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A New RP-HPLC for Simultaneous Estimation of Ornidazole and Ofloxacin in Bulk and Its Tablet Dosage Form

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A B S T R A C T

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 Ammonium acetate buffer (pH 3.0 adjusted with ortho phosphoric acid): Acetonitrile in the gradient mode. The column used was Intersil ODS-3V (250 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 254 nm. The retention times of Ofloxacin and Ornidazole were found to be 4.292 and 8.921 min respectively. The method was validated in the terms of its linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. 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.

Keywords: Ofloxacin, Ornidazole, combination, Ammonium acetate buffer, Acetonitrile

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A R T I C L E I N F O

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1. Introduction

Ofloxacin (OFL) is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin (Figure 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 [1]. 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 (Figure 2) used as anti-infective agent. It is not official in any Pharmacopoeia. Literature survey reveals that ornidazole is estimated by voltammetry[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 a simple, accurate and reproducible HPLC method 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.

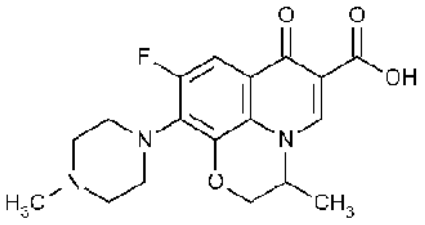

Figure 1: Chemical Structure of Ofloxacin

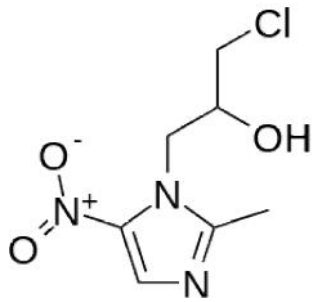

Figure 2: Chemical Structure of Ornidazole

2. Materials and Methods

Chemicals, reagents and Instrumental Conditions

Standard bulk drug sample Ofloxacin and Ornidazole 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 Waters HPLC (Double pump) with Rheodyne 7725i type injector with 20µl loop capacity and SPD M20A, Diode Array Detector. The wavelength of detection chosen was 254 nm. A reverse phase Intersil ODS-3V (250 mm x 4.6 mm i.d, 5µm) was used for the analysis. The mobile phase comprising of a mixture of Ammonium acetate buffer (pH 3.0 adjusted with ortho phosphoric acid): Acetonitrile in the gradient mode. The gradient programming was shown in table 1.The flow rate was 1ml/min. The injection volume was 20 µL.

Table 1: Gradient program for Ofloxacin and Ornidazole

Time(min)	Flow(ml/min)	Buffer%	ACN%
0.01	1 ml/min	70.0%	30.0%
4.00	1 ml/min	70.0%	30.0%
8.00	1 ml/min	35.0%	65.0%
12.00	1 ml/min	35.0%	65.0%
15.00	1 ml/min	70.0%	30.0%
20.00	1 ml/min	70.0%	30.0%

Standard Solution Preparation:

Transfer an accurately weighed quantity of about 60 mg of Ofloxacin working standard and 150 mg of Ornidazole working standard in to 100 ml volumetric flask add 75 ml of diluents and sonicate to dissolve the content, and make up to the volume with diluents and further dilute 5 ml in to 50 ml with diluent, mix.

Sample Solution Preparation:

Accurately weigh and transfer powdered tablet Equivalent to 60 mg of Ofloxacin [285.18 mg] and 150 mg of Ornidazole [285.18 mg] into a 100 ml clean dry volumetric flask add about 75 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluents. Further dilute 5 ml in to 50 ml with diluents, mix.

Assay procedure:

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 60 mg of Ofloxacin [285.18 mg] and 150 mg of Ornidazole [285.18 mg] 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.

Method Validation:

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines [11, 12].

3. Results and Discussion

The chromatograms of standard and sample solutions are presented in Fig. 3 and 4. The accuracy of the method was determined by recovery studies were carried out and the percentage of recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate. The precision procedure was satisfactory. The concentration range from 50-150 µg/ml for Ornidazole and Ofloxacin were examined by the assay procedure and the calibration curves were plotted (Table 2).

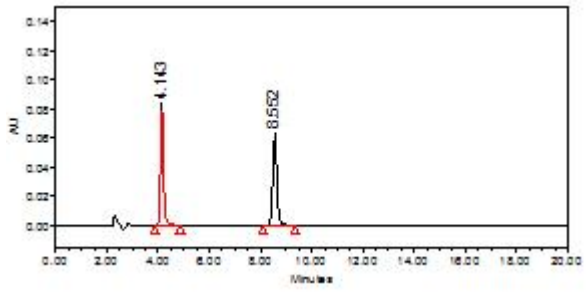

Figure 3: Standard chromatogram of Ornidazole and Ofloxacin

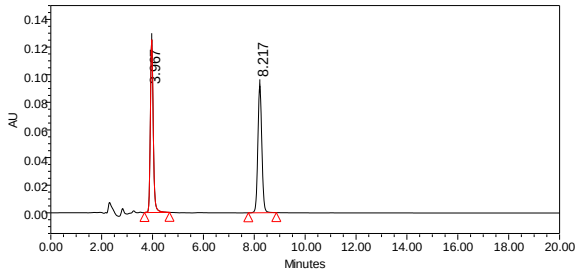

Figure 4: Standard chromatogram of Ornidazole and Ofloxacin

The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve shows linearity, which justifies the use of single point calibration and the proximity of maximum points to the calibration line demonstrated that the method has accurate linearity to the concentration to the analyte. The retention time of ofloxacin and ornidazole was found to be 4.292 and 8.921 min respectively. The limit of detection (LOD) for Ornidazole and Ofloxacin was found to be 5 µg/ml and 6 µg/ml respectively. The limit of quantification (LOQ) for Ornidazole and Ofloxacin was found to be 15 µg/ml and 18 µg/ml, respectively. Robustness of the method was determined by making slight changes in the chromatographic conditions. After that there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table–2). Experimental results reveal that, the present developed RP-HPLC method is simple, accurate, selective, precise, rugged, robust, linear and rapid for the estimation of Ornidazole and Ofloxacin in combination form of dosage formulation. Hence, this method can be applied for the quality control of raw materials, formulations and dissolution studies.

Table 2: Summary of results of method validation for Ornidazole and Ofloxacin

S. No	Parameter	Requirement	Results		Acceptance
	Parameter		Result		Limit
			Ofloxacin	Ornidazole	
1.	System suitability	RT	4.292	8.921	
		Tailing factor	1.0	1.10	NMT 2
		Plate count	6246.966	13589.046	NLT 3000
		Assay value	102.92%	101.83%	100 ± 5.0%
2.	Accuracy	% recovery	99.79 %	99.92%	100 ± 2.0%
3.	Precision	%RSD	0.90	1.39	NMT 2%
4.	Specificity	No interference	Pass	Pass	No interference
5.	Linearity	Correlation coefficient	0.9990	0.9997	NLT 0.999
6.	Range	concentration	50ppm-150ppm	50ppm-150ppm	Nil
7.	Intermediate precision	%RSD	1.18	1.46	NMT 2%

4. Conclusion

It is concluded that the proposed methods in the present investigation are simple, sensitive, accurate and precise and can be successfully applied for the routine estimation of ornidazole and ofloxacin in combined dosage forms.

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