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RESEARCH ARTICLE

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# SIMULTANEOUS QUANTIFICATION OF OFLOXACIN AND ORNIDAZOLE FROM COMBINED PHARMACEUTICAL DRUG FORMULATION BY HPLC.

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## ABSTRACT

In present study, a successful attempt has been made to develop a simple, sensitive and validated HPLC method for the simultaneous determination of ofloxacin and ornidazole from combined pharmaceutical drug formulation. Chromatographic separation of ofloxacin and ornidazole was achieved on C-18 column Water: ACN: TEA at a wavelength 230 nm. The method was validated in the terms of its linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Linearity of the method was found to be in the concentration range of 4-40 µg/mL for ofloxacin and 10-100 µg/mL for ornidazole with correlation coefficient greater than 0.999 for both the analytes. The total eluting time for the both components is less than eight minutes. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of ofloxacin and ornidazole in combined pharmaceutical drug formulations.

## KEYORDS

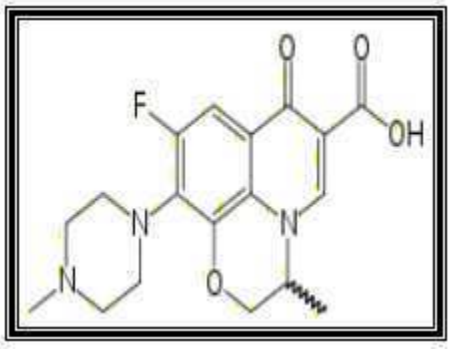
HPLC, Ofloxacin, Ornidazole, Pharmaceutical drug formulations.

## INTRODUCTION

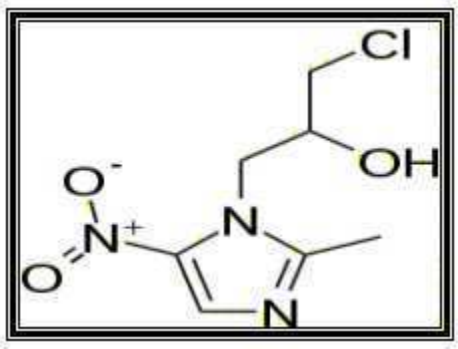
In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are the most common. Drugs with antifungal and antiprotozoal activity have been used in the treatment of the same. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections. Ornidazole, $C_7H_{10}ClN_3O_3$ that is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, is used as an antiprotozoal drug. (Molecular weight:- 219.625 g/mol)

Ofloxacin $C_{18}H_{20}FN_3O_4$ that is (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.$0^{5,13}$]trideca-5(13),6,8,11-tetraene-11-carboxylic acid is used as a antibacterial drugs. (Molecular weight: - 361.368 g/mol). It is highly effective for bacterial and protozoan infections and is available in the tablet form. Few Chromatographic such as HPLC [1-2], HPTLC [3], and Derivative and Extractive spectrophotometric methods [4-7] (Please Check it seems the words are repeated here) have been reported for the simultaneous determination of ofloxacin and ornidazole with other combinations.Very little attention has been paid to the use of electro analytical techniques.[8] A literature survey has revealed very few chromatographic methods. In the present work we have focused on deciding the optimum chromatographic conditions for the simultaneous determination of ofloxacin and ornidazole in combined pharmaceutical drug formulations and successfully developed totally new validated method for the same.

## STRUCTURE




**Ofloxacin**




**Ornidazole**

## MATERIAL AND METHODS

***Chemicals and reagents***

Standard Ofloxacin and Ornidazole were obtained from local pharmaceutical company with claimed purity 99.8%. All the solutions were prepared in double distilled water. All the necessary reagents used i.e. water and Acetonitrile (HPLC grade). Mobile phase was filtered using 0.45 µm cellulose acetate filters made by Millipore whereas; Whatmann's filter paper No.41 (purchased from local market) were used in the preparation of sample solution.

***Apparatus and chromatographic conditions***

The Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump inline vacuum degassing and PDA Detector (at wavelength 230 nm) with Empower 2 software.

| | |
|---|---|
| Chromatographic Mode | Isocratic |
| Column | Inertsil, C18, 150mm length x 4.6 mm ID 5µ particle size |
| Wavelength | 230 nm |
| Column oven temperature | 25°C |
| Sample oven temperature | 25°C |
| Flow rate | 1.0 ml/min |
| Mobile Phase: | Water: ACN: TEA Filter and Degas |

***PRAPARATION OF STANDARD SOLUTION***

Weigh accurately 20 mg of Ofloxacin WS and 50 mg Ornidazole WS, transfer it into a 100 ml volumetric flask, add 30 ml of mobile phase and sonicate to dissolve. Allow it to cool at room temperature, mix well and make up to the volume with mobile phase. The working standard solution 20 µg/mL of ofloxacin and 50 µg/mL of ornidazole were prepared by diluting 5 ml of this solution in to a 50 ml volumetric flask, mix and dilute up to the volume with mobile phase.

