International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN HYDROCHLORIDE AND ORNIDAZOLE BY RP-HPLC

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ABSTRACT

In the present study an RP-HPLC method was developed and validated for the simultaneous determination of Moxifloxacin hydrochloride and Ornidazole.The chromatographic system was equipped with Phenomenex C18 column and PDA detector set at 294nm, in conjunction with a mobile phase of phosphate buffer and Acetonitrile in the ratio of 68:32 (pH 4.3, adjusted with orthophosphoric acid) at a flow rate of 1.5 ml/min and the injection volume set at 20 µl with 5 minutes of runtime. The described method was linear over a concentration range of 10.0-50.0 µg/ml and 12.5 and 62.5 µg/ml for the simultaneous estimation of Moxifloxacin hydrochloride and Ornidazole with a with good linearity response of 0.9986 and 0.9981 respectively. The retention time of Moxifloxacin hydrochloride was 2.6 min ± 0.02 and of Ornidazole was 4.00 min ± 0.02. The results of analysis were validated. The results of the study showed that the proposed RP-HPLC method was simple, rapid, precise and accurate, which is useful for the routine determination of Moxifloxacin hydrochloride and Ornidazole in bulk drug and in pharmaceutical dosage form.

Key words: *Moxifloxacin Hydrochloride, Ornidazole, RP-HPLC and Acetonitrile.*

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INTRODUCTION

Moxifloxacin hydrochloride is a synthetic fluroquinolone drug[1] used as broad spectrum antibacterial agent used in the treatment of anaerobic bacteria[2]. It is chemically 1'S,6;S)-1-Cyclopropyl-7-(2,8-diazabicyclo[4.3.0]non-8-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride. Ornidazole is a 5-nitro imidazole derivative used as anti infective agent and Chemically ornidazole 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propan-2-ol[3]. Clinically a combination of fluroquinolones and nitro immidazole is being used in the treatment of various infections[4]. One such combination is

ofloxacin and ornidazole and it has gained increasing acceptance in bacterial and protozoal infections[5]. In view of this increase in demand for the use of combined fluoroquinolone and nitromidazole in therapy a combination of moxifloxacin hydrochloride and ornidazole could be an alternative choice in treating infections caused by bacteria and protozoa.

A literature survey revealed that only a few methods based on HPLC[6], spectrometry[7]T were reported for the simultaneous determination of ofloxacin and ornidazole from dosage forms and in biological fluids but no single method is reported so for the simultaneous estimation of moxifloxacin hydrochloride and ornidazole as this combination is not available in the market. Hence in the present study a physical mixture of moxifloxacin hydrochloride and ornidazole was being taken for simultaneous estimation by RP-HPLC method. This present investigation describes a rapid, accurate and precise RP-HPLC method of moxifloxacin hydrochloride and ornidazole in combination using 25 mM phosphate buffer and Acetonitrile with a pH 4.3 adjusted with orthophosphoric acid in the ratio of 68: 32%v/v. The column used was Phenomenex C18, at a flow rate of 1.5 ml / min and photo diode array detector (PDA) was employed in the study at 294 nm.

MATERIALS AND METHODS

Apparatus and Chromatographic Conditions:

A Shimadzu Prominence High performance liquid chromatographic instrument provided with phenomenex C-18 column (250 mm x 4.6 mm, 5µ packed with octadecyl silane chemically bounded to porous silica or ceramic micro particle) and PDA (G1314A) detector was employed in the study. A 20 µl Hamilton injection syringe was used for sample injection and data were collected using LC solution 21 CFR software.

Reagents and solutions:

HPLC grade Acetonitrile, AR grade disodium hydrogen orthophosphate, HPLC grade water (milli-pore) was used in the study.

A binary mixture of disodium hydrogen orthophosphate buffer and Acetonitrile in the ratio of 68: 32%v/v was used as a mobile phase at a pH 4.3 adjusted with ortho phosphoric acid and it is also used as a diluent for preparing the working solution of drugs. The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed using degasser (G1379A and G1322A) before use. The flow rate of mobile phase was 1.5 ml / min and the column temperature was 25^0C. The peaks were detected at 294 nm (Table No. 1).

