WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 03, 703-712. Research Article ISSN 2277– 7105

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BALOFLOXACIN AND ORNIDAZOLE IN THEIR COMBINED DOSAGE FORM

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Article Received on
25 Dec. 2016,

Revised on 15 Jan. 2017,
Accepted on 05 Feb. 2017

DOI: 10.20959/wjpr20173-7885

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ABSTRACT

A RP-HPLC method is developed for simultaneous estimation of Balofloxacin and Ornidazole in combined tablet dosage form. Balofloxacin comes in the category of antibacterial and antibiotic and it is used in infective ophthalmitis and sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, intra abdominal infection. Ornidazole comes in category of antiprotozoal, antibiotic and anti microbial and it is used in intestinal and hepatic amoebiasis, giardiasis, trichomoniasis, bacterial vaginosis, anaerobic infections. The mobile phase used was a combination of the mobile phase composed of phosphate buffer: acetonitrile (70:30%, v/v), pH-3.1 adjusted with trimethylamine. The eluent detection was carried out by using photodiode array detector at 308 nm and flow rate at 1.0 ml/min. The linearity range for Balofloxacin and Ornidazole was found to be in the range of 2.5-7.5µg/ mL, and 12.5-37.5µg /mL respectively. Correlation co-efficient of Balofloxacin and Ornidazole was found to be 0.9903 and 0.9971 respectively. The method used was Cosmosil packed MS II column C18 at ambient temperature. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. The methods were found to be simple, accurate, economical, robust and reproducible, which can be employed for routine analysis of the drugs in combined dosage form.

KEYWORDS: Balofloxacin, Ornidazole, RP-HPLC method, simultaneous estimation.

INTRODUCTION

Balofloxacin is chemically known as (±)-1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[3-(methylamino)-1-piperidinyl]-4-oxo-3-quinolinecarboxylic acid and it is used in infective

ophthalmitis and sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, intra abdominal infection.[1] Chemical structure of Balofloxacin is shown in figure 1. Ornidazole is chemically known as 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propan-2-ol and it is used in intestinal and hepatic amoebiasis, giardiasis, trichomoniasis, bacterial vaginosis, anaerobic infections.[2] Chemical structure of Ornidazole is shown in figure 2.Literature survey reveals that spectrophotometric method[3,4,5,6,7,8,9], HPTLC[10], RP-HPLC[11, 12] methods was reported for estimation of Balofloxacin alone and with other combination in Bulk and Dosage form. Literature survey reveals that by spectrophotometric[17,18,19,20,21], HPLC,[22,23,24] methods was performed for estimation of Ornidazole alone and with other combination in Bulk, and Dosage form. As Per our best effort of literature review, it reveals that no analytical method has been reported for simultaneous estimation of Balofloxacin and Ornidazole by HPLC in combined dosage form. So, the aimed was to develop and validate accurate and precise method for simultaneous estimation Balofloxacin and Ornidazole.

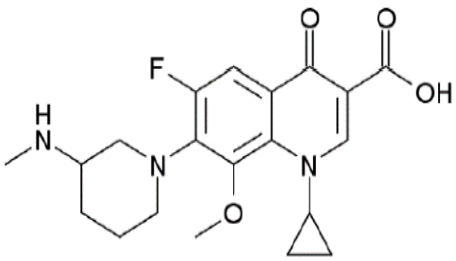

Fig: 1. BALOFLOXACIN

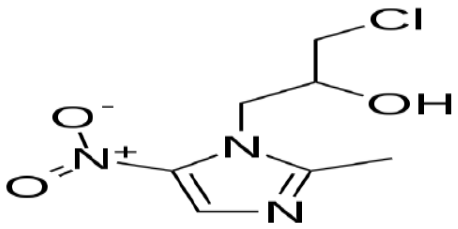

Fig. 2. ORNIDAZOLE

MATERIAL AND METHOD

The present work was carried out on High pressure liquid chromatographic method LC - 20AT SHIMADZU Cosmosil packed MS II column C18. Detector consists of photodiode array detector; the liquid phase column used was RP-C18 at ambient temperature. Diplobal-oz (Balofloxacin100mg and Ornidazole 500mg) obtained from were obtained from Biogen pharmaceutical Co.

REAGENTS AND CHEMICALS

Balofloxacin and Ornidazole were obtained as gift sample from Biogen pharmaceutical Co. Solvents and reagents were used of HPLC grade were obtained from Spectrochem, Mumbai, India.

EXPERIMENTAL CONDITIONS

The HPLC system was operated isocratically at flow rate of 1.0 ml/min. at 25°C ± 0.5°C for 10 min. The mobile phase found to be most suitable for analysis was phosphate buffer: acetonitrile (70:30%, v/v), pH-3.1 adjusted with trimethylamine, the detection was carried out at 308 nm.

