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Preformulation studies on combination of ornidazole and doxycycline in pharmaceutical dosage forms: Infra-red spectroscopy and simultaneous ultra-violet method development

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ABSTRACT

Combination of antibiotics is generally preferred for the treatment of chronic infections by covering broad range of microbes. In this study we have evaluated to explore the feasibility of combination of ornidazole (OZ) and doxycycline (DX) to be administered together in a formulation. The compatibility between both drugs was studied by Infra-red spectroscopic study. Further, two UV method was developed and validated for simultaneous estimation of OZ and DX when present in combined dosage form. UV method is economical, less time consuming and can be used for routine analysis over HPLC method. The UV method included Verodt's method (Method I) and Q-analysis method (Method II). Method I involve solving of simultaneous equations for obtained absorbances at the λ_{max} of both the drugs i.e. 319 nm and 274 nm for OZ and DX respectively in phosphate buffer pH 6.8. Method II is based on measurement of absorbance at iso-absorptive point of both the drugs (292 nm) and λ_{max} of OZ (319nm). The linearity was observed in the concentration range of 5-25µg/ml (R^2>0.999) for OZ and 5-30µg/ml (R^2>0.999) for DX at all the selected wavelengths. Both methods were compared for accuracy and precision for inter-day and intra-day variability. Limit of detection (LOD) and limit of quantitation (LOQ) were also determined to evaluate the sensitivity of the method. The method was validated and found to comply with ICH guidelines. Developed proposed method was successfully applied for the simultaneous estimation of OZ and DX when combined in synthetic mixture for routine analysis.

Keywords: Ornidazole, Doxycycline, Ultraviolet spectroscopy, Iso-absorptive point

INTRODUCTION

Ultraviolet (UV) spectroscopic method of analysis is extensively used in the analysis of pharmaceutical ingredients probably due to its sensitivity, simplicity and cost-effectiveness. Several promising methods for the estimation of multicomponent in pharmaceutical formulations have been developed using this approach.

Ornidazole is a 5-nitroimidazole derivative active against protozoa and anaerobic bacteria (Fig. 1). It is official in Indian Pharmacopoeia. It is used in bacterial periodontitis, vaginosis [1] and postoperative crohn's disease [2]. Its mechanism of action involves destruction of DNA structure and strand leading to protein synthesis inhibition and cell death of susceptible organisms. Literature survey reveals that UV-visible spectrophotometric [3], HPLC, RP-HPLC [4] and HPTLC [5] methods are available for determination of OZ from pharmaceutical and biological formulation.

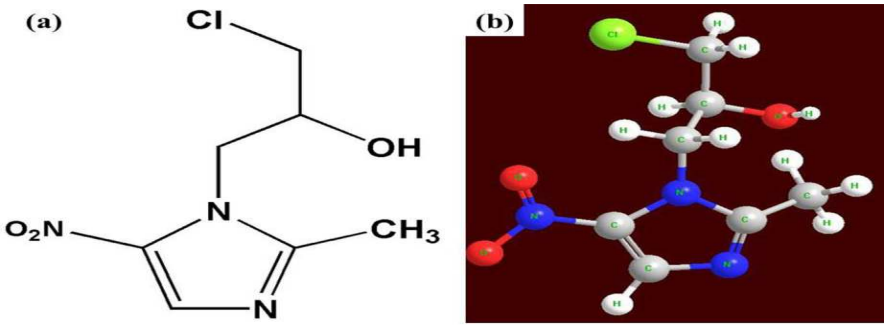

Figure 1: Structure of ornidazole; a) two dimensional view b) three dimensional view

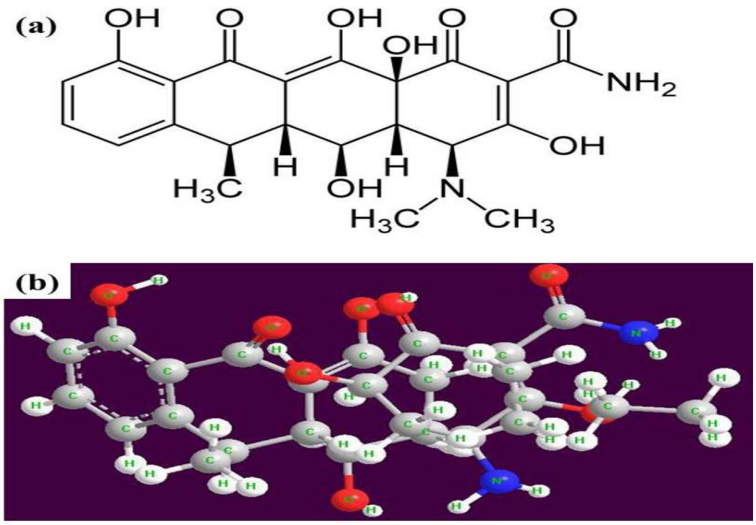

Figure 2: Structure of doxycycline; a) two dimensional view b) three dimensional view

