# METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OFLOXACIN AND ORNIDAZOLE IN TABLET DOSAGE FORM BY RP-HPLC

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**Abstract**

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Ornidazole in combination. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70: 30%v/v. The column used was Phenomenex $C_{18}$, (250 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 293 nm. The described method was linear over a concentration range of 5-50 µg/ml and 12.5-125 µg/ml for the assay of Ofloxacin and Ornidazole respectively. Gatifloxacin (50 µg/ml) was used as internal standard. The retention times of Ofloxacin, Ornidazole and Gatifloxacin were found to be 2.1, 2.5 and 5.5min respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Ofloxacin and Ornidazole were found to be 5 and10 µg/ml *10* and 25 µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Ornidazole bulk drug and in its pharmaceutical dosage form.

**Keywords:** Ofloxacin, Ornidazole and Gatifloxacin.

## 1. Introduction

Ofloxacin (OFL) is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin[1] a fluorinated carboxy quinolone, is a racemate, (±)- 9-fluro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine- 6-carboxylic acid. It is official in BP[2], USP[3], and EP[4]. The assay procedure mentioned in these pharmacopoeias uses non aqueous titration for estimation of ofloxacin. Literature surveys reveal Spectrophotometric method atomic absorption spectrometry, spectroflurometry[5-6], HPLC[7] and microbiological method[8] for its determination. Ornidazole (ORN)[1] is a 5-nitroimidazole derivative used as antiinfective agent. It is not official in any Pharmacopoeia. Literature survey reveals that ornidazole is estimated by voltametry[9] and HPLC[10]

methods for its determination in dosage forms and biological fluids. Ofloxacin and ornidazole in combined tablet dosage form is available in the market, has gained increasing acceptance in diarhoea, bacterial and protozoal infections. This paper presents two simple, accurate and reproducible spectrophotometric methods for simultaneous determination of ofloxacin and ornidazole in tablet dosage form. So far, no method has been reported for estimation of OFL and ORN in combined dosage form by HPLC, hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of OFL and ORN in combination, using 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70: 30%v/v. The column used was Phenomenex $C_{18}$, (250 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 293 nm.

## 2. Experimental

### 2.1. Chemicals, reagents and Instrumental Conditions

Standard bulk drug sample Ofloxacin and Ornidazole and Gatifloxacin were provided by Micro Laboratories Ltd., Bangalore. Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC (Double pump) with Rheodyne 7725i type injector with 20µl loop capacity and SPD M20A, Prominence Diode Array Detector. The wavelength of detection chosen was 293 nm. A reverse phase Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) was used for the analysis. The mobile phase comprising of a mixture of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with phosphoric acid in the ratio of 70: 30%v/v with a flow rate of 1ml/min. The injection volume was 20 µL.

### 2.2. Preparation of stock, working standard solutions, and sample solution

A stock solution of OFL and ORN (100 µg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-mL volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 µg/mL. The internal standard solution was prepared by taking 10 mg of gatifloxacin in a 100 ml standard flask. It is dissolved by adding 25 ml of mobile phase, shaken for few minutes to get a clear solution and the final volume was made up to 100 ml. For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10-mL volumetric flasks to get a concentration of 5,10,20,30,40 and 50 µg/ml of ofloxacin, 12.5, 25, 50,75,100 and 125 µg/ml of ornidazole and 50 µg/ml of gatifloxacin (Internal Standard).

### 3.1 Method validation

#### 3.1.1 Linearity

The developed method has been validated as per ICH guidelines[10]. Every 20 µL of the working standard solution of OFL in the mass concentration range of 5 to 50 µg/mL, and that for ORN in the mass concentration range of 12.5 to 125 µg/mL were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of OFL and ORN were obtained by plotting the peak area ratio versus the applied concentrations of OFL and ORN. The linear regression coefficients were found to be 0.9998 and 0.9991for OFL and ORN respectively.

#### 3.2.2 Precision

Repeatability of the method was checked by injecting replicate injections of the solution 10 µg/mL and 25 µg/mL of OFL and ORN respectively and the RSD was found to be 0.326% and 0.435%. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter- day precision). The results obtained for intra-day precision (RSDs) were 0.879 %& 0.945 % respectively, at $n = 6$, for both OFL and ORN. The inter-day precisions (RSDs) were 0.254 % and 0.364 %, respectively, at $n = 6$, for both OFL and ORN.

#### 3.2.3 Accuracy

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, results are shown in Table 1.

