Reverse Phase High Performance Liquid Chromatography Method for Quantification of Ofloxacin in Tablets

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A rapid, selective and precise HPLC method for quantification of ofloxacin in tablets has been developed. The chromatographic resolution of ofloxacin was achieved using acetonitrile:0.1%v/v triethylamine (20:80, pH-4.0) as the mobile phase, in an isocratic run on a chromatographic system (Waters) equipped with Waters 600 pump controller, 2487 dual λ absorbance detector, Waters[32] millennium chromatography manager software and C8 kromasil 5μ (4.6x150 mm) column. The flow rate was 1 ml/min and ofloxacin was monitored spectrophotometrically at 280 nm. Ornidazole was used as an internal standard.

Ofloxacin (OFX) belongs to fluoroquinolone group of antimicrobial agents. Chemically, it is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo -7H-pyrido(1,2,3-de-)-1,4-benzoxazine-6-carboxylic acid[1] A review of literature shows RP HPLC methods for OFX using C18 column[2-4]. In the present study, a new RP HPLC method for quantification of OFX in tablets has been developed using C8 column. OFX and ornidazole (ORN) were provided as gift samples by Ranbaxy Laboratories Limited, Toansa (Punjab). Acetonitrile, water and triethylamine were of HPLC grade and procured from Qualigens Fine Chemicals, Mumbai. Citizen CX-100 digital balance was used for weighing the materials. A Systronics pH meter model 335 was used for measuring the pH. Chromatographic system was Water 600 pump controller, 2487 dual λ absorbance detector equipped with Waters[32] millennium chromatography manager software. The chromatographic separation of OFX was achieved using kromasil C8 5 μ (4.6x150 mm) column (Flexit Jour Laboratories Private Limited, Pune).

Chromatographic variables were optimized to achieve precise and reproducible separation (Table 1). Standard stock solution of OFX was prepared by dissolving 25 mg of drug in 50 ml of mobile phase. Stock solution of ORN was prepared by dissolving 50 mg of drug in 50 ml of mobile phase to get concentration of 1000 μg/ml. The stock solution of OFX and ORN were mixed to give various dilutions containing OFX (5, 10, 20, 40, 80, 100, 150 μg/ml) and ORN (100 μg/ml). Twenty microlitres of each dilution was injected

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameters	Optimized condition
Chromatograph	Waters millennium 600 pump controller, Dual λ UV detector
Mobile phase	Acetonitrile:0.1 %v/v triethylamine (20:80), pH adjusted to 4.0 with phosphoric acid
Column	Kromasil C 8 (150x4.6 mm), 5 μ
Flow rate	1ml/min
Detection	UV at 280 nm
Injection volume	20 μl
Temperature	Ambient
Retention time of OFX	3.4 to 3.6 min
Retention time of ORN	8.3 to 8.6 min
Run time	10 min

OFX is ofloxacin, ORN is ornidazole

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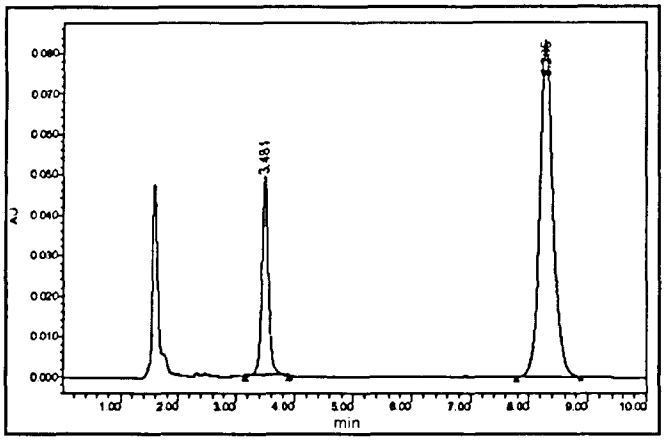

Fig. 1: Chromatogram showing peaks of ofloxacin and ornidazole

into the chromatographic system. OFX was found to elute at 3.4 min and ORN at 8.3 min (fig. 1). The calibration data is given in Table 2. The peak area ratio of OFX to ORN and concentration of OFX was found to exhibit linear correlation (r= 0.999). The plot was linear with the equation of line being, Y=0.054X+0.045. To determine the system suitability, tests were carried out by injecting standard solution, and parameters such as limit of detection (LOD), limit of quantification (LOQ), theoretical plates and tailing factor were determined (Table 3).

TABLE 2: CALIBRATION DATA OF OFLOXACIN

Concentration (μg/ml)		Ratio of AUC of ofloxacin to IS
Ofloxacin	Ornidazole (IS)	
5	100	0.286
10	100	0.559
20	100	1.133
40	100	2.263
80	100	4.437
100	100	5.555
150	100	8.158

All values are averages of three determinations

TABLE 3: RESULTS OF LINEARITY AND SYSTEM SUITABILITY

Parameters	Ofloxacin
Concentration range(μg/ml)	5.0 to150
LOD (μg/ml)	0.2075
Theoretical plates	3503
Tailing factor	0.41
Resolution between the peaks of OFX and IS	8.00

TABLE 4: ANALYSIS OF TABLETS CONTAINING OFLOXACIN AND RECOVERY STUDIES

Pharmaceutical Formulation	Amount of ofloxacin (mg)		Percentage recovery
	Labelled	Found	
Tablet 1 Zenflox (Mankind)	200	198.50	99.25
Tablet 2 Zo (FDC)	200	199.25	99.81

Twenty tablets of OFX were weighed and powdered, powder equivalent to 100 mg OFX was transferred to 100 ml volumetric flask containing mobile phase, vortexed for 25 min and 5 ml of filtrate was pipetted out into a 50 ml volumetric flask. To this was added 5 ml of stock solution of ORN (1000 μg/ml) as internal standard(IS). The solution was filtered and 20 μl of this solution was injected into the HPLC system to obtain a chromatogram and the concentration of OFX corresponding to the ratio of AUC of OFX and AUC of IS in the formulations was calculated from standard graph. Recovery studies were conducted to determine the selectivity, reproducibility and accuracy of the analytical method. A fixed amount of preanalysed sample was taken and standard drug was added, recovery studies gave results between 99.3 to 99.8 % (Table 4). The results of recovery studies indicate that method is accurate. No significant peaks were observed from the tablet excipients. As the mobile phase consists of only 20% acetonitrile, and the run time and flow rate is 10 min and 1.0 ml/min, respectively, the method is rapid and economical.

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