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Research Article

# Development and validation of RP-UPLC method for simultaneous estimation of metronidazole and ofloxacin in their combine dosage form

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**ABSTRACT**

This research manuscript describe simple, sensitive, accurate, precise and repeatable RP-UPLC method for the simultaneous determination of Metronidazole (MET) and Ofloxacin (OFL) in suspension dosage form. The sample was analyzed by reverse phase C18 column (Purospher Star 100×2.1 mm, Merck Specialities) as stationary phase and Phosphate Buffer: Acetonitrile (85:15, v/v) as a mobile phase and $P^H$ of 3. 0 adjusted by Triethylamine and ortho-phosphoric acid at a flow rate of 0. 4 ml/min. Quantification was achieved with PDA detector at 318 nm. The retention time for Metronidazole and Ofloxacin was found to be 1.131 and 1.658 minute respectively. The linearity for both the drugs was obtain in the concentration range of 5-35 µg/ml and 2. 5-17.5 µg/ml with mean accuracies 99.73 ± 0.05 and 99.13 ± 0.41 for Metronidazole and Ofloxacin, respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from suspension excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

**Keywords:** Combined dosage forms; Metronidazole; Method validation; Ofloxacin; RP-UPLC

## INTRODUCTION

Metronidazole (MET), an antiprotozoal drug is widely used in treatment of invasive amoebiasis. Chemically it is 2- (2-methyl-5-nitro-1H-imidazol-1-yl) ethanol. (fig. 1) and Ofloxacin (OFL), an antimicrobial drug chemically is (RS)-9-fluoro-3-methyl-10- (4-methylpiperazin-1-yl)-7-oxo-2, 3-dihydro-7H-pyrido[1, 2, 3, -de]-1, 4 benzoazeine-6-carboxylic acid (fig. 2), both drug are official in (Indian pharmacopeia, British Pharmacopeia and United states Pharmacopeia) The combination of MET and OFL is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry (Jadhav G. P., et al., 1998; Maliwal D., et al., 2008; Mashru R. C., et al., 1998;) conductometry (Tuncel M., et al., 1992;) HPLC (Kasabe A. J., et al., 2009; Krishnan., et al., 2002; Argekar A. P., et al., 1996; Zhong L, et al., 2007; Du Y. X., et al., 1994; Xu J., et al 1993; Ohkubo T., et al., 1992;), and LC-MS (Tuerk J., et al., 2006;)have been reported for estimation of MET and OFL in their individual and combined dosage forms with other drugs. There is no reported method for simultaneous estimation of MET and OFL in their combined dosage form by RP-UPLC. This prompted the present work. The aim of the present work is to develop a simple, rapid, accurate and precise RP-UPLC method for simultaneous estimation of MET and OFL in their marketed formulation which is more rapid, sensitive, accurate and precise method than the RP-HPLC method.

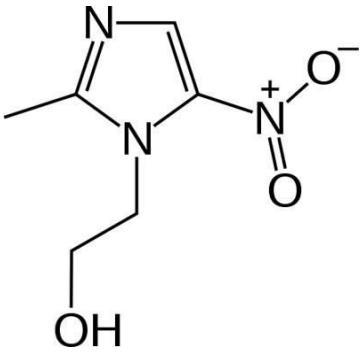


**Figure 1: Structure of Metronidazole**

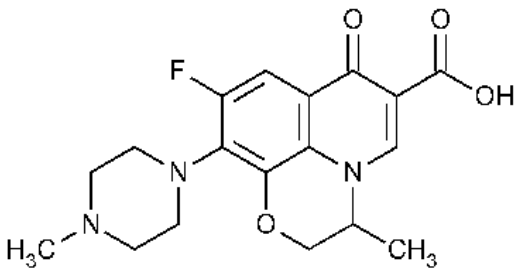


**Figure 2: Structure of Ofloxacin**

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**Table 1: Regression analysis data and summary of validation parameter for the proposed RP-UPLC method**