Table No. 1: Chromatographic Conditions:

Flow Rate	**1.5ml/minute**
Wavelength	**200- 800 nm**
Injection Volume	**20 µl**
Column temp	**25 °C**
Runtime	**5 minutes**
Columns	**Phenomenex C18, 250 X 4.6 mm; 5µ**

Drug samples:

The reference samples moxifloxacin hydrochloride (99.08%) and ornidazole (99.59%) require for the study were procured from Strides Arco lab and Drug testing laboratory Bengaluru as gift samples.

Preparation of working standard solutions:

Moxifloxacin hydrochloride:

About 100 mg of moxifloxacin hydrochloride were weighed accurately and transferred into 100 ml volumetric flask, dissolved and made upto to the mark with mobile phase. This solution was suitably diluted with a mobile phase to get a working standard solution of 100 µg/ml of moxifloxacin hydrochloride.

Ornidazole:

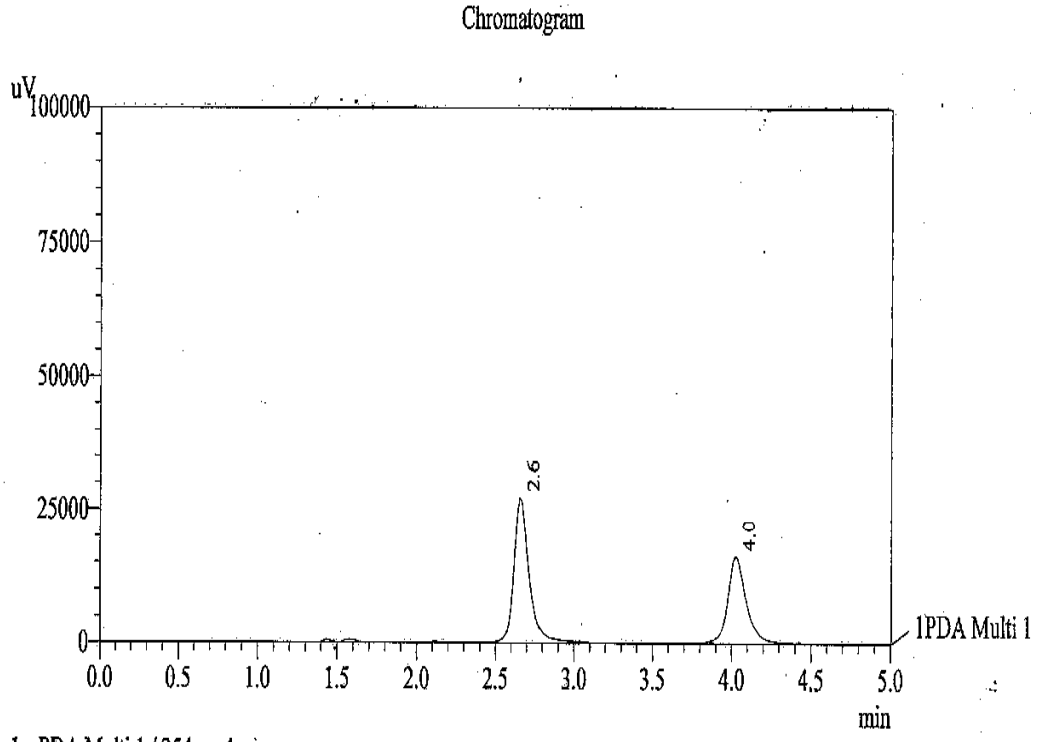
About 125 mg of ornidazole were weighed accurately and transferred into 100 ml volumetric flask, dissolved and made upto to the mark with mobile phase. This solution was suitably diluted with a mobile phase to get a working standard solution of 125 µg/ml of ornidazole.

