IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 7, Issue 9 Ver. II. (Sep. 2014), PP 32-35
www.iosrjournals.org

Development & validation of RP-UPLC method for simultaneous estimation of ofloxacin and ornidazole in their combine dosage form including stress study

Sevak Manan R*[1], Patel Nirav B[2], Patel Kamlesh N[3], Desai Hemant T[4]
[*1]*Assistant Professor at Ganpat University, Mehsana, Kherva, Gujarat, India.*
[2]*Nirlife Healthcare (Healthcare Division of Nirma), Ahemdabad, Gujrat, India.*
[3]*Virgo Uap Pharma Pvt. Ltd., Ahmedabad, Gujarat, India.*
[4]*PhD Guide, Kadi University, Gandhinagar, Gujarat, India.*

Abstract: *This research manuscript describes simple yet sensitive, speedy, accurate, precise, repeatable & reproducible RP-UPLC method for the analysis of Ofloxacin and Ornidazole in combined infusion form. The sample was analyzed by RP-UPLC instrument using reverse phase C18 column (Purospher Star 100×2.1 mm, Merck Specialities) as stationary phase and Phosphate Buffer: Acetonitrile (70:30 v/v) as a mobile phase [where P^H of of the buffer was adjusted to 2.5 by using Tri ethyl amine (1ml / lit buffer) and ortho-phosphoric acid] at a flow rate of 0. 4 ml/min. TUV detector was used for the detection at 294 nm. The retention time for Ofloxacin and Ornidazole was found to be 0.648 and 1.158 minute respectively. The linearity for both the drugs was obtained in the concentration range of 2-14 µg/ml and 5-35 µg/ml. The method was successfully applied to pharmaceutical formulation because no significant interferences from infusion excipient were found. The method retained its accuracy and precision when certain variations in method parameters were applied.*

Keywords: *Ofloxacin and Ornidazole; Combined dosage form; Method validation; RP-UPLC.*

I. Introduction

Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class- considered to be a second-generation fluoroquinolone. Chemically, Ofloxacin is a fluorinated carboxyquinolone, is the racemate, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (fig. 1) and Ornidazole is Chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (fig. 2). Ornidazole is useful for some protozoan infections and mainly used in poultry industry.

Figure 1: Chemical Structure of Ofloxacin

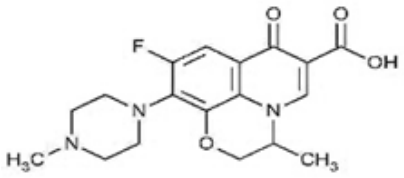
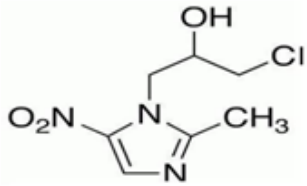


Figure 2: Chemical Structure of Ornidazole



Ofloxacin is official in Indian pharmacopeia[1] and United States Pharmacopeia[2]and british pharmacopoeia[3]. But no official method is available for the analysis of Ornidazole. The combination of Ofloxacin and Ornidazole is widely used in treatment of various G.I. tract infections. Literature search reveals that various analytical methods like spectrophotometric[4,5,6] , HPLC [7,8] and HPTLC [9] methods are available for the estimation of Ofloxacin and Ornidazole in combine dosage form. There is no reported method for estimation of Ofloxacin and Ornidazole in their combined dosage form by RP-UPLC. This prompted the present work. The method was developed and validated as per ICH [10,11 & 12] and usp[13] guideline. The aim of the present work is to develop a simple yet quick, accurate and precise RP-UPLC method for estimation of Ofloxacin and Ornidazole in their marketed formulation which is more efficient method than the available RP-HPLC method.

II. Reagents And Materials

Ofloxacin and Ornidazole standards were obtained from Nirlife (Healthcare division of Nirma), Ahmedabad, Gujarat, India. The combination product (ZENFLOX-OZ, mankind) was procured from market. Acetonitrile (HPLC grade from Finar Reagent, Ahemedabad, India), KH_2PO_4, Tri ethyl amine and ortho phosphoric acid (acquired form FINAR chemicals Pvt. Ltd., Ahmedabad, India) was used in the study. High purity Water for injection was used in the study.

