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Stability indicating method development & validation on RP-UPLC for simultaneous estimation of levofloxacin and ornidazole in their combine dosage form

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Abstract: *This research manuscript explains simple yet sensitive & speedy, accurate, precise, repeatable & reproducible RP-UPLC method for the analysis of Levofloxacin and Ornidazole in combine pharmaceutical 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 (65:35 v/v) as a mobile phase [where P^H of of the buffer was adjusted to 2.5 by using Tri ethylamine (1ml for 1 lit buffer) and ortho-phosphoric acid] at a flow rate of 0. 44 ml/min. TUV detector was used for the detection at 294 nm. The retention time for Levofloxacin and Ornidazole was found to be 0.537 and 0.938 minute respectively. The linearity for both the drugs was obtained in the concentration range of 2-14 µg/ml and 4-28 µg/ml. The method was successfully applied to pharmaceutical formulation because no significant interferences from suspension excipient were found. The method retained its accuracy and precision when certain variations in method parameters were applied.*

Keywords: *Combined dosage forms; Levofloxacin and Ornidazole; Method development & validation, RP-UPLC,Stability Study.*

I. Introduction

The combination of Levofloxacin and Ornidazole is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe of life threatening bacterial infections which have failed to respond to other antibiotic classes. It is sold under various brand names, such as Levaquin and Tavanic. Levofloxacin is an antibiotic that stops multiplication of bacteria by preventing the reproduction and repair of their genetic material, DNA. Levofloxacin is chemically a chiral fluorinated carboxyquinolone, is the pure (-) (S)-enantiomer of the racemic drug substance ofloxacin. The chemical name is (-)-(S)-9-fluoro2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4benzoxazine-6-carboxylic acid hemihydrate. (fig. 1) and Ornidazole is useful for some protozoan infections and mainly used in poultry industry. Chemically it is α-(Chloromethyl)-2-methyl-5-nitro-1H-imidazole-1-ethanol; 1-(3-Chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole; Madelen; NSC 95075; Ornidal; Ro 7-0207; Tiberal (fig. 2).

Figure 1: Chemical structure of levofloxacin

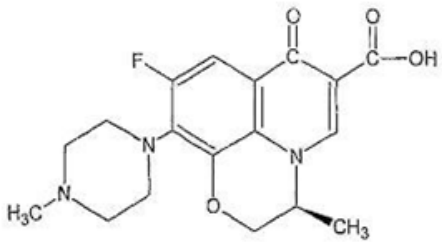
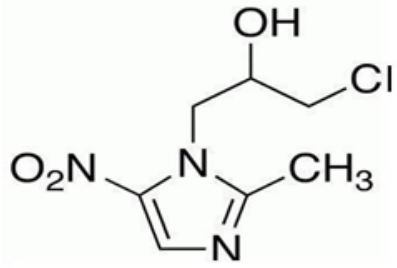


Figure 2: Chemical structure of Ornidazole



Literature search reveals that various analytical methods like HPTLC[1], HPLC[2,3] and Spectroscopy [3,4] are available for the estimation of Levofloxacin and Ornidazole in combine dosage form. There is no reported method for estimation of Levofloxacin and Ornidazole in their combined dosage form by RP-UPLC. This encouraged the present work. The method was developed and validated as per ICH [5,6 &7] and usp[8] guideline. The aim of present work is to develop a simple yet rapid, accurate and precise RP-UPLC method for estimation of Levofloxacin and Ornidazole in their combine marketed formulation which is more efficient method than the RP-HPLC method.

