Simultaneous RP-HPLC Estimation of Gatifloxacin and Ornidazole in Tablet Dosage Forms

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The proposed method is a simple, accurate, precise, specific and rapid method for the simultaneous estimation of gatifloxacin and ornidazole in bulk and tablet formulations. A column in isocratic mode, with a mobile phase consisting of acetonitrile: 0.025 M potassium dihydrogen phosphate buffer (50:50 v/v) with 0.5% v/v of triethylamine and its pH adjusted to 3.0 with glacial acetic acid were used. The flow rate was set at 1.0 ml/min and UV detection was carried out at 300 nm. The retention time of gatifloxacin and ornidazole were 2.89±0.017 min and 4.21±0.022 min, respectively. Linearity of gatifloxacin and ornidazole were found in the range of 2-24 µg/ml and 5-60 µg/ml, respectively. The developed HPLC method was extended for dissolution studies. The dissolution testing was performed at 50 rpm and 100 rpm in 0.1 N HCL as dissolution medium by paddle method.

Key words: Gatifloxacin, ornidazole, simultaneous estimation, RP-HPLC method

Gatifloxacin (GF), a fluoroquinolone derivative, has antimicrobial activity[1-3]. Chemically, GF is 1-cyclopropyl-6-fluro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3 quinolinecarboxylic acid[1,2]. Ornidazole (OZ), a 5-nitroimidazole derivative has antiamoebic and antimicrobial activity[1-3]. Chemically, OZ is 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol[1,2]. GF and OZ are available as a combined tablet dosage form in the ratio of 2:5. The literature revealed HPLC[4-8], HPTLC and UV methods for the analysis of GF and OZ as single component systems. The present report describes a precise, accurate, specific and sensitive RP-HPLC method as per ICH guidelines[9] for the simultaneous estimation of GF and OZ in tablets as well as for application to dissolution testing of tablet formulations.

MATERIALS AND METHODS

High performance liquid chromatograph instrument of Hitachi make pump L–7100, a quaternary gradient system equipped with universal Rheodyne injector with injection volume 20 µl, Hitachi L–7400 UV detector and C–18 column was used. Reference standards of GF and OZ were obtained from Emcure Pharmaceutical Ltd., Pune. Tablets of two different brands, T1 (Diragyl-Nicholas) and T2 (Garnid-Intas) having combination of GF (200 mg) and OZ (500 mg) were used. HPLC grade acetonitrile, water and triethylamine were used for the study. Potassium dihydrogen orthophosphate AR and glacial acetic acid AR grade were used.

Preparation of mobile phase:
Mobile phase was prepared by mixing 500 ml of acetonitrile with 500 ml of 0.025 M KH_2PO_4 buffer and 0.5% v/v triethylamine and its pH was adjusted to 3.0 with glacial acetic acid. The mobile phase was sonicated for 15 min and then it was filtered through a 0.45 µ membrane filter paper.

Preparation of standard stock solution:
About 10 mg GF and 10 mg OZ were accurately weighed and taken in 100 ml volumetric flasks separately and dissolved in the mobile phase. The volume was adjusted upto the mark with mobile phase to give stock solutions of 100 µg/ml of GF and OZ separately.

Preparation of sample solution:
Twenty tablets were weighed and finely powdered. Tablet powder equivalent to 10 mg of GF and 25 mg

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of OZ was transferred to a 100 ml volumetric flask and dissolved in 50 ml of mobile phase. The solution was kept in an ultrasonic bath for 20 min and filtered through 0.22 μ membrane filter paper. The sample solution was further diluted with mobile phase in the ratio of 2:5 containing 8,10 and12 μg/ml of GF and 20, 25, and 30 μg/ml of OZ, respectively.

Chromatographic conditions:

The optimum composition of the mobile phase containing acetonitrile:0.025 M potassium dihydrogen orthophosphate buffer (50:50 v/v) with 0.5%v/v of triethylamine and its pH adjusted to 3.0 with glacial acetic acid was selected as it was found to ideally resolve the peaks of GF and OZ. The flow rate was set to 1.0 ml/min and UV detection was carried out at 300 nm. All determinations were performed at ambient column temperature**.**

Linearity:

Aliquots of standard stock solutions of GF and OZ stock solution were taken in 10 ml volumetric flasks and diluted upto the mark with mobile phase in such a way that final concentrations of GF and OZ were in the range of 2-24 μg/ml and 5-60 μg/ml, respectively. Triplicate injections of 20 μl were made two times for each concentration of each drug separately and chromatographed under the conditions as described above. Evaluation of two drugs was performed with the UV detector set at 300 nm and peak areas were recorded. The plots of peak area Vs respective concentration of GF and OZ were found to be linear in the range of 2-24 μg/ml and 5-60 μg/ml with coefficient of correlation (r^2) 0.9997 and 0.9994 for GF and OZ, respectively.