Doxycycline hyclate (DX), is a semisynthetic tetracycline derivative (Fig. 2), possess broad spectrum activity against gram positive and gram negative pathogens. It is frequently used in prophylaxis of malaria and treatment of chronic prostatitis, sinusitis, syphilis, chlamydia, pelvic inflammatory disease, acne, rosacea, and rickettsial infections [6]. DX is basically a bacteriostatic antibiotic which may become bactericidal at high concentrations. DX enters bacterial cell by passive diffusion as well as active carrier mediated transport. It then reversibly binds to the 30S ribosomal subunit of susceptible organisms thereby, preventing the binding of aminoacyl transfer RNA. This inhibits protein synthesis and bacterial cell growth. It has also been reported that DX inhibits matrix metalloproteinases mainly by disrupting the active zinc domains [7]. DX is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), Japanese Pharmacopoeia (JP) and European Pharmacopoeia (EP). Literatures have revealed use of spectrophotometric and RP-HPLC [8], Liquid chromatography/Mass spectrometery [9], capillary electrophoresis [10], liquid chromatographic method [11] and derivative UV method [12] for estimation of DX in single dosage form.

The combination of OZ and DX offers a wide range of antibacterial activity and their drug profile are summarized in Table 1. Such combinations of drugs are clinically proven effective in cases of acute pelvic inflammatory disease [16] recurrent periodontitis [17] and amoebiasis [18]. Currently, two marketed preparations Avidox-OZ® manufactured by Avalanche Pharmaceuticals and Moraceae Pharmaceuticals (P) Ltd. and DOX-M-OZ® are available in the market [19]. A very few reports are there on the simultaneous estimation of OZ and DX, though the method of analysis by RP-HPLC method [20], and few UV methods [21, 22] are available. These few available methods are validated using methanol, distilled water and mobile phase in case of HPLC. For their applicability in pharmaceuticals a simple method developed at physiological pH conditions is required. Overall UV method is most popular method over other method for routine analysis due to its considerable advantages such as its economical, provides quick analysis, less tedious and recordable. These beneficial points generated the need of development of UV method for OZ and DX for daily lab analysis where HPLC and other methods can be avoided. However,

extensive literature surveys indicated lack of well validated UV method for the estimation of both the drugs in phosphate buffer pH 6.8.

Table 1. Drug profile of ornidazole and doxycycline

Parameters/Drugs	Ornidazole	Doxycycline hyclate
Formula	$C_7H_{10}ClN_3O_3$	$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$
Molecular Weight	219.65 g/mol	1025.89 g/mol
IUPAC Name	1-Chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol	Hydrochloride hemiethanol hemihydrate of (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl- 1,11-dioxo-1,4,4a,5,5a,6,11, 12a-octahydrotetracene-2-carboxamide
Appearance	Crystalline white solid	Crystalline yellow solid
Solubility	4330 mg/L at 25 ºC in water. It is soluble in water, ether, ethanol and chloroform [13]	630 mg/L at 25 °C in water. Doxycycline salt is soluble 1 in 3 of water and 1 in 4 of methanol. It is sparingly soluble in ethanol, and practically insoluble in chloroform and in ether. It dissolves in aqueous solutions of alkali hydroxides and carbonates [14].
Boiling Point	443.2 ºC at 760 mmHg	685.2 °C at 760 mmHg
Melting Point	85-90 ºC	200-209 °C
pKa	2.4±0.1 [13]	3.02 ± 0.3; 7.97 ± 0.15; 9.15 ± 0.3 [15]
Storage	Room temperature	Room temperature away from direct sunlight
Application	Antibacterial and antiprotozoal activity	Antibacterial activity and Matrix Metalloproteases (MMPs) inhibitor

Therefore, the present research investigates the feasibility of placing both drugs in fixed dose combination. Infra-red analysis was performed to assess compatibility between drugs. Moreover, two simple, precise, economical methods were developed, validated and compared for simultaneous estimation of both drugs by UV spectrophotometric method. Methods were validated as per guidelines of International Chemical Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Q2R1 [23]. The obtained results were subjected to statistical analysis.

EXPERIMENTAL SECTION

Materials/Chemicals

Ornidazole (OZ) and doxycycline (DX) were received as gift sample from ENDOC Lifecare Pvt. Ltd. (Gujrat, India) and Ranbaxy Laboratories Ltd. currently known as Sunpharma Industries Ltd. (Gurgaon, India) respectively. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. All dilutions were performed in standard Class 'A' borosilicate volumetric glassware. Millipore water (Millipore Direct Q® 3UV) was used during the entire procedure.

Fourier Transform Infrared analysis

Infrared red spectroscopic analysis of both the pure drugs (ornidazole and doxycycline) and their physical mixture (1:1) was done by using pelletization technique (FTIR, Shimadzu-8400, Japan) to determine any possible interaction between both drugs when present in combination. In this a small pellet of samples was prepared with potassium bromide. Samples were scanned in the region of 4,000-400 cm^{-1} with a resolution of 4 cm^{-1} for 20 scans.