II. Reagents And Materials

Levofloxacin and Ornidazole standards were obtained from Nirlife, Healthcare division of Nirma. Ahmedabad, Gujarat, India. The combination product M LEVO-OZ Suspension 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 using Empower 2 software. Separation was achieved by Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Merck, Germany) as a stationary phase with Phosphate Buffer: Acetonitrile (65:35 v/v) as a mobile phase at a flow rate of 0.44 ml/min, Injection volume is 1µl and detection wavelength was 294 nm in TUV detector. Column temperature was 50^{o}C and Sample temperature was 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 100mL 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 Triethylamine (1 ml / lit of buffer solution) and ortho-phosperic acid], from the prepared buffer solution 650ml is mixed with 350 ml of actonitrile in 1000 ml volumetric flask to make a mobile phase ratio buffer: acetonirile 65:35% v/v respectively. This mobile phase was used as diluents & also was used throughout study.

V. Preparation Of Standard Stock Solution

An accurately weighed Levofloxacin (10 mg) and Ornidazole (20 mg) were transferred into two different 200 mL volumetric flask, dissolved in 200 mL mobile phase produce concentration of Levofloxacin (50 µg/ml) and Ornidazole (100 µg/ml).

VI. Preparation Of Mixed Standard Working Solution

Accurately weighed Levofloxacin (10 mg) and Ornidazole (20 mg) were transferred to 200 mL volumetric flask, dissolved in 200 mL mobile phase to produce concentration of Levofloxacin (50 µg/ml) and Ornidazole (100 µg/ml).

Fig.3 linearity of Levofloxacin

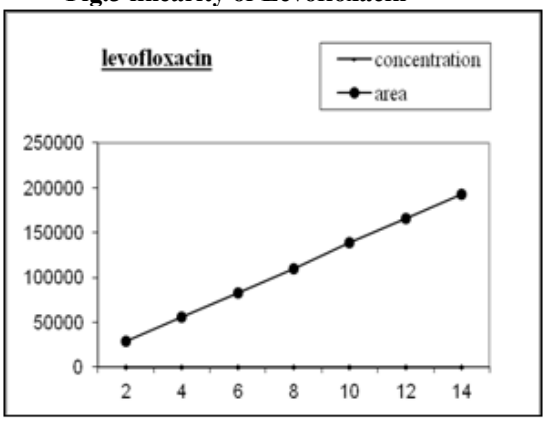
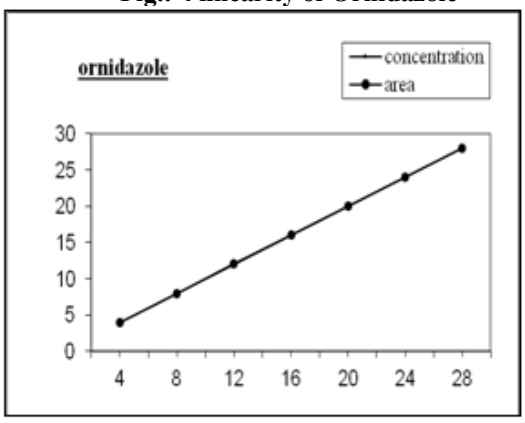


Fig.: 4 linearity of Ornidazole



VII. Preparation Of Calibration Curve

Aliquots (1.25,2.5,5.0,7.5,10.0 & 12.5 ml) of mixed standard working solutions (equivalent to 2.5,5,10,15,20 & 25 µg/ml Levofloxacin and 5,10,20,30,40 and 50 µg/ml of Ornidazole, each) 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 by following the previously described chromatographic condition 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.

VIII. Method Precision (Repeatability)

The precision of the method was checked by repeatedly injecting (n=6) injections of Levofloxacin (10ppm): Ornidazole (20ppm) without changing the parameters.

IX. Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of Levofloxacin and Ornidazole by the standard addition method. Known amounts of standard solutions of Levofloxacin and Ornidazole were added at 80%, 100% and 120 % level to pre -quantified sample solutions of Levofloxacin and Ornidazole (10 and 20 µg/ml respectively). The amounts of Levofloxacin 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 assay of Levofloxacin and Ornidazole in combine marketed formulation, M LEVO-OZ Suspension, Lable claim : levofloxacin-125mg/10ml and ornidazole-250mg/10ml was taken. From this suspension 2 ml solution was taken and transferred to 50 mL volumetric flask, dissolved in mobile phase and sonicated for 30 min. Then 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 phase. From this 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 Levofloxacin (10 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 suspension formulation.