***Preparation of sample solution***

Two commercial brands containing of ofloxacin and ornidazole in combination were procured. Each brand contained a label claim of 200 mg of ofloxacin and 500 mg of ornidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder (90 mg) equivalent to 20 mg of ofloxacin and 50 mg of ornidazole was accurately weighed, transferred into 100ml standard flask; add 30 ml of mobile phase and sonicate to dissolve. Allow it to cool at room temperature, mix well and the mixture was vortexed for 10 mins, the solution was filtered through Whatman filter paper no 41.and finally volume of the solution was made up to 100 mL with mobile phase. Appropriate volume of aliquot was diluted with the mobile phase to obtain a solution containing 20 µg/mL of ofloxacin and 50 µg/mL of ornidazole.

***Analytical method validation:*** [9-10]

***System suitability***

System suitability tests are used to ensure reproducibility of the equipment. System suitability has been checked by recording Theoretical plates and Tailing factor for both OF and ONZ which is given in **Table.1**

***Specificity***

The specificity of method was confirmed by observing the chromatograms of both the combined standard solution and the drug sample solutions. The chromatograms obtained from the drugs sample solution were found to be identical to those obtained for standard solution. The addition of the standard solution to the drug sample solution did not change the characteristics of chromatograms. This gives the validity of method for the determination of both the drugs from combined pharmaceutical formulation.

*Lod and loq*

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ of ofloxacin were 0.2µg/mL and 0.6 µg/mL. And ornidazole was found to be 0.5 µg/mL and 1.5 µg/mL respectively is given in Table.1

*Intraday and interday precision*

The intraday and interday precision was used to study the variability of the method. It was checked by recording the chromatograms of sample solutions of ofloxacin and ornidazole at three different levels i.e. 80% ,100% and 120% both at intraday (five times within 24 hour) and interday (two times each. during 3 days intervals) to check the precision. The mean % RSD for intraday and interday precision for ofloxacin found to be less than 1% for both OF and ONZ is given in **Table.1.**

*Linearity and range*

The linearity for ofloxacin and ornidazole was observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 4µg/mL to 40 µg/mL for ofloxacin and 10 µg/mL to 100 µg/mL for ornidazole. The calibration curves were constructed with concentration (C) against peak area. The slope, Intercept, regression equation and correlation coefficient for the OF and ONZ was obtained and it is given in Table.1 and Figure

**Table. 1**

***Method validation parameters for the determination of Ofloxacin and Ornidazole.***

| **Parameters** | **Values** | |
|---|---|---|
| | **Ofloxacin** | **Ornidazole** |
| **System suitability**<br>Theoretical Plates-<br>Tailing Factor- | <br>More than 5000<br>1.4 | <br>More than 8000<br>1.1 |
| Linearity range (µg/mL) | 4 to 40 µg/mL | 10 to 100 µg/mL |
| Slope (m) [a)] | 508,642.9345 | 186,979.70368 |
| Intercept(c) [a)] | 10,430.3471 | 7,585.77054 |
| Correlation coefficient ($R^2$) | 1.0000 | 0.9999 |
| LOD (µg/mL) | 0.2 µg/mL | 0.5 µg/mL |
| LOQ (µg/mL) | 0.6 µg/mL | 1.5 µg/mL |
| Intraday precision (n=5) | 0.79% | 0.62% |
| Interday precision (n=5) | 0.81% | 0.58% |
| Assay | 98% to 102% | 98% to 102% |
| Recovery | 98% to 102% | 98% to 102% |

***Assay***

The developed chromatographic method was used for simultaneous determination of ofloxacin and ornidazole from two different brands of formulations. The sample solutions were analyzed by the developed method described above. Chromatograms were recorded under the optimum experimental conditions. Resulting peak area of ofloxacin and ornidazole were measured and the amount of ofloxacin and ornidazole calculated using already constructed calibration graph. Result of assay studies are given in **Table.2**

**Table 2**

***Result of Assay studies of Ofloxacin and Ornidazole***

| Brand Name | OFNOF (Aristo) | | O2 (Medley) | |
|---|---|---|---|---|
| | **Ofloxacin** | **Ornidazole** | **Ofloxacin** | **Ornidazole** |
| **Labeled claim (mg)** | 200mg | 500mg | 200mg | 500mg |
| **Drug found in mg** | 198.9mg | 499.5 mg | 200.9mg | 501.0 mg |
| **% RSD (n=5)** | 0.520 | 0.751 | 0.68 | 0.89 |
| **% Assay** | 99.45 % | 99.9 % | 100.45 % | 100.2% |

***Robustness***

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition of mobile phase, change in flow rate and change in oven temperature. The chromatographic parameters of each analyte such as retention time, tailing factor, resolution and theoretical plates were measured at each changed conditions.

