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Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE IN SYNTHETIC MIXTURE

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ABSTRACT

A simple UV spectrophotometric absorption ratio method has been developed for the simultaneous determination of Doxycycline monohydrate and Ornidazole in synthetic mixture. This method uses the ratio of absorbances at two selected wavelengths, one which is λmax of Ornidazole (311.4 nm) and other being an Isoabsorptive point of both drugs (286.6 nm) in methanol. The linearity was obtained in the concentration range of 3-27 µg/ml for both Doxycycline monohydrate and Ornidazole. The results of analysis have been validated statistically and also by recovery studies according to ICH guidelines. The method was successfully applied to synthetic mixture because no interference from the mixture excipients was found. The Method was found to be simple, sensitive, accurate and precise and was applicable for simultaneous estimation of Doxycycline monohydrate and Ornidazole in synthetic mixture.

KEYWORDS: Doxycycline monohydrate, Ornidazole, absorption ratio method, Spectrophotometric, Synthetic mixture, Validation

INTRODUCTION

Doxycycline (DOX) is chemically 4-(dimethylamino)-3,5,10,12,12 a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12 a-octahydrotetracene-2-carboxamide (Figure 1) It is a well-known Tetracycline class of antibiotic drug that is used in respiratory infections, relapsing fever, empirical therapy (for initial treatment of all mixed infection), urinary tract infection and chronic intestinal amoebiasis[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 that 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 estimation of DOX with other drugs in combination. Ornidazole (ORN) is chemically 1-chloro-3-(2-methyl-5-nitroimidazol-lyl) Propan-2-ol. (Figure 2) It is used in treatment of Amoebiasis, Giardiasis, Peptic ulcer, ulcerative gingivitis, trichomanas vagginitis and Pseudo membranous enterocolitis. It is official in IP[11]. Literature survey reveals that HPLC[12] and UV spectrophotometry[13] methods for determination of ORN in single dosage form. Literature survey also reveals HPLC[14], UPLC[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 combined mixture. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of DOX and ORN in synthetic mixture or dosage forms. The present works describes simple, sensitive, rapid, accurate, precise and cost effective Q-absorbance ratio method for simultaneous determination of both drugs in synthetic mixture.

MATERIALS & METHODS

Reagents and Materials

DOX and ORN bulk powder was kindly gifted by Astron Pharmaceuticals Pvt. Ltd. Ahmedabad, India. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no. 41 (Millipore, USA) was also used in the study.

Apparatus

A shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was also used in the study.

Preparation of standard stock solutions

Standard stock solution (100 µg/ml) of DOX and ORN was prepared separately by dissolving an accurately weighed quantity of DOX (10 mg) and ORN (10 mg) to a separate 100 ml volumetric flask and diluted up to the mark with methanol to obtain standard solution having concentration of 100 µg/ml for both drugs.

Preparation of Synthetic mixture

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

Development of Method

Q-Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an Isoabsorptive point and the

other being λmax of one of the two components. From the overlay spectra of two drugs, it is observed that DOX and ORN shows an Isoabsorptive point at 286.6 nm. The second wavelength used is 311.4 nm, which is the λmax of ORN. Working standard solutions having concentration 3, 6, 9, 12, 15, 18, 21, 24 and 27 µg/ml for both DOX and ORN were prepared in methanol. The absorbance at 286.6 nm (Isoabsorptive point) and at 311.4 nm (λmax of ORN) were measured and absorptivity coefficients were calculated by using calibration curve.
The concentration of two drugs in the mixture can be calculated using following equations

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{.....(3)}$$
$$C_Y = (A_1/aX_1) - C_X \quad \text{.....(4)}$$

Where, A_1 and A_2 are absorbances of mixture at 286.6 nm and 311.4 nm; aX_1 and aY_1 are absorptivities of DOX and ORN at 286.6 nm; aX_2 and aY_2 are absorptivities of DOX and ORN respectively at 311.4 nm; and $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$.