Linearity and construction of calibration curve:

Working dilutions of moxifloxacin hydrochloride in the range of 10-50 µg/ml and ornidazole in the range of 12.5-62.5 µg/ml were prepared by taking suitable aliquots of working standard solutions of drugs in different 10 ml volumetric flask and

diluting upto the mark with the mobile phase. 20 µl quantity of the dilutions was injected each time into the column at a flow rate of 1.5 ml/min. Each dilution was injected 5 times into the column. The drug in the elute was monitored at 294 nm and the corresponding chromatogram were recorded. From these chromatograms the mean peak (Table No. 2) areas were calculated and a plot of conc. vs the peak areas were constructed (Fig No. 1 and 2). The regression of the plot was computed by least square regression method. A linear relationship in the range was found to be 10-50 µg/ml of moxifloxacin hydrochloride and 12.5-62.5 µg/ml of ornidazole between the concentration of drugs and their respective peak areas. This regression equation was used to estimate the amount of moxifloxacin hydrochloride and ornidazole in the physical mixture (Fig No. 3).

Fig No.3: Chromatogram of Moxifloxacin HCl and Ornidazole



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Name	Ret. Time	Area	Tailing Factor	Theoretical Plate#
1	MOXI	2.6	187036	1.410	3319.895
2	ORZ	4.0	135876	1.229	5302.388
Total			322912		

Table No. 2: Linearity of Moxifloxacin HCl and Ornidazole

Sr. No.	Moxifloxacin HCl Conc. Of stock solution (100 µg/ml)			Ornidazole Conc. Of stock solution (125 µg/ml)		
	Injection volume (in µl)	Conc. (ppm) µg/ml	Area*	Injection volume (in µl)	Conc. (ppm) µg/ml	Area*
1	20	10.0	191348	20	12.5	125495
2	20	20.0	338703	20	25.0	230708
3	20	30.0	504302	20	37.5	356446
4	20	40.0	703674	20	50.0	504550
5	20	50.0	865698	20	62.5	614914

Preparation of Sample solutions and recovery studies:

From physical mixture:

Weighed accurately 10 mg of Moxifloxacin hydrochloride and 12.5 mg of Ornidazole were mixed uniformly in a mortor (based on the normal dose Moxifloxacin hydrochloride 400 mg and ornidazole 500 mg, a physical mixture of these drugs was prepared) and transferred into a clean 100 ml volumetric flask, dissolved and made upto 100 ml with mobile phase to obtain concentration of 100 µg / ml and 125 µg / ml of Moxifloxacin hydrochloride and ornidazole respectively (Stock A) and filtered through .045 µ nylon membrane filter. From the above stock A aliquots was taken into different 10 ml different volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of Moxifloxacin HCl and Ornidazole was 10.0-50.0 µg/ml and 12.5-62.5 µg/ml respectively. Detection was performed with PDA detector at 294nm, peak and peak areas were recorded for all the injection made. The slope and intercept value for calibration curve was y= 17232x-3163.6 (R2=0.9986) for Moxifloxacin HCl and that of Ornidazole was y=9914.2x-4467.5 (R2=0.9981).

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the method developed were determined by injecting progressively low concentration of the standard solutions. The LOD is the smallest concentration of the analyte that gives a measurable response of signal to noise ratio of 3. The LOD for Moxifloxacin HCl and Ornidazole were found to be 0.5 and 0.3mcg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified signal to noise ratio of 10. The LOQ was 1.0 and 0.8 mcg/ml of Moxifloxacin hydrochloride and Ornidazole respectively (Table No. 3).

Table No. 3: Validation parameters

Parameter	Moxifloxacin HCl	Ornidazole
Linearity range	10-50.0mcg/ml	12.5-62.5mcg/ml
Correlation coefficient	0.9986	0.9981
LOD	0.5mcg/ml	0.6mcg/ml
LOQ	1.0mcg/ml	0.8mcg//ml
Recovery (n=9)	98.76	100.76
Precision(%RSD)		
Intra day (n=9)	0.518	0.816
Inter day (n=9)	0.583	0.411

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments using different C18 columns on different days. Robustness is determined by making slight changes in the chromatographic conditions like change in wavelength, flow rate, mobile phase ratio and pH. It was observed that there were no marked changes in the chromatograms, which indicates that the RP-HPLC method developed is rugged and robust.

