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Quantative Estimation and Validation of Ofloxacin and Ornidazole in Tablet Dosage Form by Hydrotropic Solubilization Phenomenon

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ABSTRACT

Three new, simple, accurate, cost effective, safe, sensitive spectrophotometric methods have been developed for simultaneous estimation ofloxacin and ornidazole in tablet dosage form by using aqueous solution of 2.0 M sodium benzoate solution, as a hydrotropic agent. Sodium benzoate solution and additives of tablet did not interfere in analysis, as sodium benzoate did not show any absorbance above 300nm. In 2.0M sodium benzoate solution, ofloxacin and ornidazole shows maximum absorbance at wavelength 332 nm and 320 nm respectively and isobestic point was observed at 303nm. Beer's law was obeyed in the concentration range 5-35 µg/ml for ofloxacin and 5-40µg/ml for ornidazole. Method-I is based on simultaneous equation method and method II is based on determination of Q-value. Method-III involves estimation of both drugs by two wavelength method, where difference in absorbance at 332 and 307nm (difference was zero for ornidazole) were used for estimation of ofloxacin and difference at 320 and 341nm(difference was zero for ofloxacin) were used for estimation of ornidazole. Results of analysis for methods were tested and validated for various parameters according to ICH guidelines, hence can be adopted for the routine analysis of ofloxacin and ornidazole in tablet dosage form.

Keywords: Hydrotropic Solubilization, Ofloxacin, Ornidazole, Simultaneous equation method, Absorbance ratio method, Dual wavelength spectrophotometry

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INTRODUCTION

Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs such as alteration in pH of solvent, co-solvents, complexation etc. Hydrotropic Solubilization is one of them employed for estimation[1]. The term "hydrotrophy" originally put forwarded by Newberg to describe the increase in the solubility of the solute by the addition of fairly high concentration of alkali metal salts of various organic acids. According to Newberg, the solublizing agent, are anionic organic salts. Saleh and El-Khordagui made an attempt to extent the definition of the term hydrotropic agent to included cationic and nonionic organic compounds bearing the essential structural features of Newberg's hydrotropes. Model planar cationic compounds such as p-aminobenzoic acid hydrochloride, procaine hydrochloride and neutral molecules such as resorcinol and pyrogallol confer typical hydrotropic properties.

Winsor considered hydrotrophy as a solubilization phenomenon, with the solute dissolved in oriented clusters are the hydrotropic agents.

The mechanism of this effect is still not clear. Some workers believe that the mechanism is complexation with a weak interaction existing between the hydrotropic agent and the solute. However, complexation is not able to explain all hydrotropic systems, still other reason the phenomenon must be due to a change in solvent character because of the large amount of additive particularly at hydrotropic concentration (i.e. >1M).

Sodium salicylate, sodium benzoate, sodium lauryl sulphate, sodium glycinate, sodium gentisate, nicotinamide, urea sodium acetate, sodium citrate and, niacinamide have been employed as a hydrotropic agent.

Literature survey revealed that many methods are available for the determination of ofloxacin and ornidazole in combination with other drugs[2-4] and for combination[5-7]. Till now simultaneous equation absorbance ratio and dual wavelength spectrophotometry are not explained for this combination using hydrotropic solubilization phenomenon as these methods are simple, accurate, cost effective, safe and sensitive.