METHOD VALIDATION

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 3-27 µg/ml for both DOX and ORN. Accurately measured standard stock solutions of both DOX and ORN (0.3, 0.6, 0.9, 1.2 1.5, 1.8, 2.1, 2.4, and 2.7 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbance of solution were measured at Isoabsorptive point (286.6 nm) and at λmax of ORN (311.4 nm). The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions (n = 6) of DOX and ORN (15 µg/ml for both drugs) without changing the parameters of the proposed method.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of DOX and ORN (6, 9 and 12 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% recovery study)

The accuracy of the method was determined by calculating the recoveries of both DOX and ORN by the standard addition method. Known amounts of standard solutions of DOX and ORN were at added at 80, 100 and 120 % level to prequantified sample solutions of DOX (5 µg/ml) and ORN (25 µg/ml). The amounts of DOX and ORN were estimated by applying obtained values to the respective regression line equations.

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 as designated by International Conference on Harmonization (ICH) guidelines[18]

$$LOD = 3.3 \times \sigma/S, \quad LOQ = 10 \times \sigma/S$$

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

Analysis of DOX and ORN in synthetic mixture

DOX (10 mg) and ORN (50 mg) standard drug powder were accurately weighed and then mixed with commonly used formulation additives like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 10 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. The solution was suitably diluted with methanol to get a final concentration of 5 µg/ml of DOX and 25 µg/ml of ORN. The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 286.6 nm (Isoabsorptive point) and 311.4 nm (λmax of ORN) respectively, and ratio of absorbance were calculated, i.e. A_2/A_1. Relative concentration of both drugs in the synthetic mixture was calculated by using above equation (3) and (4).

TABLE 1: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
DOX	I	5	80	99.08 ± 0.72
	II	5	100	101.1 ± 0.90
	III	5	120	100.7 ± 1.20
ORN	I	25	80	100.2 ± 1.45
	II	25	100	98.80 ± 0.78
	III	25	120	99.43 ± 0.41

TABLE 2: ANALYSIS OF DOX AND ORN IN SYNTHETIC MIXTURE BY PROPOSED METHOD (n=6)

Synthetic mixture	Label claim (mg)		Amount found (mg) ± S.D. (n = 6)		% Label claim ± S.D. (n = 6)	
	ORN	DOX	ORN	DOX	ORN	DOX
1	500	100	499.8 ± 1.65	100.4 ± 0.66	99.97 ± 0.33	100.4 ± 0.66

TABLE 3: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED METHOD

PARAMETERS	ORN	DOX	ISOABSORPTIVE POINT
Wavelength (nm)	311.40	311.40	286.60
Beer's law limit (µg/ml)	3 - 27	3 – 27	3 - 27
Regression equation (y = a + bc)	Y = 0.039x – 0.003	Y = 0.020x – 0.013	Y = 0.022x – 0.002
Slope (b)	0.039	0.020	0.022
Intercept (a)	0.003	0.013	0.002
Correlation Coefficient (r2)	0.999	0.998	0.999
Method precision (Repeatability) (% RSD, n = 6),	0.48	0.97	0.92
Interday (n = 3) (% RSD)	0.68 – 1.51	0.71 – 1.68	1.24 – 1.43
Intraday(n = 3) (% RSD)	0.57 – 0.86	0.23 – 1.52	0.68 – 1.45
LOD (µg/ml)	0.04	0.29	0.33
LOQ (µg/ml)	0.12	0.89	1.00
Accuracy (%Recovery ± SD, n=3)	99.32 ± 0.97	100.26 ± 1.04	
Assay (% ± SD)	100.46 ± 0.88	100.43 ± 0.43	

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

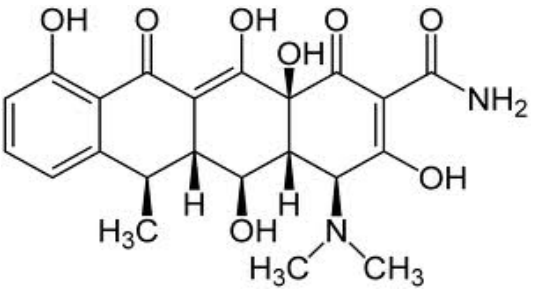

Figure 1: Chemical structure of Doxycycline (DOX)