Specificity:

The specificity of the RP-HPLC method was determined by complete separation of GF and OZ as shown in fig. 1 with parameters like retention time (t_R), resolution (R_S) and tailing factor (T). Here tailing factor for peaks of GF and OZ was less than 2% and resolution was satisfactory. The average retention time±standard deviation for GF and OZ were found to be 2.89±0.0179 and 4.21±0.0228, respectively, for six replicates. The peaks obtained for GF and OZ were sharp and have clear baseline separation.

Precision of the assay[11-13]:

From the standard stock solutions, mixed standards containing GF and OZ in the ratio of 2:5 was

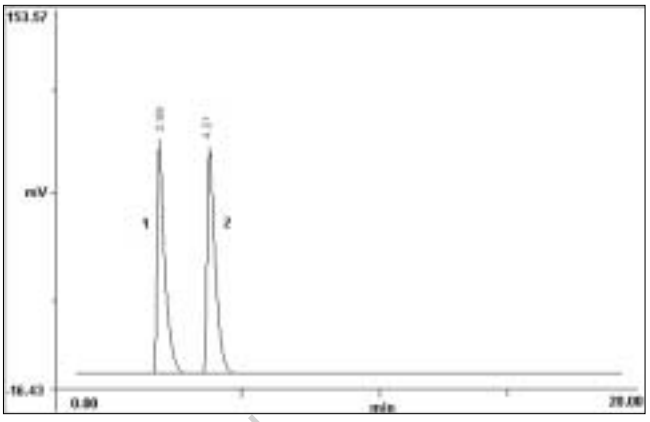

Fig. 1: Typical chromatograms of a mixture of standard GF and OZ
1. indicates peak of gatifloxacin (GF, 10 μg/ml) and 2. indicates peak of ornidazole (OZ, 25 μg/ml)

prepared. Also sample solution was diluted to mixtures containing GF and OZ in the ratio 2:5. Standard and sample solutions (n=6) were injected using a universal Rheodyne injector with injection volume of 20 μl. From the peak area of GF and OZ present in the pure mixture, the amount of each drug present in the samples (n=6) was determined. Statistical evalution of tablet analysis was carried out (Table 1). The intraday and interday precisions were determined and results of which are given in Table 2.

Accuracy:

Recovery studies were carried out by applying the standard addition method. A known amount of standard GF and OZ corresponding to 80%, 100%, and 120% of the label claim was added to preanalysed sample of tablet dosage form separately. The recovery studies were carried out six times, at each level of recovery. The results of studies along with its evaluation are given in Table 3.

Limit of detection (LOD) and limit of quantitation (LOQ)[9,10]:

The LOD and LOQ were separately determined (Table 4) based on the standard calibration curve. The residual standard deviation of the regression line

TABLE 1: STATISTICAL EVALUATION OF TABLET ANALYSIS

Tablet formulation	Drug	% Mean*	±SD	% RSD	SE
T1	GF	100.63	0.1632	0.1621	0.0666
	OZ	100.17	0.1505	0.1502	0.0868
T2	GF	99.88	0.5441	0.5447	0.2221
	OZ	99.83	0.3233	0.3238	0.1320

*Mean of six determinations (n=6) T1 and T2 are two different brands of tablet formulations. GF and OZ denotes gatifloxacin and ornidazole respectively

TABLE 2: INTRA DAY AND INTER DAY PRECISION

Tablet formulation	Drug	Intra day precision* (Mean % ±SD)	Inter day precision* (Mean % ±SD)	% RSD Intra day	% RSD Inter day
T1	GF	99.87±0.10	101.27±0.16	0.098	0.163
	OZ	99.66±0.11	100.95±0.15	0.108	0.145
T2	GF	99.30±0.03	100.98±0.38	0.032	0.376
	OZ	99.63±0.07	100.60±0.19	0.075	0.190

*Mean of six determinations (n=6), T1 and T2 are two different brands of tablet formulations. GF and OZ denotes gatifloxacin and ornidazole respectively

TABLE 3: STATISTICAL DATA FOR RECOVERY STUDIES

Tablet formulation	Drug	Level of % recovery	% mean*	±SD	% RSD	SE
T1	GF	80	100.12	0.2316	0.2313	0.0945
	OZ	80	100.01	0.2065	0.2064	0.0843
	GF	100	100.10	0.3033	0.3029	0.1238
	OZ	100	99.98	0.2004	0.2004	0.0818
	GF	120	100.27	0.2338	0.2338	0.0954
	OZ	120	99.98	0.2658	0.2650	0.1085
T2	GF	80	99.95	0.7436	0.7439	0.3035
	OZ	80	99.73	0.3680	0.3690	0.1502
	GF	100	100.07	0.5278	0.5274	0.2154
	OZ	100	99.80	0.1883	0.1886	0.0768
	GF	120	99.82	0.4262	0.4269	0.1740
	OZ	120	99.97	0.2215	0.2215	0.0904