UV Spectrophotometric analysis

Spectrophotometric analysis was performed on a UV double beam spectrophotometer (Shimadzu-1700 Tokyo, Japan) using 1 cm quartz cells with a slit width of 2 nm and a scan rate of 100 nm/min. Absorbance was measured and spectra was plotted against the solvent blank over the wavelength range of 200-400 nm. Spectrophotometric determinations were made using visible spectrophotometer connected to a baseline computer. Digital balance (Shimadzu AUW-220, Japan) was used for weighing the samples. Digital pH-meter (Perfit India) was used for adjusting pH of the working buffers.

Preparation of standard and sample solution of OZ and DX

Preparation of Phosphate buffer*:* A Phosphate buffer solution (pH 6.8) was prepared according to standard methods given in Indian Pharmacopoeia, 2014.

Stock solutions: Stock solutions of 100 µg/ml were prepared for OZ and DX in Phosphate buffer pH 6.8.

Calibration curve preparation

Standard solutions of OZ and DX were prepared by serial dilution from the corresponding stock solutions to obtain a concentration range of 5-30 µg/ml in phosphate buffer pH 6.8. The resulting solutions were scanned individually in the range of 200-400 nm to determine the wavelength of maximum absorbance (λ_{max}) for both the drugs. The calibration curves were plotted between observed absorbance (A) and corresponding concentrations of drugs at their

absorption maxima. The overlain zero order spectra of OZ and DX shows the absorption maxima of OZ and DX (Fig. 3).

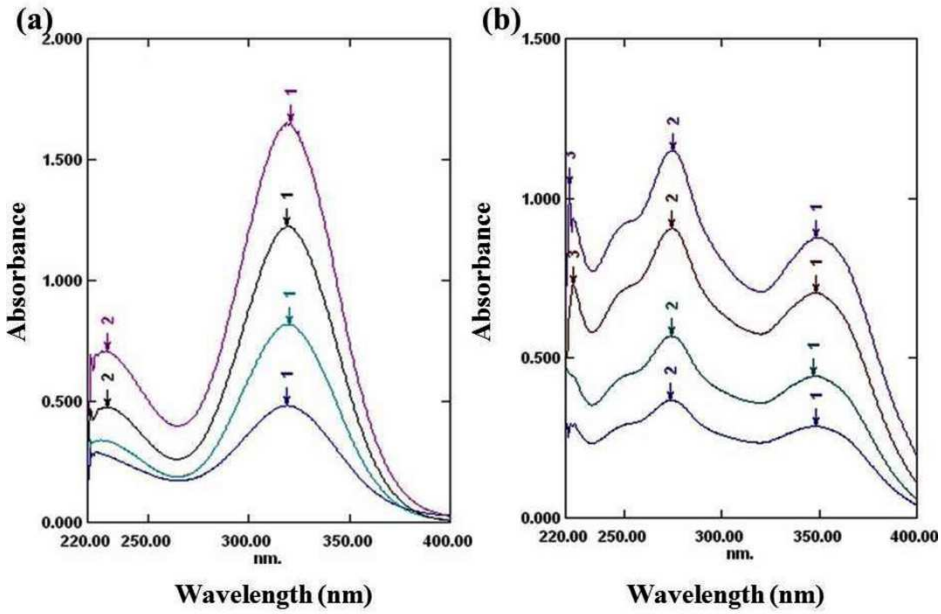

Figure 3: Zero order UV spectra; a) Ornidazole (OZ) b) Doxycycline hyclate

Development of UV method:

Method I

Vierordt's method was used which is also known as simultaneous equation method. It is based on recording of absorbance at two selected absorption maxima wavelengths *i.e*. 319 nm (λ_{max} of OZ) and 274 nm (λ_{max} of DX) (Fig. 3). This method can be applied only when OZ and DX have well distinguished absorption maxima. Following equation can be used to quantify the concentrations of OZ and DX [24].

$$\text{OZ cocentration} = \frac{\text{A274} \times \text{aDX319} - \text{A319} \times \text{aDX274}}{\text{aOZ274} \times \text{aDX319} - \text{aOZ319} \times \text{aDX274}} \times 100$$

$$\text{DX cocentration} = \frac{\text{A319} \times \text{aOZ274} - \text{A274} \times \text{aOZ274}}{\text{aOZ274} \times \text{aDX319} - \text{aOZ319} \times \text{aDX274}} \times 100$$

Where, A319 and A274 are absorbances of mixture at 319nm and 274nm respectively; aOZ319 and aOZ274 are absorptivity coefficients of OZ at 319 nm and 274nm, respectively; aDX319 and aDX274 are absorptivity coefficients of DX at 319 nm and 274 nm, respectively. Absorptivity at respective wavelengths was calculated using Beer's lambert Law.

$$A=\text{Log } (I_0/I)=\epsilon cl$$

Where, I_0=intensity of light incident upon sample cell, I=Intensity of light leaving sample cell, c=molar concentration of solute, l=length of sample cell, ϵ=molar absorptivity