Recovery Studies:

To study the accuracy and reproducibility of the proposed method recovery studies were carried out. A fixed amount of sample mixture was taken

and standard drugs were added at 80%, 100% and 120% levels. Each level was injected nine times. The contents of Moxifloxacin HCl and Ornidazole found by the proposed method are shown (Table No. 3). The lower values of RSD indicate that the method is accurate. The mean recoveries of Moxifloxacin HCl and Ornidazole were in the range of 99.0-103.0% and 101.3-102.8% respectively.

RESULTS AND DISCUSSION

The present study was carried out in developing a rapid, accurate and precise HPLC method for the analysis of Moxifloxacin hydrochloride and Onidazole in physical mixture. A binary mixture of disodium hydrogen orthophosphate buffer and Acetonitrile in the ratio of 68:32%v/v was used as mobile phase at pH 4.3. The retention time observed for Moxifloxacin hydrochloride was 2.6 min and that of ornidazole was 4.0 min. Each of the samples was injected 6 times and the same retention was observed in all cases. The peak areas of Moxifloxacin hydrochloride and ornidazole were reproducesable as indicated by low coefficient of variation. The slope and intercept value for calibration curve was y= 17232x-3163.6 (R2=0.9986) for Moxifloxacin HCl and that of Ornidazole was y=9914.2x-4467.5 (R2=0.9981). The deliberate changes in the method have not much effected the peak tailing, theoretical plates and the % assay. This indicated the robustness of the method and the results are presented in (Table No. 4). System suitability parameters were studied with the 6 replicates standard solution of the drugs and the calculated parameters are within the acceptance criteria. The tailing factor, the number of theoretical plates and HETP or all in the acceptable limits.

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of Moxifloxacin hydrochloride and Onidazole in quality control laboratories with short analysis time.

Table No. 4: Results of validation for the proposed analytical method

Sl. No.	parameter	Acceptence Crtieria	Result observed for Moxifloxacin HCl	Result observed for ornidazole
1	Linearity	Percentage curve fitting should be more than 99.7%	Range 10-50.0 µg/ml %CF 99.86	Range 12.5-62.5µg/ml %CF 99.81
2	LOD	S/N=3	0.5 mcg/ml	0.3mcg/ml
3	LOQ	S/N=10	1.0mcg/ml	0.8mcg/ml
4	Precision of the method	Relative standarad deviation within 2%	0.68%	0.70%
5	Precision of the system	Relative standarad deviation within 2%	0.34%	0.52%
6	Accuracy	Percentage recovery to be found within the range of 97-103%	98-102%	99-101%
7	Robustness	1.change in pH of buffer	At 4.2, 104.4%	99.56%
			At 4.4, 100.3%	99.8%
		Change in mobile phase	66:35 -99.4%	97.3%
			68:32-102.2%	102.9%
		(Phosphate buffer:ACN)	70:37-103.3%	103.1%
		Change in flow rate	At 1.4ml/min	101.76%
			At 1.6ml/min	102.50%
8	Specificity	No interference of peak	Mobile phase with excipients did not show any peak from 1-10min	

CONCLUSION

Proposed study describes new and simple RP-HPLC method for the simultaneous estimation of Moxifloxacin hydrochloride and Onidazole. The method validated was found to be simple, sensitive, accurate and precise. Therefore the proposed method can be used for quantification of Moxifloxacin HCl and Ornidazole in the physical mixture of these drugs.

ACKNOWLEDGEMENT

The authors are to M/s Strides Arco lab and Drugs testing laboratories, Bengaluru for providing gift samples of drugs. The authors are also thankful to Principal, Govt. College of Pharmacy, Bengaluru, for providing laboratory facilities to carry out the research work.

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