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Research Article

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SPECTROPHOTOMETRIC ESTIMATION OF DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE IN SYNTHETIC MIXTURE BY SIMULTANEOUS EQUATION METHOD

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ABSTRACT

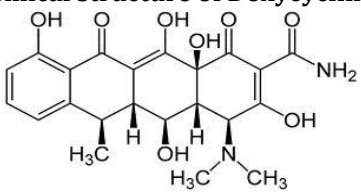
A simple, accurate, rapid and precise spectrophotometric method has been developed for simultaneous estimation of Doxycycline monohydrate and Ornidazole in bulk and synthetic mixture. This method was based on UV spectrophotometric determination of two drugs, using Simultaneous equation method. It involves λmax 270nm (For Doxycycline) and 310.6nm (For Ornidazole) for the analysis in methanol. The linearity was observed in the concentration range of 4-36 µg/ml for Doxycycline and 4-36 µg/ml for Ornidazole. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Doxycycline and Ornidazole in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Keywords: Simultaneous equation method; Ornidazole; Doxycycline monohydrate; Recovery; Validation.

INTRODUCTION

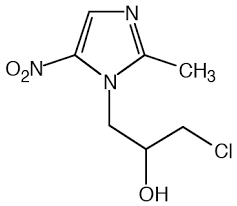
Doxycycline (DOX) is chemically 4-(dimethylamino)-3,5,10,12,12a - pentahydroxy - 6 – methyl - 1,11 – dioxo -1, 4,4a,5,5a,6,11,12a – octahydrotetracene - 2 - carboxamide (Figure **1**) is a well-known antibiotic drug[1]. It is official in Indian Pharmacopoeia[2], British Pharmacopoeia[3], United State Pharmacopoeia[4], European Pharmacopoeia[5] and Japanese Pharmacopoeia[6]. Literature survey reveals HPLC[7] and UV spectrophotometry[8] methods for estimation of DOX in single dosage form. Literature survey also reveals HPLC[9] and UV spectrophotometry[10] methods for determination of DOX with other drugs in combination.

Figure 1. Chemical structure of Doxycycline (DOX)



Ornidazole (ORN) is chemically 1-chloro-3-(2-methyl-5-nitroimidazol-lyl) Propan-2-ol. (Figure **2**). Ornidazole (ORN) is official in IP[11]. Literature survey reveals HPLC[12] and UV spectrophotometry[13] methods for determination of ORN in single dosage form.

Figure 2. Chemical structure of Ornidazole (ORN)



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Literature survey also reveals HPLC[14-15], UV spectrophotometry[16] and HPTLC[17] method for the determination of ORN with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of DOX and ORN in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of DOX and ORN in synthetic mixture or dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on Simultaneous equation method for simultaneous estimation of both drugs in their combined synthetic mixture.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

DOX and ORN bulk powder was kindly gifted by Torrent Research Centre, Gandhinagar, India and Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India respectively. Methanol (AR Grade, S D Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

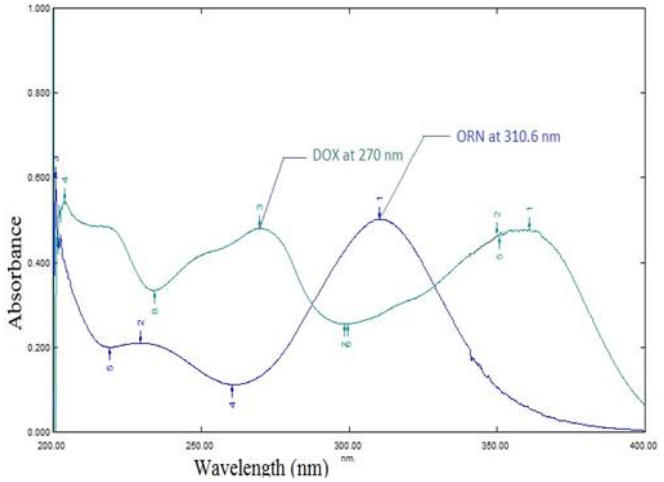
An accurately weighed quantity of standard DOX (10 mg) and ORN (10 mg) powder were weighed and transferred

to 100ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of DOX and ORN.