Method II

Q-analysis method is modification of Vierordt's method and is also referred as iso-absorptive point or absorbance ratio method in some literatures. It is based on the principle of iso-absorptive point where compounds obeying Beer's law exhibits constant value of ratio of absorbance's at particular two wavelengths. Iso-absorptive point is constant value and is independent of concentration and path length. It was determined by overlapping the UV spectra of both the drugs and is obtained as 291.85 nm ≈ 292 nm (Fig. 4). For quantitation of drugs in mixture absorbance was measured at two wavelengths. One being λ_{max} of OZ and other being wavelength corresponding to iso-absorptive point. The calibration curve of both drugs was plotted at these wavelengths and the absorptivity values were calculated using Beer's lambert Law. Following equations were used to calculate the OZ concentration and DX concentration in mixture.

$$\text{OZ concentration} = \frac{\frac{A319}{A292} - \frac{aDX319}{aDX274}}{\frac{aOZ319}{aOZ292} - \frac{aDX319}{aDX274}} \times \frac{aOZ292}{A292}$$

$$\text{DX concentration} = \frac{\frac{A319}{A292} - \frac{aDX319}{aDX274}}{\frac{aOZ319}{aOZ292} - \frac{aDX319}{aDX274}} \times aDX292/A292$$

Where, A292 and A319 are absorbance of mixture at 292 nm and 319 nm respectively; aOZ319 and aOZ292 are absorptivity coefficients of OZ at 319 nm and 292 nm, respectively; aDX319 and aDX292 absorptivity coefficients of DX at 319 nm and 292 nm respectively.

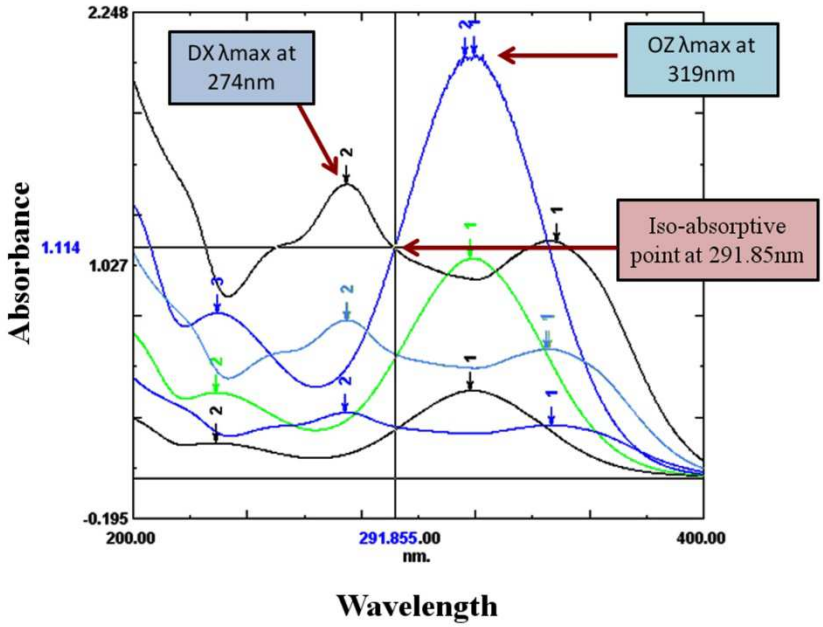

Figure 4: Overlay spectra of ornidazole (OZ) and doxycycline (DX) illustrating λ_{max} of both the drugs and their iso-absorptive point

Method validation

Validation is an important part of quality assurance program and aims to demonstrate that the analytical method is suitable for the intended proposal and it is safe to run. Method validation was performed following ICH specifications for linearity, accuracy, precision, detection limit and quantitation limit, and mixture analysis [23].

Linearity and range

In analysis, linearity refers to the ability of obtained results to remain directly proportional to the analyte concentration. Mathematically, the concentration range over which Beer's Lambert law is obeyed is linearity. The range of an analytical procedure is the interval between the upper and lower concentration or amounts of analyte in the sample (including these concentrations) for which it exhibits suitable level of precision, accuracy and linearity [23]. Linearity and range of analyte at different wavelengths was determined from corresponding calibration curve of drugs.