Table 1: Recovery Studies of OFL and ORN (n=6)

| Drug | Concentration of Std Solution used (µg/mL) | Concentration of Sample Solution Added (µg/mL) | Amount Found (µg/mL) | % Recovery | % RSD |
|---|---|---|---|---|---|
| OFL | 10 | 10 | 19.90 | 99.50 | 0.324 |
| | 10 | 20 | 30.14 | 100.46 | 0.687 |
| | 10 | 30 | 40.87 | 102.17 | 0.652 |
| ORN | 25 | 25 | 49.69 | 100.53 | 0. 517 |
| | 25 | 50 | 74.82 | 99.38 | 0.674 |
| | 25 | 75 | 99.83 | 99.83 | 0. 285 |

### 3.2.4 Specificity

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both OFL and ORN from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

### 3.2.5 Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

### 3.2.6 LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 $\partial/S$ and 10 $\partial/S$, respectively as per ICH guidelines, where $\partial$ is the standard deviation of the response ($y$-intercept) and $S$ is the slope of the calibration plot.

The results of validation parameters and System suitability parameters were shown in Table 2.

Table 2:

| Validation Parameters | OFL | ORN |
|---|---|---|
| Linearity range ( µg / ml) | 5-50 | 12.5-125 |
| r | 0.9998 | 0.9991 |
| LOD (ng /ml) | 5 | 10 |
| LOQ (ng /ml) | 10 | 25 |
| Intra day (% RSD)* | 0.879 | 0.945 |
| Inter day (% RSD)* | 0.254 | 0.364 |
| Repeatability (% RSD)* | 0.3251 | 0.4250 |
| Accuracy | 99.50 – 102.17 | 99.38-100.53 |
| Peak purity index | 1.0000 | 1.0000 |
| Resolution factor ( $R_s$) | - | 19.618 |
| Asymmetry factor ($A_s$) | 0.95 | |
| No. of theoretical plates (N) | 3059 | 12500 |
| Capacity factor ($K'$) | - | 0.330 |
| High equivalent to theoretical plates (HETP) | 49.029 | 11.999 |
| Tailing factor | 1.149 | 1.140 |
| Separation factor | 7.902 | |

**Each value is a mean of six observations.**

### 3.2.7 Analysis of Formulation

Twenty tablets of OFL and ORN in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 20 mg of OFL and 50 mg of ORN was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then what Mann's filter paper No-41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 10 µg/mL of OFL and 25 µg/mL of ORN, which was falling in the Beer's-Lamberts range with 50 µg/mL internal standard, showed good results and was selected for the entire analysis. The results of tablet analysis ($n$ = 6) were found to be 99.81 and 99.44 for OFL and ORN respectively. From the typical chromatogram of OFL, ORN and Gatifloxacin (Internal standard) it was found that the retention time of OFL was 2.1 min, Gatifloxacin was 2.5 min and ORN was 5.5 min, which were well-resolved peaks with a resolution factor of 3.3 for Gatifloxacin and 19.618 for ORN.

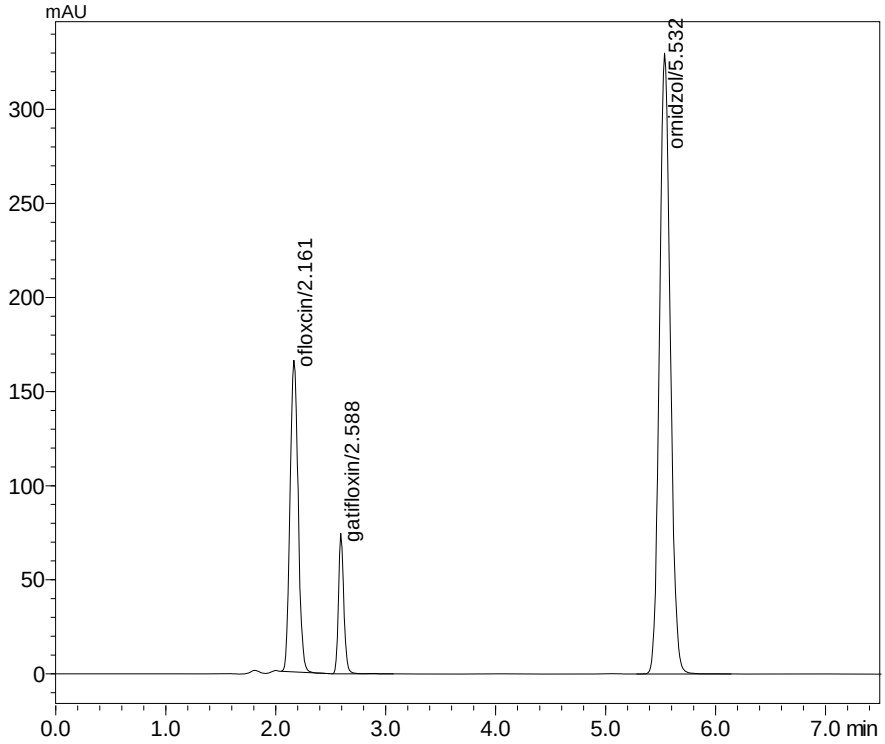


Fig-1 Typical Chromatogram of OFL, Gatifloxacin and ORN in Sample Solution

As there was no interference of impurities and also no change in the retention time, the method was found to be specific and the respective peak purity curve and overlay UV Spectrum were shown in Fig 2, 3, 4 & 5.