| Parameters | RP-UPLC method | |
|---|---|---|
| | Metronidazole | Ofloxacin |
| Concentration range (µg/ml) | 5-35 | 2.5-17.5 |
| Slope | 8694 | 23429 |
| Intercept | 2885 | -3149 |
| Correlation coefficient | 0.999 | 0.998 |
| LOD[a] (µg/ml) | 0.260 | 0.025 |
| LOQ[b] (µg/ml) | 0.789 | 0.077 |
| Accuracy | 99.73 ± 0.05 | 99.13 ± 0.41 |
| Repeatability (% RSD[c], n = 6) | 0.229 | 0.414 |
| Precision (%RSD) | | |
| Intraday (*n* = 3) | 0.028-0.18% | 0. 078-0.529% |
| Interday (*n* = 3) | 0.062-0.568% | 0. 148-0.244% |

a=Limit of Detection, b=Limit of Quantitation, c=relative standard deviate

**Table 2: Recovery data for the proposed method**

| Drug | Level | Amount of sample taken (µg/ml) | Amount of Standard spiked (%) | Mean % Recovery ± S. D (n=6) |
|---|---|---|---|---|
| Metronidazole | I | 10 | 80 % | 99. 65±0.18 |
| | II | 10 | 100 % | 100±0.13 |
| | III | 10 | 120 % | 99. 57±0.52 |
| Ofloxacin | I | 5 | 80 % | 99. 58±0.14 |
| | II | 5 | 100 % | 99. 86±0.30 |
| | III | 5 | 120 % | 99. 5±1.0 |

## MATERIALS & METHODS

### Apparatus

The chromatography was performed on a Water (Acquity) RP-UPLC instrument equipped with PDA detector and Em-power 2 software, Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Marck, Germany) was used as stationary phase. Mettler Toledo analytical balance (Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used in the study.

### Reagents and materials

Metronidazole and Ofloxacin bulk powder was obtained from Nirlife, Healthcare division of Nirma. Ahmedabad, India. The commercial fixed dose combination product was procured from the Nirlife. Acetonitrile, Methanol, $KH_2PO_4$ (Finar Reagent, Ahemedabad, India) used were of HPLC grade. Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

### Chromatographic Condition

Seperation was achieved by using Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Marck, Germany) as stationary phase with Phosphate Buffer: Acetonitrile (85:15, v/v) as a mobile phase and $P^H$ of 3.0 adjusted by Triethylamine and ortho-phosperic acid at a flow rate of 0.4 ml/min and detection wavelength was 318nm in PDA detector

### Preparation of mobile phase

0.68 gm Dihydrogen ortho phosphate was weighed accurately in 1000mL volumetric flask. To it add about 70mL of Water and sonicate further make up the volume up to mark with water, further this buffer solution is mix with 150 ml of actonitrile in 1000 ml volumetric flask to make a mobile phase ratio buffer:acetonirile (85:15%v/v) and adjust $p^H$ 3 by using ortho phosphoric acid and triethylamine this mobile phase used as a diluents also was used throughout study

### Preparation of standard stock solution (100µg/mL)

An accurately weighed Metronidazole (10 mg) and Ofloxacin (5 mg) were transferred into two different 100 mL volumetric flask, dissolved in 50 mL methanol and sonicate after this diluted up to mark with methanol to get concentration of Metronidazole (100µg/mL) and Ofloxacin (50µg/mL)

### Preparation of mixed standard working solution (10µg/mL)

Accurately weighed Metronidazole (10 mg) and Ofloxacin (5 mg) were transferred to 100 mL volumetric flask, dissolved in 50 mL methanol and diluted up to mark with methanol to get concentration of Metronidazole (100µg/mL) and Ofloxacin (50µg/mL)

### Preparation of calibration curve

Aliquots (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 ml) of mixed standard working solutions (equivalent to 5, 10, 15, 20, 25, 30, and 35µg/ml of Metronidazole and 2. 5, 5, 7. 5, 10, 12. 5, 15, and 17. 5 µg/ml Ofloxacin, each) were

**Table 3: System suitability test parameters for Metronidazole and Ofloxacin for the proposed RP-UPLC method**

| Parameters | Metronidazole ± RSD (n = 6) | Ofloxacin ± RSD (n = 6) |
|---|---|---|
| Retention time (min) | 1.131 ± 0.253 | 1.65 ± 0.161 |
| Tailing factor | 1.21 ± 0.354 | 1.26 ± 0.225 |
| Theoretical plates | 6302 ± 1.475 | 6857 ± 0.937 |
| Resolution | 7.54 ± 0.71 | |