*Mean of six determinations (n=6) T1 and T2 are two different brands of tablet formulations. GF and OZ denotes gatifloxacin and ornidazole respectively

or the standard deviation of y-intercepts of regression lines may be used to calculate LOD and LOQ. LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

***In vitro* evaluation of tablets[14]:**

In vitro evaluation of tablets containing GF and OZ was performed using dissolution studies. Parameters for dissolution testing (dissolution medium and speed) were optimized using 0.1 N HCL as the dissolution media at 50 rpm as well as 100 rpm using USP apparatus type 2. Two different brands of tablets containing GF and OZ were taken for dissolution testing. Dissolution study of tablets was carried out in 900 ml of 0.1 N HCL, maintained at 37±0.5° at a speed of 50 and 100 rpm. Ten milliliters samples were withdrawn at time intervals of 5, 10, 15, 20, 25 and 30 min. The volume of dissolution media was adjusted to 900 ml by replacing each 10 ml aliquot withdrawn with 10 ml of 0.1 N HCL. From this sampled solution, 1 ml solution was further diluted to 10 ml with mobile phase. The sample solutions were analysed as per the procedure for tablet formulations. The concentrations of GF and OZ in the samples were determined by the proposed RP-HPLC method.

RESULTS AND DISCUSSION

Analysis of tablets containing GF and OZ was carried out by using the optimized mobile phase containing acetonitrile: 0.025 M KH_2PO_4 buffer (50:50 v/v) with 0.5% v/v of triethylamine and its pH adjusted to 3.0 with glacial acetic acid. UV detection was carried out at 300 nm. System suitability tests were carried

TABLE 4: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	Gatifloxacin	Ornidazole
Linearity range (µg/ml)	2-24	5-60
Slope±SD	1010108±2006.47	408456.1±2129.31
Intercept±SD	21293±122.87	46301±492.71
Regression coefficient (r2)±SD	0.9997±0.00016	0.9994±0.00012
Retention time (min.)±SD	2.89±0.017	4.21±0.022
Tailing factor	1.25	1
Resolution factor	-	4.7
Limit of detection (µg/ml)	0.0004	0.004
Limit of quantification (µg/ml)	0.0012	0.012
Theoretical plates	37108.8	113434

TABLE 5: AVERAGE % DRUG RELEASE IN 0.1 N HCL

Speed (RPM)	Time (min)	Average % release of drug* GF T1	GF T2	OZ T1	OZ T2
50	5	23.58	26.73	37.36	37.71
	10	43.23	47.54	64.89	66.35
	15	65.14	77.43	83.32	87.06
	20	86.76	99.14	91.88	99.19
	25	99.64	-	99.32	-
100	5	28.04	31.82	40.18	44.59
	10	53.64	53.77	69.69	73.61
	15	82.14	87.67	89.46	91.57
	20	99.41	99.29	99.68	99.61

*Mean of three determinations (n=3) T1 and T2 are two different brands of tablet formulations. GF and OZ denotes gatifloxacin and ornidazole respectively

out using freshly prepared standard stock solution of GF and OZ and the parameters obtained with 20 µl injection volume are summarized in Table 4. The low % RSD value for intraday and interday precision revealed that the proposed method is robust and rugged. The results obtained by the proposed method were close to the label claim of both drugs. The lower values of % RSD in Table 2 indicate that the method is precise and accurate. The mean percentage recoveries of GF and OZ reveal no interference of excipients in tablets (Table 3). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.0004 µg/ml and 0.0012 µg/ml for GF and 0.004 µg/ml and 0.012 µg/ml for OZ. The LOD and LOQ showed that the method is sensitive for GF and OZ. Two different brands, T1 and T2 of tablets were taken for dissolution studies. Brand T1 showed almost 100% dissolution of GF and OZ in 25 min. at 50 rpm and in 20 min. at 100 rpm while brand T2 showed almost 100% dissolution of GF and OZ in 20 min. at 50 as well as at 100 rpm as shown in Table 5. It was found that brand T2 showed faster dissolution than brand T1. The proposed method is simple, specific, precise and accurate for simultaneous estimation of gatifloxacin and ornidazole in tablets. The developed method can also be applied for dissolution testing of tablet formulations.

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