Preparation of standard stock solution of Balofloxacin (1 µg/mL)

Accurately weighed quantity of Balofloxacin 50 mg was transferred into 50 mL volumetric flask, dissolved and diluted up to mark with methanol. 100µg/mL of Balofloxacin solution was prepared by diluting 5 mL of above solution to 50 mL with methanol. 10µg/mL of Balofloxacin solution was prepared by diluting 5 mL of stock solution (100µg/mL) to 50 mL with methanol.

Preparation of standard stock solution of Ornidazole (10µg/mL)

Accurately weighed quantity of Ornidazole 50 mg was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with methanol. 100µg/mL of Ornidazole solution was prepared by diluting 5 mL of above solution to 50 mL with methanol.10µg/mL of Ornidazole solution was prepared by diluting 5 mL of stock solution (100µg/mL) to 50 mL with methanol.

Preparation of Sample solution

Twenty tablets were weighed and powdered. The tablet powder equivalent to 10 mg of Balofloxacin was transferred to 100ml conical flask, dissolved and sonicated for 20 min and diluted up to mark with methanol. The solution was filtered through 0.45 µ filter paper and first few ml of filtrate were discarded. From this solution take 1 ml and diluted up to 10 ml (10µg/ml).

PROCEDURE

Chromatographic Condition

Chromatographic separation was achieved by using mobile phase consisted of phosphate buffer: acetonitrile (70:30%, v/v), pH-3.1 adjusted with trimethylamine flowing through RP-C18 column at a constant flow rate of 1.0 ml/min for 10 min. A RP-C18 column was used as the separation phase. Detection was carried out using a photo diode array detector at 308 nm.

Linearity

To establish the linearity a series of dilutions ranging from 2.5-7.5 µg/ml for Balofloxacin and 12.5-37.5 µg/ml for Ornidazole were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

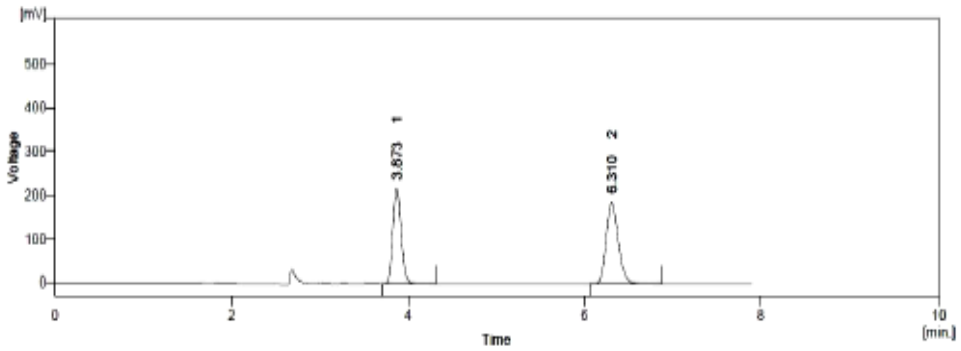

Fig 3 HPLC chromatogram of standard solution of Balofloxacin 2.5µg/ml and Ornidazole 12.5µg/ml

Method validation

Accuracy

Accuracy was performed by recovery study. Accuracy was performed by spiking Balofloxacin and Ornidazole at three different concentration levels 80,100 and 120 % to the assay sample, with a constant concentration of 2.5 µg/ ml for Balofloxacin and 12.5 µg/ ml for Ornidazole. Peak Area was calculated and % recovery was determined.

Precision

The precision of method was determined by repeatability, intraday and interday precision. Repeatability was determined by estimating Balofloxacin (5µg/mL) and Ornidazole (25µg/mL) six times was expressed as % relative standard deviation (% R.S.D).

Intraday Precision

Three different concentration of Balofloxacin (2.5, 5 and7.5µg/mL) and Ornidazole (12.5, 25and 37.5µg/mL) were analyzed on the same day three times at different time interval and % R.S.D was calculated.

Interday Precision

Three different concentration of Balofloxacin 2.5,5 and7.5µg/mL) and Ornidazole (12.5, 25and 37.5µg/mL) were analyzed on three different days and % R.S.D was calculated.

Robustness

Robustness of the method was evaluated by changing flow rate, ratio of mobile phase composition and pH and their effects on retention time (R_t) and tailing factor (T) were studied.

The following parameters were changed one by one

1) Flow rate of mobile phase (± 0.2 ml/min), 0.8 ml/min and 1.2 ml/min
2) Ratio of mobile phase composition (± 2 %) phosphate buffer: acetonitrile(28:72 %v/v and 32:68 %v/v)
3) pH of mobile phase (± 0.2 unit)

The accuracy, precision and robustness were determined by analyzing a set of laboratory sample (n=5) with each of the five concentrations ranging from 2.5-7.5 µg/ml and 12.5-37.5 µg/ml for Balofloxacin and Ornidazole respectively.