III. Instruments And Condition

The fast liquid chromatography was performed using waters' UPLC system with TUV detector. Chromatogram and data were recorded by means of Empower 2 software. Separation was achieved by using Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Merck, Germany) as a stationary phase with Phosphate Buffer: Acetonitrile (70:30 v/v) as a mobile phase at a flow rate of 0.4 ml/min, Injection volume is 1µl and detection wavelength was 294 nm in TUV detector. Column temperature is 50^{o}C and Sample temperature is taken 20^{o}C. Weigh machine of Essetoreka Company - model AR- 2140, Ph meter of Systronics company- model 362 and sonicator of Toshcon company- model SW1 were used in the study.

IV. Preparation Of Mobile Phase

6.8 gm KH_2PO_4 was weighed accurately in 1000mL volumetric flask. To it about 70mL of Water is added, sonicated and further make up the volume up to mark with water [and P^H of the buffer is adjusted to 2.5 by Tri ethylamine (1 ml / lit of buffer solution) and ortho-phosperic acid], from the prepared buffer solution 700ml is mixed with 300 ml of actonitrile in 1000 ml volumetric flask to make a mobile phase ratio buffer:acetonirile 70:30% v/v respectively. This mobile phase was used as diluents & also was used throughout study.

V. Preparation Of Standard Stock Solution

An accurately weighed Ofloxacin (10 mg) and Ornidazole (25 mg) were transferred into two different 200 mL volumetric flask, dissolved in 200 mL mobile phase produce concentration of Ofloxacin (50 µg/ml) and Ornidazole (125 µg/ml).

VI. Preparation Of Mixed Standard Working Solution

Accurately weighed Ofloxacin (10 mg) and Ornidazole (25 mg) were transferred to 200 mL volumetric flask, dissolved in 200 mL mobile phase to produce concentration of Ofloxacin (50 µg/ml) and Ornidazole (125 µg/ml).

VII. Preparation Of Calibration Curve

Aliquots (1,2,3,4,5,6 &7 ml) of mixed standard working solutions (equivalent to 2,4,6,8,10,12 & 14 µg/ml Ofloxacin and 5, 10, 15, 20, 25, 30, and 35 µg/ml of Ornidazole respectively) were transferred in a series of 25 ml volumetric flasks, and the volume was made up to the mark with mobile phase. 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.3&4), each response was average of three determinations.

Fig.3 linearity of Ofloxacin

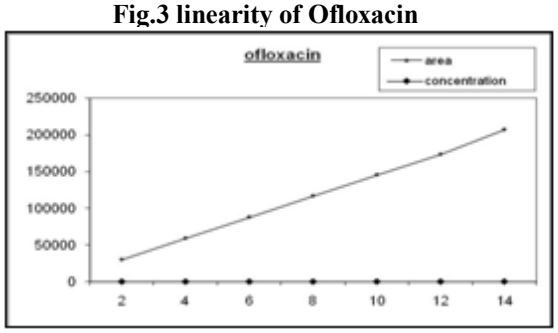
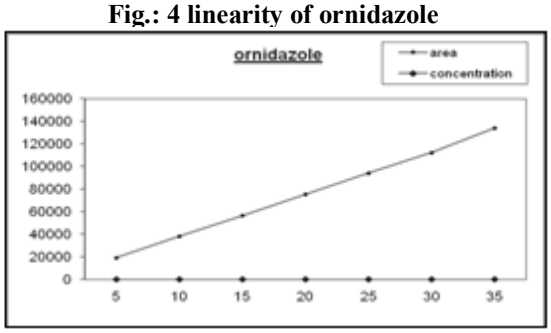


Fig.: 4 linearity of ornidazole



VIII. Method Precision (Repeatability)

The precision of the method was checked by repeatedly injecting (n=6) injections of Ofloxacin (8 ppm): Ornidazole (20ppm) without changing the parameters.

IX. Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of Ofloxacin and Ornidazole by the standard addition method. Known amounts of standard solutions of Ofloxacin and Ornidazole were added at 80%, 100% and 120 % level to pre-quantified sample solutions of Ofloxacin and Ornidazole. The amounts of Ofloxacin and Ornidazole were estimated by applying obtained values to the respective regression line equations.