Accuracy

ICH guidelines define accuracy as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the experimental value [23]. It is a measure of exactness of analytical method. It is expressed as % recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

$$\%\ Recovery = \frac{Calculated\ concentration}{Theoretical\ Concentration} \times 100$$

Precision

Precision is defined by ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Precision was evaluated with respect to both repeatability

(intra-day precision) and intermediate precision (inter-day precision). Intra-day precision was determined by repeating the experiments on the same day. Inter-day precision was studied by repetition of the assays on two different days to obtain reproducibility for each method [23]. The precision was expressed in %RSD (relative standard deviation) and calculated by following equation:

$$\%RSD = \frac{Standard\ deviation\ of\ values}{Mean\ value} \times 100$$

Limit of detection and limit of quantitation

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily exactly quantified. The limit of quantitation (LOQ) is the lowest amount of analyte in a sample which can be quantitated with precision and accuracy. For each determination, y intercept was calculated and the standard deviation (SD) of the y intercept was computed. From these values, LOD and LOQ were calculated on the basis of response and slope (S) of the regression equation obtained from the linearity studies as follows;

$$LOD = 3.3\ (SD/S)$$

$$LOQ = 10\ (SD/S)$$

Analysis of synthetic mixture of OZ and DX

The synthetic mixture of OZ and DX was prepared in the ratio of the mixed standard solutions (5:10, 10:15, 15:20, 20:25) and were scanned from 200-400 nm. The absorbances of final sample solution were measured at 319, 274 and 292 nm and corresponding drug concentrations were determined by Method I and Method II. All the determinations were performed in triplicate.

RESULTS AND DISCUSSION

FTIR spectral analysis

Infrared spectral analysis of OZ and DX was performed to characterize both the drugs. Table 2 and Figure 5, shows the characteristics peaks of OZ and DX and their physical mixture. The infrared spectra of OZ exhibited transmittance peaks at 3165 cm^{-1}, 3088 cm^{-1}, 1154 cm^{-1}, and 832 cm^{-1} owing to presence of O-H stretching, aromatic C-H stretching, aliphatic C-H stretching and C-Cl group respectively. Further, peaks at 1540 cm^{-1} and 1361 cm^{-1} corresponds to asymmetric N=O stretching and symmetric N=O stretching. Peaks at 2963 cm^{-1}, 1618 cm^{-1}, 1600 cm^{-1}, 3282 cm^{-1}, 3454 cm^{-1}, 3300 cm^{-1}, in infrared spectra of DX are attributed to C-H stretching, C-C stretching, aromatic C=C bonds, C-O bond, primary O-H group and N-H group respectively. These characteristic peaks of both the drugs are well preserved in physical mixture of OZ and DX (1:1) indicating absence of any interactions between both the drugs. Therefore, both drugs can be administered together in a combination.

Table 2. FTIR characteristic peaks of ornidazole and doxycycline

Peak position (cm^{-1})	Group responsible
Ornidazole	
3165	O-H stretching
3088	Aromatic C-H stretching
1540	asymmetric NO_2 stretching
1268 and 1361	symmetric NO_2 stretching
1154	C-H stretching
830	C-N and NO_2 stretching.
832	C-Cl stretching
Doxycycline	
2964	C-H stretching
1618	C-C stretches
1672	C-O group
1600 and 1400	aromatic C=C bonds
1245	C-O bond
3282	primary $–NH_2$
3454	Primary -OH group
3300	-NH group

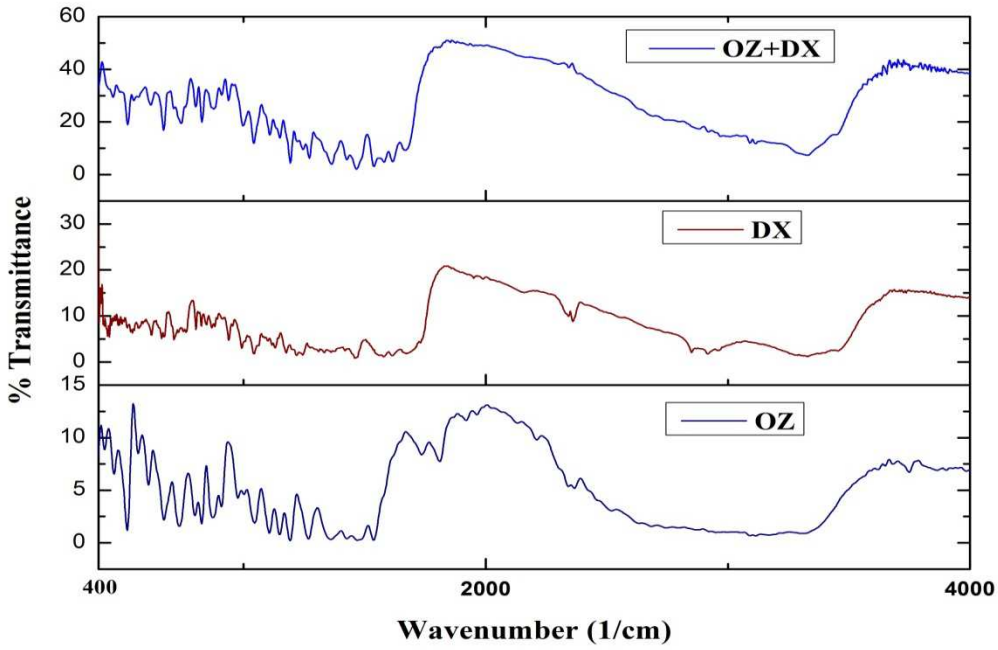

Figure 5: FTIR spectra of ornidazole (OZ), doxycycline hyclate (DX), and physical mixture (OZ+DX)

Method development

The individual and overlapping UV spectra of OZ and DX in Phosphate buffer pH 6.8 are shown in Figure 3 and 4. UV spectra of OZ showed two absorption peaks at 319 nm and 227 nm while DX exhibited three absorption peaks at 345 nm, 274 nm and 222 nm (Fig. 3). Wavelength of maximum absorbance λ_{max} was chosen at 319 nm for OZ and 274 nm for DX which is useful for quantitative estimation of both the drugs when present individually.