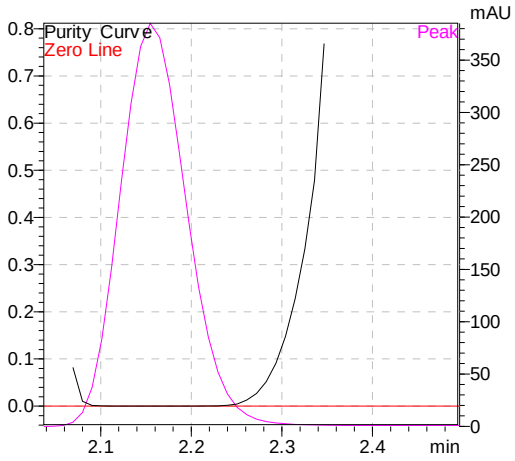


Fig-2 Peak Purity Curve of Ofloxacin

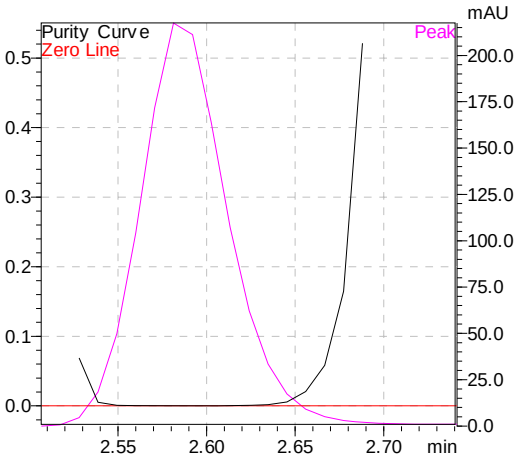


Fig-3 Peak Purity Curve of Ornidazole

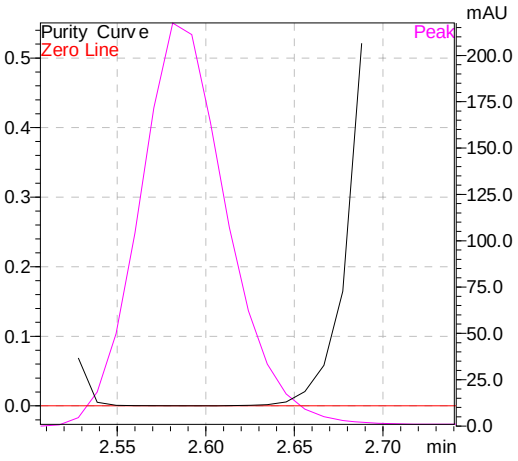


Fig-4 Peak Purity Curve of Gatifloxacin

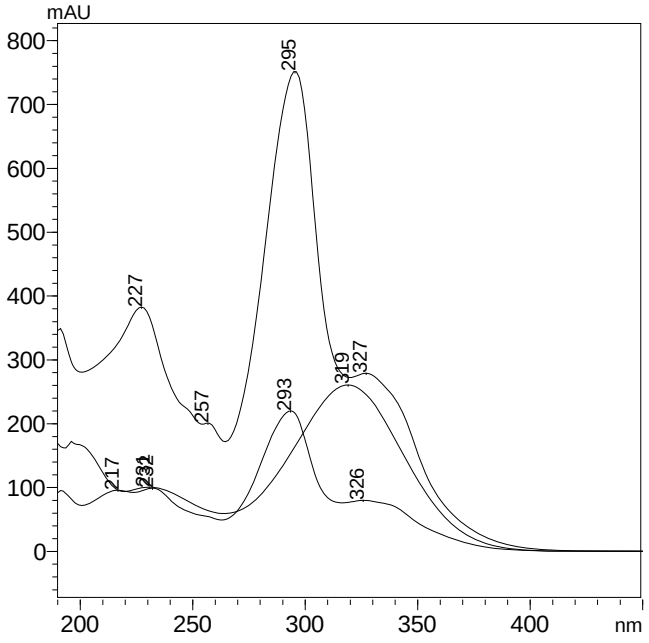


Fig – 5 Overlaid UV Spectrum of OFL, ORN and Gatifloxacin

The results analysis was shown in Table – 3.

Table 3: Analysis of Formulation:

| Drugs | Labelled Amount (mg) | Amount taken for assay ($\mu$g/ml) | *Amount found(mg) | % label claim |
|---|---|---|---|---|
| Ofloxacin | 20 | 10 | 9.981±0.153 | 99.81 |
| Ornidazole | 50 | 25 | 24.86±1.025 | 99.44 |

Each value is a mean of six observations

## 4. Conclusions

The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for OFL and ORN in the concentration ranges of 5–50 µg/mL and 12.5–125 µg/mL respectively. The correlation coefficient for OFL was found to be 0.9998 and that for ORN was 0.9991. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of OFL and ORN estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of OFL and ORN simultaneously, from its pharmaceutical formulations and bulk drug.

## 4. References

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