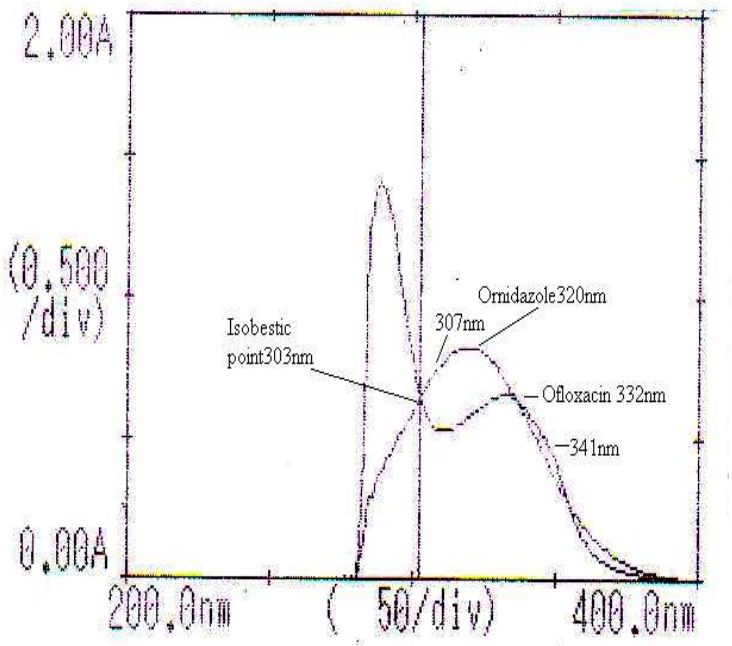
MATERIALS AND METHODS

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells is used. Pure sample of ofloxacin was obtained as gift samples from M/s Alkem Laboratories Limited, Mumbai, India and ornidazole was obtained as gift samples from Zest Pharma,275,Sector-F,Sanwer Road, Indore. The commercially available tablets, Oflomac-OZ (Label claim: Ofloxacin-200mg, ornidazole- 500mg) is procured from local market.

Preparation of standard stock, calibration curve and binary mixture solutions

The standard stock solutions of ofloxacin and ornidazole were prepared by dissolving 50mg of each drug in 40ml of 2.0M sodium benzoate solutions and final volume was adjusted with distilled water in 100ml of volumetric flask. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of each drug.

Working standard solutions were scanned in the entire UV range of 400-200 nm to determine the λ max of both drugs. The λ max of ofloxacin and ornidazole were found to be 332 nm and 320nm respectively and from overlain spectra (Fig.1)



Figuer-1 Overlain spectra of ofloxacin and ornidazole

It is evident that isobestic point was obtained at 303 nm. Eight working standard solutions for both drugs having concentration 5, 10, 15, 20, 25, 30,35, 40µg/ml were prepared in distilled water from stock solution. The absorbances of resulting solutions for both drugs were measured at 320, 332nm for method I, 303,332nm for method II and absorbance difference at 332,307nm for ofloxacin and 320,341nm for ornidazole were calculated for method III and plotted a calibration curve against concentration to get the linearity and regression equation of both drugs.

Six mixed standards solutions with concentration of ofloxacin and ornidazole in µg/ml of 30:5,25:10, 20:15,15:20,10:25,5:30 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions.

Method-I (Simultaneous equation method)

Simultaneous equation method of analysis was based on the absorption of drugs (ofloxacin and ornidazole) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations was 332 nm and 320 nm, λmax of ofloxacin and ornidazole respectively. The absorbances of both the drugs were measured at 332 nm and 320 nm. The absorptivity values E (1%, 1cm) determined for ofloxacin at 332 nm and 320nm were 325(ay_2) and 287 (ay_1) while respective values for ornidazole were 356 (ax_2)and 411(ax_1). These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$C_{ofloxacin} = \frac{(A_1 x356) - (A_2 X\,411)}{-31403} \text{.....Eqn.1} \quad C_{ornidazole} = \frac{(A_2 x287) - (A_1 x325)}{-31403} \text{.....Eqn.2}$$

Where $C_{ofloxacin}$ and $C_{ornidazole}$ are concentration of ofloxacin and ornidazole respectively in g/100mL. A_1 and A_2 are the absorbance of the mixture at 320nm & 332 nm respectively.

$$A_1 = 411xC_{ornidazole} + 287xC_{ofloxacin} \text{ and } \quad A_2 = 356xC_{ornidazole} + 325xC_{ofloxacin}$$

Method-II (Absorbance ratio method)

Absorbance ratio method of analysis was based on the absorbance's at two selected wavelengths, one of which is an iso-bestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig.1) 303 nm (Iso-bestic point) and 332 nm (λmax of ofloxacin) were selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorbances of both drug measured at 303 and 332 nm. The absorptivity values E (1%, 1cm) determined for ofloxacin at 332 and 303nm were 325(ax_2) and 312.11(ax_1) while respective values for ornidazole were 356(ay_2) and 323.30(ay_1). These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$C_{ofloxacin} = \frac{Q_M - 1.1011}{-0.0599} x \frac{A_1}{312.11} \text{.....Eqn.3} \qquad C_{ornidazole} = \frac{Q_M - 1.0412}{0.0599} x \frac{A_1}{323.30} \text{.....Eqn.4}$$