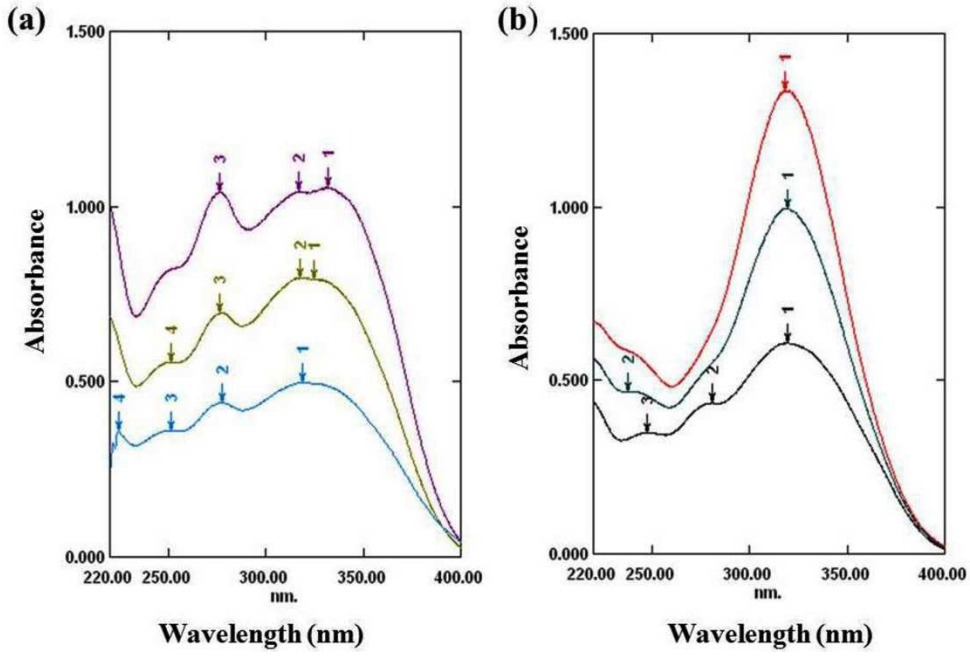

Figure 6: Overlay UV spectra of mixture of ornidazole (OZ) and doxycycline (DX) at the ratio of a) 1:1 (blue), 1:2 (green), 1:3 (violet); b) 1:1 (black), 2:1 (green), and red (3:1)

Nevertheless, OZ and DX are UV active drugs and shows well defined absorption maxima that are well separated from each other. But, they cannot be determined quantitatively by direct UV method in physical mixture due to masking of λ_{max} of one drug by the other. As obtained in Figure 6, with the increasing concentration of one drug the λ_{max} of second drug in lower concentration starts disappearing. For instance, when DX concentration was increased, λ_{max} of OZ got omitted and DX absorption maxima peak were more prominently observed (Fig. 6a). This effect can

be more clearly observed in Fig. 6b where complete disappearance of λ_{max} of DX has occurred at the ratio of 2:1 and 3:1 (OZ:DX). Therefore, physical mixture of these two drugs cannot be estimated by direct UV method. Therefore, two simple indirect UV methods had been developed and validated in phosphate buffer pH 6.8.

Calibration curves of both drugs were plotted at 319 nm, 274 nm and 292 nm. The drugs obeyed Beer's Lambert law and showed linearity in the concentration range of 5-25µg/ml and 5-30µg/ml for OZ and DX respectively at all wavelengths (for Method I and Method II) (Fig. 7).

