Simultaneous Spectrophotometric Estimation of Gatifloxacin and Ornidazole in Mixture

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Two simple, rapid, accurate and economical methods have been developed for the estimation of gatifloxacin and ornidazole in the mixture. Gatifloxacin has absorbance maxima at 286.2 nm and ornidazole has absorbance maxima at 319 nm in distilled water. The linearity was observed in the concentration range of 2-14 μg/ml for gatifloxacin and 2-20 μg/ml for ornidazole. First method is based on the simultaneous equations and second method is based on Q-absorbance ratio. Absorbances at isoabsorptive point 299.2nm and at the λ-max of ornidazole. These methods were validated statistically. The recovery studies confirmed the accuracy of the proposed methods.

Gatifloxacin (GAT), 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxilic acid[1] has broader spectrum of antibacterial activity than the older fluoroquinolones and shows good activity against gram +ve and gram –ve microorganisms[2]. Ornidazole (ORN), 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole[1] is used as an antiinfective agent. ORN is used in combination with other fluoroquinolone in the treatment of PID and intraabdominal infection[8]. Gatifloxacin is the newer fluoroquinolones so in future its combination with ORN may be used for the same purpose. These two drugs are not official in any pharmacopoeia; hence no official method is available for the estimation of GAT and ORN in formulations. HPLC[3] and LC/ESI-MS/MS[4] methods have been reported for the estimation of GAT in dosage forms and from human plasma. HPLC[5], chemiluminescence[6], HPTLC (www.camag.com) and Spectrophotometric[7] methods have been reported for the estimation of ORN alone as well as in combination. In this study, two simple, rapid, accurate and economical methods have been developed for simultaneous estimation of GAT and ORN in combinations. GAT (100 mg) and ORN (100 mg) were accurately weighed and sonicated and dissolved in distilled water to give stock solution having concer ration of 1000 μg/ml. From these stock solutions, working standard solutions having concentration 20 μg/ml each were prepared by appropriate dilutions. Working standard solutions were scanned in the entire UV range to determine the λ-max. The λ-max of GAT and ORN were found to be 286.2 nm and 319 nm, respectively. Six standard dilutions of each drugs were prepared having concentrations of 2, 4, 6, 8, 10, 12 and 14 μg/ml for GAT and 2, 4, 8, 12, 16 and 20 μg/ml for ORN separately from the working standard. The absorbances of these standard solutions were measured at 286.2 nm and 319 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve. Two simultaneous equations were formed using these absorptivity coefficient values.

$A_1=756 \times C_G+200 \times C_O$ $A_2=293 \times C_G+415 \times C_O$, where C_G and C_O are concentration of GAT and ORN, respectively in g/100 ml in the sample solution. A_1 and A_2 are the absorbances of the mixture at 286.2 nm and 319 nm, respectively. Solving these two equations the concentration of C_G and C_O can be readily found out.

Absorbance ratio method uses the ratio of absorbances at two-selected wavelengths one at Isoabsorptive point and other being the λ-max one of the two components. From the overlain spectra of two drugs, it is evident that GAT and ORN have isoabsorptive point at 299.2 nm and the λ-max of ORN

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TABLE 1: ASSAY AND RECOVERY STUDIES RESULTS OF GATIFLOXACIN AND ORNIDAZOLE IN SYNTHETIC MIXTURE.

Drug	Label amount (µg/ml)	Method I (n=7)			Method II (n=7)		
		% Found±SD	% Recovery ±SD	% CV	% Found ±SD	% Recovery ±SD	% CV
GAT	4	100±0.75	101±0.35	0.751	100±1.46	101±0.57	1.415
ORN	5	103±1.05	101±1.39	1.022	102±0.51	102±0.96	0.491
GAT	6	101±0.67	98.4±0.34	0.667	99.6±0.98	99.6±2.24	0.971
ORN	7.5	102±0.90	98.4±0.59	0.875	102±0.36	100±0.33	0.347

is at 319 nm. Six standard solutions of each drug having concentration 2, 4, 6, 8, 10 and 12 µg/ml were prepared separately in distilled water and the absorbances at 299.2 nm (isoabsorptive point) and 319 nm (λ-max of ORN) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using equations $C_G=Q_M-Q_Y/Q_X-Q_Y \times A_1/aG_1$, $C_O=A_1/aO_1-C_G$, where A_1 and A_2 are absorbances of mixture at 299.2 nm and 319 nm, and aG_1 and aO_1 are absorptivity of GAT and ORN respectively at 299.2 nm, aG_2 and aO_2 are absorptivity of GAT and ORN respectively at 319 nm and $Q_M=A_2/A_1$, $Q_Y=aO_2/aO_1$ and $Q_X=aG_2/aG_1$.

The synthetic mixture of the combination of both the drugs were prepared in the ratio of 4:5 (GAT:ORN) considering the dosage strength of other fluoroquinolones and imidazoles in combination[9] formulations available in market. From the synthetic mixture a stock solution containing GAT (20 µg/ml) and ORN (25 µg/ml) was prepared. Appropriate dilutions of the stock were prepared and the concentration of GAT and ORN determined. The absorbances at selected wavelengths in both proposed method were recorded. The concentration of GAT and ORN were worked out utilizing the equations developed. The diluted solutions were also used for the recovery studies.

In method I, two wavelengths of respective absorbance maxima i.e. 286.2 nm for GAT and 319 nm for ORN were used for the analysis of the drugs. The criteria for obtaining maximum precision[10], by this method, were calculated and found to be out side the range of 0.1-2.0. In absorbance ratio method (method II), the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength[10], which was fulfilled in case of both these drugs. The two wavelengths used for the analysis of both drugs were 299.2 nm (isoabsorptive point) and 319 nm (λ-max of ORN).

The validation parameters were studied at all the three wavelengths for both the methods. Accuracy was determined by calculating the recovery and the mean was determined. Precision was calculated as repeatability (Standard deviation and relative standard deviation) and inter and intra day variation (%CV) for both the drugs. Both the methods were successfully used to estimate the amounts of GAT and ORN present in the synthetic mixture that was prepared. The results obtained were in well agreement with the corresponding labeled amount (Table 1). By observing the validation parameters, both the methods were found to be specific, accurate and precise. Hence both the methods can be employed for routine analysis of these two drugs in combinations.

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