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1},\ Q_X = \frac{ax_2}{ax_1} = 1.0412,\ Q_Y = \frac{ay_2}{ay_1} = 1.1011$$

Where $C_{ofloxacin}$ and $C_{ornidazole}$ are concentration of ofloxacin and ornidazole respectively in g/100mL. A_1 and A_2 were the absorbance of the sample at 303 nm and 332 nm respectively, and $A_1 = 312.11xC_{ofloxacin} + 323.30xC_{ornidazole}$ and $A_2 = 325xC_{ofloxacin} + 356xC_{ornidazole}$

Method-II (Dual wavelength spectrophotometry)

In this method, two wavelength were selected for each drug in a way so that the differrnce in absorbance is zero for one drug at a time. As per (Fig.1) the spectrum of ofloxacin showed that the absorbance of ofloxacin is identical at 320 and 341nm, so these two wavelength were selected for analysis of ornidazole and same as in 332 and 307nm absorbance of ornidazole were identical, so these two wavelength were selected for analysis of ofloxacin. All the mixed standards were scanned at these selected wavelength separately using quantitative mode of the instrument and the values of difference in absorbance were extrapolated in the regression equation to get the concentration. The absorbances difference at respected wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$C_{ofloxacin} = \frac{Y_1 - 0.0056}{0.0051} \text{..... Eqn.5} \qquad C_{ornidazole} = \frac{Y_2 - 0.0064}{0.0132} \text{.....Eqn.6}$$

Where $C_{ofloxacin}$ and $C_{ornidazole}$ are concentration of ofloxacin and ornidazole respectively in µg/ml. Y_1 and Y_2 were absorbance difference between 332,307nm and 341,320nm respectively.

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of ornidazole was transferred to 100 ml volumetric flask and dissolved in 40 ml of 2.0M sodium benzoate with frequent shaking for 15minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of ornidazole and corresponding concentration of ofloxacin. This solution contains ofloxacin and ornidazole in the proportions of 1:2.5 For three methods- 2.0 ml of solution was transferred in 10ml volumetric flask and diluted with distilled water to obtain final concentration of 8µg/ml of ofloxacin and 20 µg/ml of ornidazole For method –I absorbance of the sample solution viz. A_1 and A_2 were recorded at 320 and 332 nm respectively and concentration of two drugs in the sample were determined using Eqn.1 and 2. For method-II, absorbances of the sample solution viz. A_1 and A_2 were recorded at 303nm (iso-bestic point) and 332nm, λmax of ofloxacin respectively and ratio of absorbance were calculated viz. A_2/A_1. Concentration of two drugs in the sample were calculated using Eqn.3 and 4. For method-III absorbance of the sample solution is recorded at 332,307nm for analysis of ofloxacin and 341,320nm for analysis of ornidazole. Difference was calculated and concentration of ofloxacin and ornidazole is calculated by using regression Eqn 5 and 6. Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation was reported in Table 2.

VALIDATION[8]

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 2.

Precision

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The result of statistical evaluation are given in Table 2.

Intermediate Precision- (Inter-day and Intra-day precision)

The inter-day and intra-day precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The result of the same are presented in Table 3.

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method-I,II and III, the Beer- Lambert's concentration range was found to be 5-35 µg/ml for ofloxacin and 5-40 µg/ml for ornidazole. The linearity data for both methods are presented in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ of ofloxacin and ornidazole by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISSCUSION

Linearity range for ofloxacin and ornidazole were found to be 5-35 µg/ml and 5-40 µg/ml at respective selected wavelengths and coefficient of correlation were found 0.9999, 0.9999, 0.9991,0.9991 for ofloxacin at 320,332,303,332-307nm and 0.9996, 0.9996, 0.9983,0.9987 for ornidazole at 320,332,303,341-320 nm respectively (Table 1).