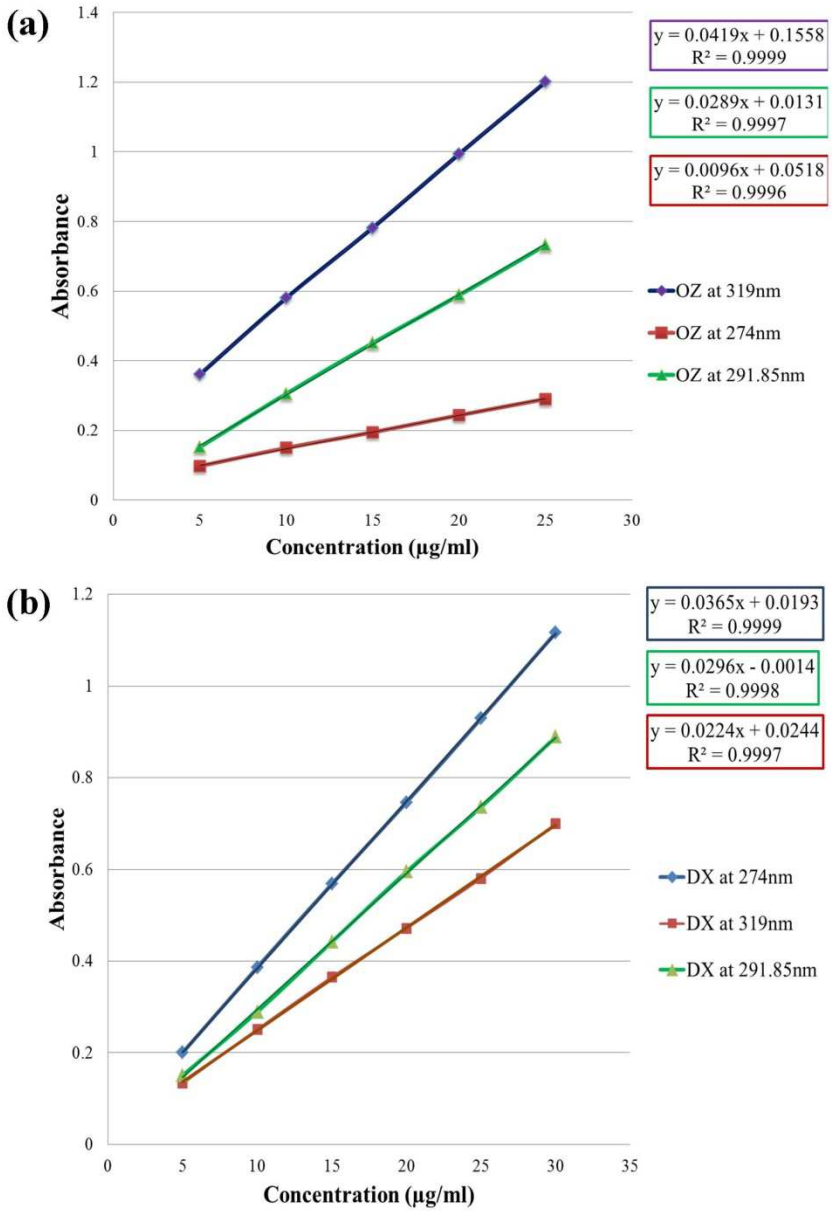

Figure 7: Calibration curve of a) OZ and b) DX at 319, 274 and 291.85 nm

The values of regression coefficients (R^2) and linear equations at the corresponding wavelengths were determined from the calibration curve. The regression equation for OZ was calculated as y=0.0428x+0.0236 (R^2=0.9998), y=0.289x+0.0131 (R^2=0.9997) and y=0.0173x+0.0076 (R^2=0.9994) at 319nm, 292nm and 274nm respectively. The regression equation for DX was calculated as y=0.0365x+0.0193 (R^2=0.9999), y=0.0296x+0.0014 (R^2=0.9998) and y=0.0224x+0.0244 (R^2=0.9997) at 274 nm, 292nm and 319 nm respectively (Fig. 7). The values of R^2 were more than 0.999 indicating very good linearity. The overlay spectra of both the drugs showed iso-absorptive point at 292nm, which falls in between the absorption maxima of both drugs (Fig. 4). The absorptivity values A (1%, 1cm) and molar absorptivity for OZ and DX were determined at selected wavelengths from the corresponding calibrations curves and are shown in Table 3.

Table 3. Absoptivity and Molar absorptivity of drugs at respective wavelengths

Optical parameters	Ornidazole			Doxycycline		
	319nm	274nm	292nm	274nm	292nm	319nm
Absorptivity	468.88	142.84	299.73	372.17	233.17	296.33
Molar absorptivity (L/mole/cm)	1.02×10^4	3.14×10^4	1.02×10^4	1.65×10^4	1.04×10^4	1.31×10^4

For simultaneous equation method wavelengths selected for quantitation were 319nm (λ_{max} of OZ) and 274nm (λ_{max} of DX). However for Q-analysis method iso-absorptive point at 292nm and 319nm (λ_{max} of OZ) was selected for analysis.

Validation of the proposed methods was carried out [23]. Intra-day and inter-day accuracy was calculated and reported in terms of % recovery of drugs from prepared mixture. The average intra-day % recovery was calculated as 99.086 % and 100.32 % (Method I), 100.18 % and 100.33% (Method II) for OZ and DX respectively which indicates high accuracy of both the methods (Table 4 and 5).