XI. Intermediate Precision (Reproducibility)

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

The interday precision was 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 Levofloxacin (8,10 and 12 µg/ml) and Ornidazole (16, 20 and 24 µg/ml).

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

Parameters	RP-UPLC method	
	Levofloxacin	Ornidazole
Concentration range (µg/ml)	2-14	4-28
Slope	13719.97	3311.65
Intercept	742.38	-192.05
Correlation coefficient	1.0000	0.9999
LOD[a] (µg/ml)	0.122	0.221
LOQ[b] (µg/ml)	0.370	0.671
Accuracy	99.95%	99.89%
Repeatability (%RSD[C], n=6)	0.219	0.211
Intraday (n=3) (%RSD[C])	0.563	0.784
Interday (n=3) (%RSD[C])	0.448	0.627

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)
Levofloxacin	I	8	80%	100.13
	II	10	100%	101.08
	III	12	120%	100.81
Ornidazole	I	16	80%	99.91
	II	20	100%	100.04
	III	24	120%	99.96

Table 3: System suitability test

Parameters	Levofloxacin (n=6)	Ornidazole (n=6)
Retention time (min)	0.537	0.938
Tailing factor	0.99	0.83
Theoretical plates	2222.6	5732.0
Resolution	8.85	

Table 4: Analysis of marketed formulation of levofloxacin and ornidazole by proposed RP-UPLC

Suspension	Label claim (per 10ml)		Amount found (per 10 ml)		%Label claim	
	Levofloxacin	Ornidazole	levofloxacin	Ornidazole	levofloxacin	Ornidazole
I	125 mg	250 mg	124.7	249.8	99.76 %	99.92 %

Table 5: Robustness study

Conditions	RT (min)		Assay (%)	
Flow rate variation	Levofloxacin	ornidazole	Levofloxacin	ornidazole
0.43 ml/min	0.542	0.946	99.67	99.45
0.44 ml/min	0.532	0.930	100.21	100.05
0.45 ml/min	0.516	0.902	99.51	99.82
Column : sample temperature	Levofloxacin	ornidazole	Levofloxacin	ornidazole
48°C:20°C	0.528	0.925	99.98	99.52
48°C:22°C	0.528	0.924	100.24	99.85
50°C:20°C	0.527	0.921	99.94	100.34
50°C:22°C	0.527	0.921	99.52	99.67
52°C:20°C	0.525	0.908	100.03	99.48
Different Analyst	Levofloxacin	ornidazole	Levofloxacin	ornidazole
Analyst 1	0.525	0.912	100.35	99.56
Analyst 2	0.525	0.913	100.21	100.59
Analyst 3	0.524	0.912	99.58	99.94
Mobile Phase ratio (Buffer:ACN)	Levofloxacin	ornidazole	Levofloxacin	ornidazole
63:37 V/V	0.511	0.870	99.62	99.24
65:35 V/V	0.526	0.920	99.56	99.75
67:33 V/V	0.546	0.980	100.05	99.27

(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 Levofloxacin and Ornidazole. The results of force degradation study are explained in Table 6.

Table 6: Specificity Study

Stress condition	Time duration	%Degradation	
		Levofloxacin	Levofloxacin
Acid degradation	1 Hour	27.65	19.43
Base degradation	1 Hour	25.80	54.16
Oxidative degradation	1 Hour	33.33	65.96
Thermal degradation	1 Hour	28.42	26.88
Photo degradation	48 Hour	23.61	35.86

XIII. Conclusion

A stability indicating UPLC method has been developed and validated for the determination of Levofloxacin 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 Levofloxacin and Ornidazole in their combined pharmaceutical dosage form.

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