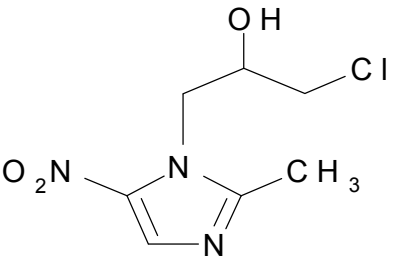

Figure 2: Chemical structure of Ornidazole (ORN)

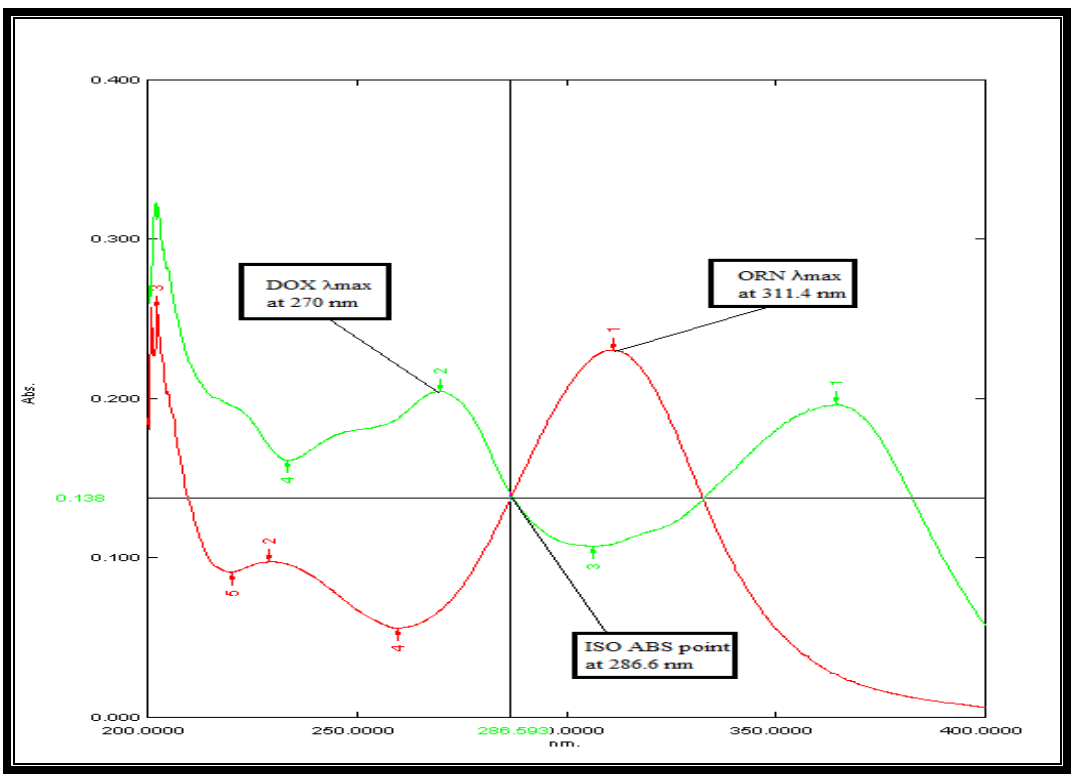

Figure 3: Overlain absorption spectra of DOX (270 nm) and ORN (311.4 nm) showing Isoabsorptive point (286.6 nm) in methanol

RESULTS & DISCUSSION

In Q-absorbance ratio method, the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength[19], which was fulfilled for both these drugs. The two wavelengths were used for the analysis of the drugs were 286.6 nm (Isoabsorptive point) and 311.4 nm (λmax of ORN) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of ORN at λmax 311.4 nm and DOX at λmax 270 nm shows Isoabsorptive point at 286.6 nm in methanol, is shown in Figure 3.

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of DOX and ORN present in the synthetic mixture. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 3. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined synthetic mixture.

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