure 1: Structure of Doxycycline Monohydrate.

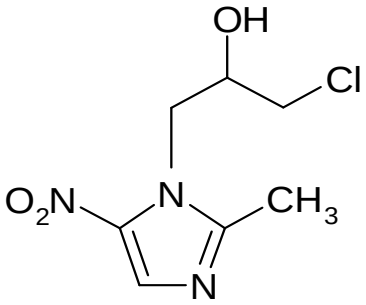

Figure 2: Structure of Ornidazole

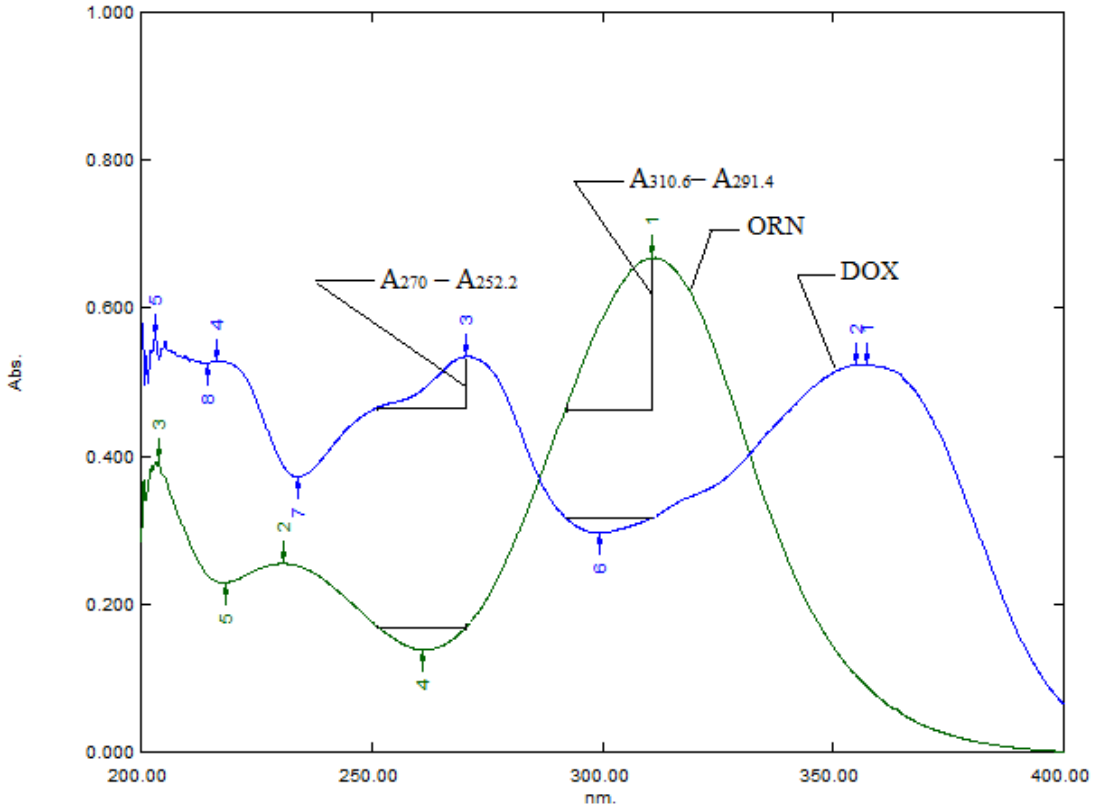

Figure 3. Overlay spectra of DOX (15 µg/ml) and ORN (15 µg/ml)

LIST OF TABLES

Table 1. Regression parameters of DOX and ORN

PARAMETERS	DOX	ORN
Wavelength (nm)	270-252.2	310.6-291.4
Beer's law limit (µg/ml)	5-35	5-35
Regression Equation Y=mX+C	Y=0.0045X-0.0016	Y=0.0142X-0.0007
Slop (m)	0.0045	0.0142
Intercept (C)	0.0016	0.0007
Correlation coefficient (r^2)	0.9994	0.9995

Table 2. Result of recovery study of DOX and ORN by developed method.

DRUG	LEVEL	Amt. Present (µg/ml)	Amt. added (µg/ml)	% Mean Recovery ± SD
DOX	I	5	4	100.43±0.38
	II	5	5	99.70±0.68
	III	5	6	100.00±0.37
ORN	I	25	20	100.27±0.26
	II	25	25	99.84±0.35
	III	25	30	100.06±0.29

Table 3. Analysis of DOX and ORN in Pharmaceutical formulation by developed method.

Sample No.	Label claim (mg/Tablet)		Amt. found (mg/Tablet)		% Label claim (%)	
	DOX	ORN	DOX	ORN	DOX	ORN
1	100	500	99.55	499.85	99.55	99.97
2	100	500	103.11	504.64	103.11	100.92
3	100	500	100.00	501.26	100.00	100.25
4	100	500	98.22	505.63	98.22	101.12
5	100	500	100.44	491.26	100.44	98.25
6	100	500	99.11	502.23	99.11	100.50
MEAN			100.07	500.87	100.07	100.17
SD			1.67	5.15	1.67	1.03

Table 4. Regression analysis data and summery of validation parameters for the developed method.