**Table 4: Analysis of marketed formulation of Metronidazole and Ofloxacin by proposed RP-UPLC method**

| Suspension | Label claim (mg/5ml) | | Amount found (mg/5ml) | | % Label claim ± SD (n=3) | |
|---|---|---|---|---|---|---|
| | Metronidazole | Ofloxacin | Metronidazole | Ofloxacin | Metronidazole | Ofloxacin |
| I | 100.0 | 50.0 | 99.97 | 99.87 | 99.97±0.54 | 99.87±1.03 |

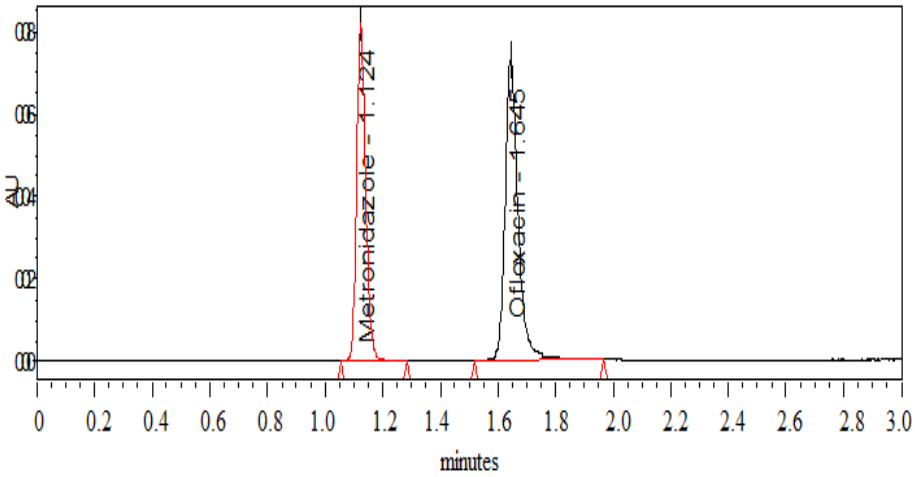


**Figure 3: Chromatogram of mixed standard solution (10.0µg/mL) of Ofloxacin (RT 1.645 min) and Metronidazole (RT 1.124 min) in methanol by RP-UPLC method**

transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with mobile phase. A each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration (fig. 4 and 5 respectively), and the regression equations were calculated. Each response was average of three determinations.

**Preparation of Marketed sample solution for Assay**

For determination of the content of Metronidazole and Ofloxaxcin in marketed suspension (Lable:MET-100mg/5ml and OFL-50mg/5ml). Take 0. 5 ml solution from suspension and transferred to 100 mL volumetric flask, dissolved in mobile phase and sonicated for 30 min. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with mobile phase. The solution was diluted up to the mark with mobile phasel. Accurately measured 2. 0 mL of solution was transferred to 10 mL volumetric flask, diluted up to the mark with mobile phase to get final working concentration of Metronidazole (20 µg/mL) and Ofloxacin (10 µg/mL). A sample solution was injected under the operating chromatographic condition as described above and responses were recorded (fig. 3). The analysis procedure was repeated three times with Suspension formulation.

**METHOD VALIDATION**

The method was validated in compliance with ICH guidelines. (Q 2 B)

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating the recoveries of Metronidazole and Ofloxacin by the standard addition method. Known amounts of standard solutions of Metronidazole and Ofloxacin were at added at 80, 100 and 120 % level to prequantified sample solutions of Metronidazole and Ofloxacin (10 and 5 µg/ml respectively). The amounts of Metronidazole and Ofloxacin were estimated by applying obtained values to the respective regression line equations.

**Method precision (repeatability)**

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Metronidazole and Ofloxacin (20 µg/ml and 10 µg/ml for both drugs) without changing the parameters.

**Intermediate precision (reproducibility)**

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of Metronidazole (10, 15, 20 µg/ml) and Ofloxacin (5, 7.5 and 10 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