***Accuracy (recovery)***

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percentage recovery was determined at different levels i.e. from 50% to 150% level. The results of recovery analysis for ofloxacin and ornidazole are shown in **(Table.3).**

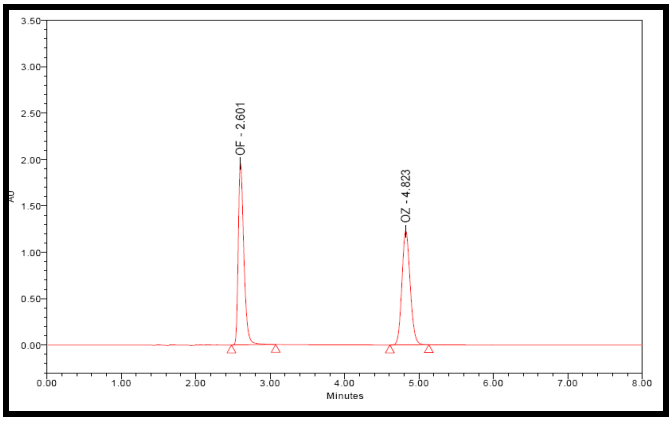
**Table 3**
***Results of Recovery studies of ofloxacin and ornidazole***

| Standard | Level | Amount of Std added | Amount of std Found | RSD (%) (n = 5) | Recovery (% ) |
|---|---|---|---|---|---|
| **Ofloxacin** | 50% | 10.0 | 9.95 | 0.55 | 99.5% |
| | 100% | 20.0 | 20.20 | 0.49 | 101 % |
| | 150% | 30.0 | 30.18 | 0.40 | 100.6% |
| | | | | **Mean** | **100.36%** |
| **Ornidazole** | 50% | 25.0 | 25.12 | 0.51 | 100.5% |
| | 100% | 50.0 | 49.90 | 0.71 | 99.8% |
| | 150 % | 75.0 | 75.67 | 0.38 | 100.9% |
| | | | | **Mean** | **100.40%** |

## RESULT AND DUSCUSSION

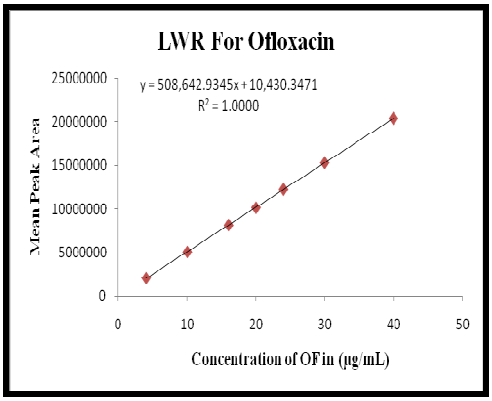
In the present work, conditions were optimized for development and validation of a simple and accurate HPLC method for simultaneous quantification of ofloxacin and ornidzole in combined pharmaceutical drug formulation. Method development was right from optimization of the condition and parameters i.e. selection of system, column, mobile phase, different composition of mobile phases have been tried. But finally Water**:** ACN**:** TEA in the ratio 700**:**300**:**2 is the most appropriate composition because both the components were eluted with good resolution and good peak shape. Under the described experimental conditions, sharp peaks that belong to OF and ONZ were obtained at retention time of 2.6min and 4.8min respectively.**(Figure.1)** The developed chromatographic method was validated using ICH guidelines. A new chromatographic method has been developed and subsequently validated for the simultaneous quantification of ofloxacin and ornidazole from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample, good reproducibility. In addition to this, proposed method is found to be more simple, economic, accurate and practical. Thus, presented method can be recommended for simultaneous determination of ofloxacin and ornidazole in routine quality control analysis in combined drug formulations.

**Figure-1**
***Chromatogram for ofloxacin and ornidazole standard***

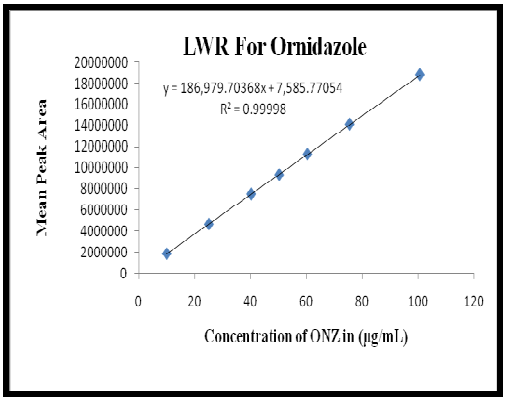



**PDA Result Table**

| | Name | RT | Area | Purity (#1) Angle | Purity (#1) Threshold | Purity Criteria | Match (#1) Spect. Name | Match (#1) Angle | Match Criteria | Match (#1) Threshold |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | OF | 2.601 | 10226510 | | | | | | | |
| 2 | OZ | 4.823 | 9447079 | | | | | | | |




**1. Ofloxacin standard**




**2. Ornidazole standard**

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