X. Preparation Of Marketed Sample Solution For Assay

For determination of the content of Ofloxacin and Ornidazole in marketed infusion (Lable claim: OFL-200mg/100ml and ORN-500mg/100ml). Take 10 ml solution from infusion and transferred to 50 mL volumetric flask, dissolved in mobile phase and sonicated for 30 min. The solution was filtered through Whatmann filter paper No. 41. The solution was diluted up to the mark with mobile phase. From that prepared solution, accurately measured 1.0 mL of solution was transferred to 50 mL volumetric flask, diluted up to the mark with mobile phase to get final working concentration of Ofloxacin (8 µg/ml) and Ornidazole (20 µg/ml). A sample solution was injected under the operating chromatographic condition as described above and responses were recorded. The analysis procedure was repeated three times with infusion formulation.

XI. Intermediate Precision (Reproducibility)

The intraday precisions of the proposed method were determined by estimating the corresponding responses for 3 different concentrations in a same day in morning, evening and night.

The interday precisions were determined by estimating the corresponding responses for 3 different concentrations in 3 different (alternative) days of a week in morning, evening and night.

Different concentrations taken for Ofloxacin were (6,8 and 10µg/ml) and Ornidazole were (15,20 and 25 µg/ml)

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

Parameters	RP-UPLC method	
	Ofloxacin	Ornidazole
Concentration range (µg/ml)	2-14	5-35
Slope	14600.46	3792.56
Intercept	314.71	-131.43
Correlation coefficient	0.9998	0.9998
LOD[a] (µg/ml)	0.051	0.139
LOQ[b] (µg/ml)	0.156	0.422
Accuracy	100.27	99.87
Repeatability (%RSD[C], n=6)	0.490	0.375
Intraday (n=3) (%RSD[C])	0.308	0.285
Interday (n=3) (%RSD[C])	0.259	0.316

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 (n=6)
Ofloxacin	I	6.4	80%	100.27%
	II	8.0	100%	99.52%
	III	9.6	120%	100.62%
Ornidazole	I	16	80%	99.88%
	II	20	100%	99.88%
	III	24	120%	100.66%

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

Parameters	Ofloxacin (n=6)	Ornidazole (n=6)
Retention time (min)	0.648	1.158
Tailing factor	1.12	0.94
Theoretical plates	3169.3	3337.5
Resolution	8.28	

Table 4: Analysis of marketed formulation of Ofloxacin and Ornidazole by proposed RP-UPLC

Infusion	Label claim (mg/100ml)		Amount found (mg/100ml)		%Label claim	
	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole
I	200	500	199.04	499.40	99.52%	99.88%

Table 5: Robustness study

Conditions	RT (min)		Assay (%)	
Flow rate variation	Ofloxacin	ornidazole	Ofloxacin	ornidazole
0.38 ml/min	0.684	1.230	99.24	99.62
0.40 ml/min	0.651	1.169	100.06	99.98
0.42 ml/min	0.622	1.114	99.68	99.46

Column : sample temperature	Ofloxacin	ornidazole	Ofloxacin	ornidazole
48°C:22°C	0.649	1.173	99.38	99.46
50°C:20°C	0.650	1.168	99.89	99.77
50°C:22°C	0.647	1.161	99.63	99.52
Ph variation	Ofloxacin	ornidazole	Ofloxacin	ornidazole
2.40	0.646	1.152	99.54	99.53
2.50	0.645	1.180	99.49	99.62
2.60	0.643	1.176	99.51	99.42
Mobile Phase ratio (Buffer:ACN)	Ofloxacin	ornidazole	Ofloxacin	ornidazole
67:33 V/V	0.604	1.050	99.34	99.62
70:30 V/V	0.642	1.156	99.92	99.88
73:27 V/V	0.716	1.335	99.67	99.74

(Each determination is the outcome of 3 repeated injections)

XII. Specificity

The specificity of the developed method was determined by injecting sample solutions which were prepared by forcibly degrading the sample in presence of stress conditions such as acid, base & oxidative medium and application of light and heat. The stability signifying ability of the method was established from the acquired chromatographic data for Ofloxacin and Ornidazole. The results of force degradation study are explained in Table 6.