PARAMETERS	DOX	ORN
Wavelength (nm)	270-252.2	310.6-291.4
Beer's law limit (µg/ml)	5-35	5-35
Regression Equation Y=mX+c	Y=0.0045X-0.0016	Y=0.0142X-0.0007
Slop (m)	0.0045	0.0142
Intercept (c)	0.0016	0.0007
Correlation coefficient (r^2)	0.9994	0.9995
Sandell's sensitivity (µg cm^{-2})	0.0852	0.0260
Method precision Repeatability (n=6, %RSD)	1.53	1.02
Interday precision (n=3, %RSD)	1.61-1.91	1.20-1.92
Intraday precision (n=3, %RSD)	0.81-1.29	0.38-0.69
LOD (µg/ml)	0.43	0.11
LOQ (µg/ml)	1.31	0.35
% Recovery ± SD (n=3)	100.04±0.36	100.05±0.21
Assay ± SD (n=3)	100.07±1.67	100.17±1.03

5. 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.

6. ACKNOWALEDGEMENT

The authors wish to thank camper Healthcare and Acme Pharmaceutical Private Limited for providing DOX and ORN pure drug powder as gift sample for research work. And we are also hearty thankful to department of Quality assurance of S.K.Patel College of Pharmaceutical Education & Research ,Ganpat University, for providing permission and all the facilities to carry out the research work.

7. REFERENCES

1. O'Neil MJ. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: Doxycycline; 2006; 3474: 606.
2. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. New Delhi: The controller of publication, 6th edition, volume 2, 2010; 1257-1260.
3. British Pharmacopoeia, The Department of Health, Social Services and Public Safety, Doxycycline hyclate, 2010; 1: 755-57.
4. USP 32, NF 27; The United States Pharmacopeia; Doxycycline, 2009; 2: 2211.
5. European Pharmacopoeia Vol. 5.0, Starboury, Council of Europe, 2008; 1760-1763.
6. Japanese Pharmacopoeia. Society of Japanese Pharmacopeia.15th ed. Shibuya Tokyo, Japan 2006; 1190-1191.
7. Snezana SM, Gordana ZM. A rapid and reliable determination of doxycycline hyclate by HPLC with UV detection in pharmaceutical samples. Journal of Serbian chemical society 2008; 73(6): 665–671.
8. Ramesh, Divya, Nagarju. Titrimetric and Spectrophotometric Determination of doxycycline monohydrate in pharmaceutical formulation. Chemical Industry & Chemical Engineering Quarterly 2010; 16(2): 139−148.

9. Salvi M.S. et al. Development and validation of RP-HPLC Method for simultaneous estimation of doxycycline hyclate and tinidazole in bulk and tablet dosage International Journal of pharmacy and pharmaceutical sciences, 2013; 5(4): 7666-7671.
10. Venugopal Darak et al, Development and validation of simultaneous Spectrophotometric estimation of Doxycycline and Tinidazole in tablet dosage forms. An international journal of pharmaceutical sciences, 2011; 2(2): 85-90.
11. Indian Pharmacopoeia, Government of India, New Delhi: The controller of publication, 6th edition volume - 3, 2010; 1823-1824.
12. Development of new dissolution test and HPLC-RP method for anti-parasitic Ornidazole coated tablets, Quim. Nova, 2010; 33(2): 478-481.
13. Lila KN, Tapan KG. Spectrophotometric Method Development and Determination of Ornidazole in Bulk and Tablet Dosage Form. International Journal of Pharmaceutical Technology and research 2011; 3(1): 153-156.
14. Pinak Patel et al. New Improved RP- HPLC Method for Determination of Norfloxacin and Ornidazole in Their Combined Dosage Form. International Journal of Research in Pharmaceutical and Biomedical Science. 2011; 2(2): 710-713.
15. Sevak Manan R. et al. Development & validation of RP-UPLC method for simultaneous estimation of Ofloxacin and Ornidazole in their combine dosage form including stress study, Journal of Applied Chemistry; 2014; 7(9): 32-35.
16. Natraj KS et al. UV Spectrophotometric Method Development and Validation of Simultaneous Estimation Of Ciprofloxacin And Ornidazole In Tablet Dosage Form, Int Res J Pharm 2013; 4(7): 178-181.
17. Gandhi Santosh V et al. HPTLC Determination of Cefuroxime Axetil and Ornidazole in Combined Tablet Dosage Form, Journal of Chromatographic Science; 2010; 48: 26-29.
18. The International Conference on Harmonization.Q2 (R1).Validation of Analytical Procedure Text and Methodology. 2005.