Table 4. Data of Intraday recovery and precision studies by Method I and Method II

Theoretical concentration (µg/ml)		Calculated Concentration (µg/ml±SD, n=6)		Intraday % recovery		Intraday Precision (%RSD)	
		Method I	Method II	Method I	Method II	Method I	Method II
OZ	5	4.91±0.091	5.04±0.072	98.21	100.80	1.835	1.428
	10	10.18±0.177	9.95±0.137	101.80	99.50	1.739	1.376
	15	14.89±0.123	15.04±0.113	99.26	100.26	0.826	0.751
DX	5	4.95±0.066	4.89±0.069	99.00	97.80	1.333	1.411
	10	10.13±0.085	10.19±0.143	101.30	101.70	0.839	1.403
	15	15.1±0.186	14.71±0.131	100.67	98.07	1.232	0.891

Table 5. Data of Interday recovery and precision studies by simultaneous equation and Q-absorption ratio methods

Theoretical concentration (µg/ml)		Calculated Concentration (µg/ml±SD, n=6)		Interday % recovery		Interday Precision (%RSD)	
		Method I	Method II	Method I	Method II	Method I	Method II
OZ	5	5.06±0.091	5.05±0.082	101.2	101.00	1.83	1.6237
	10	9.82±0.177	9.88±0.159	98.2	98.80	1.77	1.6093
	15	14.87±0.163	14.94±0.163	99.13	99.60	1.09	1.0910
DX	5	4.96±0.0.09	5.10±0.082	99.2	102.00	1.80	1.6078
	10	10.17±0.134	10.01±0.092	101.7	100.10	1.34	0.9190
	15	15.16±0.212	15.29±0.136	101.07	101.93	1.41	0.8894

Table 6. Summary of validation parameters for Method I and Method II

Methods	Parameters	Ornidazole	Doxycycline
Method I	Intraday Precision	0.9678	1.2522
	Interday Precision	1.2022	1.3890
	Linearity range (µg/ml)	5-25	5-30
	Accuracy (%Recovery)	Intra-99.96 Inter-99.84	100.20 100.15
	LOD (µg)	0.1635 at 319 nm 0.1014 at 274 nm	0.4062 at 319 nm 0.1023 at 274nm
	LOQ (µg)	0.4956 at 319 nm 0.3072 at 274 nm	1.2311 at 319 nm 0.3099 at 274 nm
Method II	Intraday Precision	0.7670	1.0715
	Interday Precision	1.0698	1.2029
	Linearity range (µg/ml)	5-25	5-30
	Accuracy (%Recovery)	Intraday-100.63 Interday-100.44	Intraday-100.50 Intraday-100.75
	LOD (µg)	0.1460 at 292 nm	0.1563 at 292 nm
	LOQ (µg)	0.4431 at 292 nm	0.4738 at 292 nm

The inter-day recovery studies have given satisfactory results with an average percentage recovery of 98.71 % and 100.65 % (Method I), 99.80 % and 101.34% (Method II) for OZ and DX respectively. The intra-day and inter-day precisions were determined and expressed as %RSD. The intra-day precisions of six replicates on the same day for all concentrations were < 2.00 for both the methods. The inter-day precisions on 3 consecutive days were ≤ 1.3 % for all concentrations for both the methods. From Tables 4 and 5, it appears that both precision and accuracy were within acceptable limits for routine drug analysis (15%). The obtained % RSD indicates good precision and sufficient sensitivity for the analysis of OZ and DX using both the methods. For UV Spectrophotometric method the

LOD and LOQ values obtained indicate drugs in microgram concentration can be quantified accurately. Table 6 gives the summary of validation parameters for both methods I and II.

Analysis of prepared synthetic mixture was performed at different concentration ratios of OZ and DX. The % recovery and %RSD were calculated and are shown in Table 7. All the values were within limit indicating suitability of both methods for the simultaneous estimation of OZ and DX.

Table 7. Analysis of synthetic mixture of ornidazole and doxycycline

Composition (µg)		Amount recovered (µg±SD)				% Recovery				%RSD			
		Method I		Method II		Method I		Method II		Method I		Method II	
OZ	DX	OZ	DX	OZ	DX	OZ	DX	OZ	DX	OZ	DX	OZ	DX
5	10	5.07±0.089	10.19±0.073	4.97±0.084	9.87±0.105	101.40	101.90	99.40	98.70	1.755	0.716	1.690	1.064
10	15	10.1±0.166	15.2±0.227	10.17±0.127	14.86±0.136	101.00	101.33	101.70	99.07	1.643	1.493	1.248	0.915
15	20	14.87±0.121	19.56±0.211	15.17±0.103	19.89±0.17	99.13	97.8	101.13	99.45	0.813	1.078	0.678	0.864
20	25	19.68±0.106	24.89±0.185	19.78±0.134	25.09±0.105	98.40	99.56	98.90	100.36	0.538	0.743	0.677	0.418

CONCLUSION

OZ and DX are compatible drugs and can be formulated in single dosage form due to coverage of broad range of microorganisms. FTIR studies showed absence of any possible interaction between both drugs. Developed UV method offers substitution of chromatographic methods which are more expensive and time consuming and cannot be used for routine analysis. Moreover, the developed method is rapid, simple, economic, reproducible, sensitive and specific for routine quality control analysis as compared to available methods. The proposed methods are highly accurate, selective and precise hence can be used for quantitative simultaneous assay of drugs in combination.

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