System Suitability Test

To study system suitability parameter, sample solutions were injected six times and chromatogram were studied for theoretical plates, resolution and tailing factor.

RESULT AND DISCUSSION

Chromatographic Method

LC15 detector was used and various trials were performed of mobile phase such as Water: Methanol; Water: Acetonitrile in various ratio were tried but proper resolution was not obtained. Then Buffer (pH 4.5): Acetonitrile (40:60, v/v) in which Balofloxacin peak was not proper and then Buffer (pH 4): Acetonitrile (70:30, v/v) where proper peaks were obtained suitable for quantitation .So, this mobile phase does not give suitable results.

Finally the system containing was phosphate buffer: acetonitrile (70:30%, v/v) was found to be satisfactory and gave two well resolved peaks for Balofloxacin and Ornidazole with retention time for Balofloxacin and Ornidazole 3.877 and 6.410 min respectively. A representative graph of this is shown in Fig.3.

Table: 1 Parameters of System Suitability

Sr No.	Parameters	Balofloxacin	Ornidazole
1.	Theoretical Plates	**1.24**	1.36
2.	Tailing Factor	**7636**	9573
3.	Resolution	**1.89**	-

Table: 2. Accuracy data Balofloxacin and Ornidazole

Assay Level	Tablet content of drug equivalent to (mg)		Standard added (mg)		Total amount (mg)	
	Balo	Orni	Balo	Orni	Balo	Orni
Blank	3	15	0	0	3	15
	3	15	0	0	3	15
	3	15	0	0	3	15
80%	3	15	2.4	12	5.4	27
	3	15	2.4	12	5.4	27
	3	15	2.4	12	5.4	27
100%	3	15	3	15	6	30
	3	15	3	15	6	30
	3	15	3	15	6	30
120%	3	15	3.6	18	6.6	33
	3	15	3.6	18	6.6	33
	3	15	3.6	18	6.6	33

Precision

Repeatability was determined by calculating % RSD for five replicates of 3µg/ml Balofloxacin and 15µg/ml of Ornidazole was found as same for both the drug that is 0.458%

Intraday precision

The % RSD of Balofloxacin and Ornidazole for intraday precision was found to be less than0.37 and 0.53 respectively.

Interday Precision

% R.S.D of Balofloxacin and Ornidazole was found to be less than 0.60 and 0.77 respectively.

Table 3: Robustness study for Balofloxacin

Parameter	Variation	Peak area	Tailing factor	Theoretical plate
Flow	0.8 ml/min	1510.51	1.22	7448

Rate	1.2 ml/min	1421.64	1.24	7636
pH	3.8	1493.54	1.26	7746
	4.2	1394.56	1.24	7749
Mobile	28:68(v/v)	1468.92	1.23	7448
Phase	32:72(v/v)	14.17.77	1.24	7637

Table 4: Robustness study for Ornidazole

Parameter	Variation	Peak area	Tailing factor	Theoretical plate
Flow rate	0.8 ml/min	1877.13	1.31	9582
	1.2 ml/min	1772.90	1.36	9214
pH	3.8	1857.29	1.34	9351
	4.2	1732.64	1.33	9573
Mobile phase	28:68(v/v)	1856.91	1.34	9351
	32:72(v/v)	1766.91	1.36	9214

Table 5: Summary of Validation Parameters of RP-HPLC

Regression analysis	Statistic value	
	Balofloxacin	Ornidazole
Regression equation	Y= 0.0398x -0.0044	Y=0.0077x-0.0015
Correlation coefficient (R_2)	0.9903	0.9971
Slope	0.0398	0.0077
Intercept	0.0044	0.0015
LOD (µg/ml)	0.90	1.90
LOQ (µg/ml)	1.80	1.16

Assay

The content of Balofloxacin and Ornidazole found in the tablets by the proposed method are shown in Table 6. And chromatograph had shown in Fig. 3.

Table 6: Assay

Drug	Lable claim (mg)	Amount estimated(mg) ± SD	Assay (%label claim) ± SD
Balofloxacin	100	95.76 ± 0.74	95.76 ± 0.74
Ornidazole	500	494.01 ± 0.58	98.80 ± 0.53

CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of Balofloxacin and Ornidazole simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage form of Balofloxacin and Ornidazole. The method was evaluated in a mass of facets, such as best condition, linear relation including coefficient of correlation, robustness, accuracy,

reproducibility and precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. Any of the developed method can be successfully used for routine quality control of Balofloxacin and Ornidazole in their combined dosage form.

ACKNOWLEDGMENTS

The authors are grateful to Bhagwan Mahavir College of Pharmacy-India, for providing the facilities to carry the experiment Biogen Pharmaceutical Company for providing gift samples of Balofloxacin and Ornidazole.

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