Table 6: Specificity Study

Stress condition	Time duration	%Degradation	
		Ofloxacin	Ornidazole
Acid degradation	1 Hour	28.74	28.87
Base degradation	1 Hour	23.91	68.31
Oxidative degradation	1 Hour	18.12	18.90
Thermal degradation	1 Hour	4.98	5.37
Photo degradation	48 Hour	13.30	10.99

XIII. Conclusion

A stability indicating UPLC method has been developed and validated for the determination of Ofloxacin and Ornidazole in combined pharmaceutical dosage forms. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust, specific and less time consuming as compared to available methods. No interference from any components of pharmaceutical dosage form or degradation products was observed, and the method has been successfully used to perform rapid and accurate analysis of Ofloxacin and Ornidazole in their combined pharmaceutical dosage form.

References

[1]. Indian Pharmacopeia Government Of India, Ministry Of Health And Family Welfare; The Indian Pharmacopoeia Commission 2007, Vol. II and III, page 854-855.

[2]. United States Pharmacopoeia, 30 National Formulary 24, 2007. page 2793.

[3]. British Pharmacopoeia, British Pharmacopoeia Comission Office, The Department of Health,The Stationery Office on behalf of MHRA, 2009. Vol. I, II &III, page 9426.

[4]. VM. Gandhi*, SB. Nair, C. Menezes and R. Narayan, Development Of UV-Spectrophotometric Method For The Quantitative Estimation Of Ofloxacin And Ornidazole In Combined Liquid Oral Dosage Form By Simultaneous Equation Method. International Journal of research in pharmacy and chemistry, 2013, 3(1) ISSN: 2231-2781.

[5]. Bhusari KP* and Chaple DR : Simultaneous Spectrophotometric Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form. Asian J. Research Chem.. 2(1): Jan..-Mar. 2009. ISSN 0974-4169.

[6]. Jawed Akhtar*, Birendra Shrivastava, Parth bhatt, Ankur Patel, Vikas Thakur : Simultaneous Estimation of Ofloxacin and Ornidazole in Synthetic Mixture by Q Analysis UV-Spectrophotometric Method. Asian Journal of Pharmacy & Life Science,Vol. 1 (1), Jan-Mar, 2011.

[7]. P. V. Rege.*1 And Ramesh Mapari : Simultaneous Quantification Of Ofloxacin And Ornidazole From Combined Pharmaceutical Drug Formulation By HPLC. International Journal of Pharma and Bio Sciences, vol.2 issue 4, oct-dec 2011, issn: 0975-6299.

[8]. B.Dhandapani*, N.Thirumoorthy, Shaik Harun Rasheed, M.Rama kotaiah and N.Anjaneyalu : Method Development And Validation For The Simultaneous Estimation Of Ofloxacin And Ornidazole In Tablet Dosage Form By RP-HPLC. International Journal of Pharma Sciences and Research (IJPSR) Vol.1 (1), 2010, 78-83.

[9]. Manisha Puranik,* D. V. Bhawsar, Prachi Rathi, and P. G. Yeole : Simultaneous Determination of Ofloxacin and Ornidazole in Solid Dosage Form by RP-HPLC and HPTLC Techniques. Indian J Pharm Sci. 2010 Jul-Aug; 72(4): 513–517.

[10]. FDA, ICH-Q1A (R2): Stability Testing Of New Drug SubstancesAnd Products, Vol. 68, U S Food And Drug Administration, Washington, DC, USA, 2nd Edition, 2003.

[11]. FDA, ICH-Q1B: Photo-Stability Testing Of New Drug Sub-Stances And Products, Vol. 62, U S Food And Drug Administration, Washington, DC, USA, 1997.

[12]. FDA, ICH-Q2 (R1): Validation Of Analytical Procedures: Text And Methodology, Vol. 60, U S Food And Drug Administration, Washington, DC, USA, 1995.

[13]. United State Pharmacopoeia, The U.S. Pharmacopeia Convention, United State Pharmacopoeia, Rockville, Md, USA, 30th Edition, 2007.