Methodology

The working standard solutions of DOX and ORN were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400nm against methanol as blank. Absorbance was measured at 270nm and 310.6nm for DOX and ORN, respectively. These two wavelengths can be employed for the determination of DOX and ORN without any interference from the other components in their synthetic formulations. (Figure **3**)

Figure 3. Overlain absorption spectra of DOX and ORN in methanol



Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[18]

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 4-36µg/ml for DOX and 4-36µg/ml for ORN. Accurately measured standard solutions of DOX (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6ml) and ORN (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of the solutions were measured at 270 and 310.6nm against methanol as blank. The calibration curves were constructed by plotting areas versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of area of solutions (*n* = 6) for DOX and ORN (12 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined 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 standard solutions of DOX and ORN (12, 16, 20 µg/ml for DOX and 12, 16, 20 µg/ml for ORN). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of DOX and ORN by the standard addition method. Known amounts of standard solutions of DOX and ORN were added at 50, 100 and 150 % level to prequantified sample solutions of DOX and ORN (6 µg/ml DOX and 30 µg/ml ORN). The amounts of DOX and ORN were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Table 2. Recovery data of DOX and ORN

Drug	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 3)
DOX	6	50	100.73±0.87
	6	100	99.16±0.82
	6	150	100.45±0.82
ORN	30	50	100.12±0.92
	30	100	99.83±0.66
	30	150	100.11±0.36

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines [18]

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of DOX and ORN from synthetic mixture

Doxycycline (10mg) and Ornidazole (50mg) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100ml volumetric flask containing 50 ml methanol and sonicated for 20min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution (0.6ml) was taken in to a 10ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of DOX (6 µg/ml) and ORN (30 µg/ml). The responses of the sample solution were measured at 270nm and 310.6nm for quantitation of DOX and ORN, respectively. The amounts of the DOX and ORN present in the sample solution were calculated by solving respective equations for DOX and ORN as follows.

$$Cx = (A2\ aY1 - A1\ aY2) / (aY1\ aX2 - aY2\ aX1)$$

$$Cy = (A1\ aX2 - A2\ aX1) / (aY1\ aX2 - aY2\ aX1)$$

Where,

Cx = Concentrations of DOX,
Cy = Concentrations of ORN,
A_1 = Absorbance at 270nm,
A_2 = Absorbance at 310.6nm,
aX_1 and aY_1 are Absorptivity of DOX and ORN respectively at 270nm,
aX_2 and aY_2 are Absorptivity of DOX and ORN respectively at 310.6nm.

Table 3. Analysis of DOX and ORN in synthetic mixture

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	DOX	ORN	DOX	ORN	DOX	ORN
I	100	500	100.18	498.61	100.18±1.04	99.72 ± 1.16

RESULTS AND DISCUSSION

The present work provides an accurate, reproducible, sensitive method for the simultaneous analysis of DOX & ORN in bulk and synthetic mixture. Linear relationships between drug concentrations were obtained over the range of at 4-36 µg/ml & 4-36 µg/ml for DOX and ORN

respectively. Under experimental conditions described assay, linearity, accuracy studies and precision, LOD and LOQ were estimated. Correlation coefficient was found to be > 0.995. The results are presented in Table **1**. The % assay was found to be 100.18 % for DOX and 99.72 % for ORN, and S.D. and R.S.D. for six determinations of sample, by this method, was found to be less than 2.0 indicating the precision of this method. No interference was observed from the pharmaceutical adjuvants /excipients.

Table 1. Regression analysis data and summary of validation parameters for DOX and ORN

Parameters	DOX		ORN	
Wavelength (nm)	270	310.6	270	310.6
Beer's law limit (µg /ml)	4-36	4-36	4-36	4-36
Regression equation (y = a + be) Slope (b) Intercept (a)	y = 0.0358x - 0.0211 0.0358 0.0211	y = 0.0222x - 0.037 0.0222 0.037	y = 0.0106x - 0.0051 0.0106 0.0051	y = 0.0401x - 0.0081 0.0401 0.0081
Correlation coefficient (R^2)	0.9995	0.9968	0.9995	0.9997
LOD[a] (µg/ml)	0.34	0.52	0.43	0.27
LOQ[b] (µg /ml)	1.04	1.59	1.32	0.82
Repeatability (% RSD[c] , n = 6)	0.54	1.32	1.90	0.32
Precision (% RSD, n = 3) Interday Intraday	 0.77-1.92% 0.08-1.18%	 0.41-1.73% 0.21-1.97%	 0.48-1.47% 0.32-1.82%	 0.19-1.92% 0.05-1.11%
Accuracy ± S. D[d]. (% Recovery, n = 3)	100.12 ± 0.83		100.02 ± 0.16	

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

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of DOX and ORN in Synthetic mixture. The method utilizes easily available and cheap solvent for analysis of DOX and ORN hence the method was also economic for estimation of DOX and ORN from Synthetic mixture. The common excipients and other additives present in the Synthetic mixture do not interfere in the analysis of DOX and ORN in this method; hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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