**Limit of detection and Limit of quantification**

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

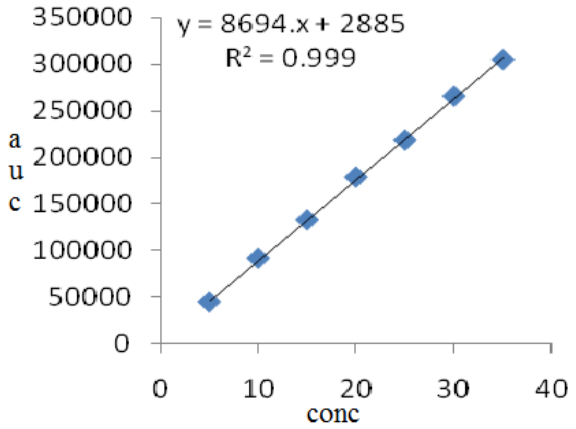


**Figure 4: Linearity of Metronidazole**

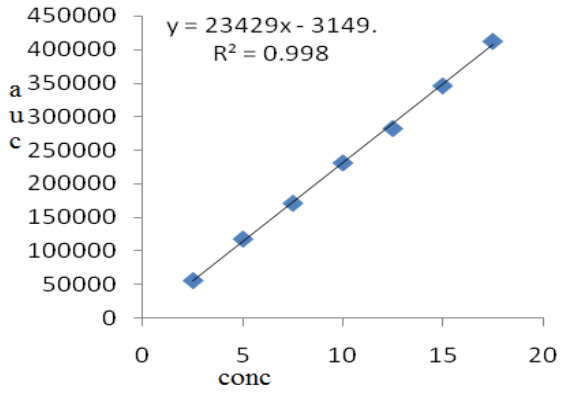


**Figure 5: Linearity of Ofloxacin**

**RESULTS AND DISCUSSION**

To optimize the RP-UPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Metroniidazole and Ofloxacin were obtained with a mobile phase comprising of Phosphate Buffer: Acetonitrile (85:15, v/v) and $P^H$ of 3. 0 adjusted by Triethylamine and orthophosperic acid at a flow rate of 0. 4 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 318 nm based on peak area. The retention time for Metronidazole and Ofloxacin were found to be 1.131 and 1.658 min, respectively (Fig. 3). Linear correlation was obtained between peak area versus concentrations of Metronidazole and Ofloxacin (Fig. 4 and 5 respectively) in the concentration ranges of concentration range of 5-35 µg/ml and 2.5-17.5 µg/ml with mean accuracies 99.73 ± 0.05 and 99.13 ± 0.41 for Metronidazole and Ofloxacin The mean recoveries obtained were 99.73 ± 0.05 and 99.13 ± 0. 41 % for Metronidazole and Ofloxacin, respectively, Table 1 and 2 which indicates accuracy of the proposed method. The % RSD values for Metronidazole and Ofloxacin were found to be <2 %, which indicates that the proposed method is repeatable. The low % RSD values of interday (0. 062-0.568% and 0.148-0.244%) and intraday (0.028-0.18% and 0.078-0.529%) variations for Metronidazole and Ofloxacin, respectively, reveal that the proposed method is precise. LOD values for Metronidazole and Ofloxacin were found to be 0. 26µg/ml and 0.0255µg/ml, respectively and LOQ values for Metronidazole and Ofloxacin were found to be 0.78µg/ml and 0.077µg/ml, respectively (Table 1). These data show that the proposed method is sensitive for the determination of Metronidazole and Ofloxacin. The results of system suitability testing are given in (Table 3). The amount of Metronidazole and Ofloxacin present in the marketed sample solutions were determined by fitting the responses into the regression equations of the calibration curve for Metronidazole and Ofloxacin, respectively and the results obtained were comparable with the corresponding labeled claim (Table 4).

**CONCLUSION**

In this proposed method the linearity is observed in the concentration range of 5-35µg/ml and 2.5-17.5 µg/ml with co-efficient of correlation, ($r^2$) = 0.999 and ($r^2$) = 0.998 for Metronidazole and Ofloxacin, respectively at 318 nm. The proposed method is simple, accurate, precise, specific, sensitive and has ability to separate drug in suspension. The method is suitable for routine analysis of Metronidazole and Ofloxacin in Suspension. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS. The prime importance was given to develop less time consuming and simple RP-UPLC-PDA method. The RP-UPLC method developed meets the system suitability criteria, peak integrity and resolution for the parent drug. Detection and quantification limits achieved, describe the method is very sensitive. High recoveries and acceptable % CV values confirm established RP-UPLC method is accurate and precise. The analytical results demonstrate the ability of the developed method to assay Metronidazole and Ofloxacin. Assay results found from the study show that the method is successfully applied for the analysis of Metronidazole and Ofloxacin in Suspension. Hence, the method is recommended for routine quality control analysis of Metronidazole and Ofloxacin.

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