Table 1: Optical Characteristics Data of Ofloxacin and Ornidazole for Method-I, II and III

Parameters	Values							
	OF	OF	OF	OF	OR	OR	OR	OR
Working λ in 2.0M sodium benzoate solution	320nm	332nm	303nm	332-307nm	320nm	332nm	303nm	341-320nm
Beer's law limit (µg/ml)	5-35	5-35	5-35	5-35	5-40	5-40	5-40	5-40
Absorptive E(1%,1cm)*	287	325	312.11	-	411	356	323.30	-
Correlation coefficient*	0.9999	0.9999	0.9991	0.9991	0.9996	0.9996	0.9983	0.9987
Intercept*	0.0002	0.0027	0.0002	0.0056	0.0035	0.0014	-0.0138	0.0064
Slope*	0.0288	0.0324	0.0313	0.0051	0.0411	0.0358	0.033	0.0132

*OF: Ofloxacin, OR: Ornidazole, *Average of six estimation*

Percentage estimation of both drugs was found in tablet dosage form were 99.60 and 99.20 in method I, 100.10and 98.85 in method II and 100.12 and 100.025 for ofloxacin and ornidazole respectively with standard deviation <2 (Table 2).

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.	Amount Added at (%)	Amount Added mg ml^{-1}	% Recovery #
I	OF	200	198.671	99.60	0.5083	0.5103	0.2075	80	160	99.89
								100	200	99.00
								120	240	100.02
	OR	500	493.879	99.20	0.2990	0.3014	0.1220	80	400	98.98
								100	500	98.50
								120	600	99.50
II	OF	200	199.250	100.10	1.2626	1.2613	0.5154	80	160	99.50
								100	200	98.95
								120	240	100.04
	OR	500	491.928	98.85	0.2010	0.2033	0.0820	80	400	99.60
								100	500	98.60
								120	600	99.00
III	OF	200	199.343	100.12	1.2323	1.2307	0.5031	80	160	100.05
								100	200	100.02
								120	240	99.50
	OR	500	497.735	100.02	0.6926	0.6924	0.2827	80	400	99.50
								100	500	98.50
								120	600	100.25

OF: Ofloxacin, OR:Ornidazole, S.D.: Standard deviation,
*COV: Coefficient of variation, S.E.: Standard error,*Average of six estimation of tablet formulation,*
 # Average of three estimation at each level of recovery

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods are in good agreement with their respective label claims, which suggested non interference of formulation additives and hydrotropic solubilizing agent sodium benzoate in estimation.(Table-2)

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for ofloxacin and tinidazole. The results were mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %COV were not more than 2.0% indicates good repeatability and intermediate precision (Table 3).

Table 3: Validation Parameters

Method	Drug	LOD*µg/ml	LOQ*µg/ml	Precision (% COV)			
				Intraday n=6	Interday*		
					First day	Second day	Third day
I	OF	0.1576	0.4778	0.6495	0.9058	0.5918	0.6884
	OR	0.1255	0.3805	0.9424	0.7321	0.9320	0.7773
II	OF	0.1576	0.4778	0.7807	0.9850	0.5031	0.5097
	OR	0.0808	0.2449	0.7135	0.5918	0.6629	0.5929
III	OF	0.0426	0.1292	0.6886	0.7272	0.6910	0.6522
	OR	0.0347	0.0105	0.5172	0.6908	0.5459	0.7667

OF: Ofloxacin, OR: Ornidazole, COV: Coefficient of variation,
** Average of six determination.*

The value of LOD and LOQ were 0.1576µg/ml, 0.4778 µg/ml for ofloxacin and 0.1255 µg/ml, 0.3805for ornidazole in method I, 0.1576 µg/ml, 0.4778 µg/ml for ofloxacin and 0.0808 µg/ml, 0.2449 µg/ml for ornidazole in method II,0.0426 µg/ml,0.1292 µg/ml for ofloxacin and 0.0347 µg/ml,0.0105 µg/ml for ornidazole respectively.

CONCLUSION

The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of ofloxacin and ornidazole in tablet dosage form by simultaneous equation method, absorbance ratio method and Dual wavelength spectrophotometry. Both drugs showed good regression values at their respective wavelengths and the results of recovery study reveled that any small change in the drug concentration in the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of

proposed methods. Hence proposed methods are new, simple, cost effective, accurate, sensitive, free from pollution and precise and can be adopted for routine analysis of ofloxacin and ornidazole in tablet dosage form. Further, as sodium benzoate does not absorb above 300 nm, a large number of drugs having λmax above 300 nm can be used for estimation by proposed methods.

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