ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LEVOFLOXACIN AND ORNIDAZOLE TABLETS BY RP-HPLC METHOD

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Abstract

Levofloxacin is a third-generation fluoroquinolone antibacterial agent with a broad-spectrum activity against Gram-positive and Gram-negative bacteria and atypical pathogens. It shows its bactericidal activity by inhibiting topoisomerase IV and DNA gyrase. Levofloxacin has shown strong antibacterial activity against Staphylococcus species, Streptococcus pyogenes, Streptococcus pneumoniae. It also possesses killing effect on resistant mycobacteria like Mycobacterium tuberculosis and Mycobacterium leprae causing tuberculosis and even Mycoplasma species of HIV infection. It is indicated in the treatment of urinary tract infection, respiratory tract infection, biliary tract infection.
Ornidazole is a 5-nitroimidazole derivative active against protozoa and anaerobic bacteria. It is converted to reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to a protein synthesis inhibition and cell death in susceptible organisms.
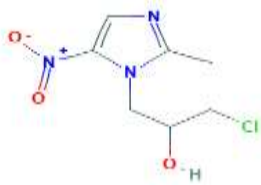
Keywords

third-generation fluoroquinolone, 5-nitroimidazole, Gram-positive, Gram-negative bacteria, topoisomerase IV, DNA gyrase, bactericide, parasitic, Hansen's Disease, Leprosy.

Molecular Structure	
Molecular	$C_{18}H_{20}FN_3O_4$
Molecular Name	(-)-(S)-9-**fluoro**-2,3-**dihydro**-3-methyl-10-(4-methyl-1-**piperazinyl**)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid
Molecular	361.368g/mol
Category	Antibacterial agent, anti-infective
Solubility	Freely soluble in glacial acetic acid and chloroform and sparingly soluble in water.
Characteristics	Yellowish white to yellow powder.
Storage	Store in a well closed container.
Storage	25^0C
CAS Registry	100986-85-4

INTRODUCTION

Chromatography is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. HPLC is the term used to describe A liquid

mobile phase is pumped under pressure through a stainless-

Molecular Structure	
Molecular formula	$C_7H_{10}ClN_3O_3$
Molecular name	1-(3-Chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole
Molecular weight	219.695g/mol
Category	Anti-protozoal antibiotic, Anti-microbial.
Solubility	Insoluble in non-polar solvents, highly soluble in moderate and polar solvents
Characteristics	White to light yellow crystalline powder
Storage	Store in a well closed container.
Melting Point	$85^0C - 90^0C$
Storage	-20^0C
CAS registry No.	16773-42-5

steel column containing particles of stationary phase with a diameter of 3-10μm. The analyte is loaded onto the head of the column via a loop value. The separation of a mixture occurs according to the relative lengths of time spent by its components in the stationary phase. HPLC is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in various dosage forms and biological fluids.

In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either be polar or non-polar.

Adsorption chromatography employs high-surface area particles that absorb the solute molecules. Usually, a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography. In adsorption chromatography, adsorption process is described by competition model and solvent interaction model.

DRUG PROFILE

Levofloxacin

Levofloxacin is a Broad-spectrum antibacterial agent. It acts by inhibiting the enzyme DNA gyrase (Topoisomerase 2) and Topoisomerase 4.

DNA gyrase helps in the formation of a highly condensed 3-dimensional structure of the DNA by its nicking and closing activity and also by introducing negative supercoil into the DNA double helix. Levofloxacin inhibits DNA gyrase which results in abnormal linkage between opened DNA and gyrase and negative super coiling is also impaired. This will inhibit

transcription of DNA into RNA and subsequent protein synthesis. Increased concentration of cyclosporin or tacrolimus reduced absorption with diagnosing, ferrous sulphate or dietary supplements containing zinc, calcium, magnesium or iron may increase plasma level of Theophylline, Increased half-life and decreased clearance of procainamide, Altered glucose levels with Anti-diabetic agents, Increased risk of CNS stimulation and seizures with NSAID'S, Increased risk of ventricular arrythmias with QT prolonging drugs like Quinidine, Sotalol.
Side effects: Nausea, Vomiting, Diarrhea, Abdominal discomfort, restlessness, Dizziness, Drowsiness.

Ornidazole: Ornidazole is a nitro-imidazole which has broad

spectrum cidal activity against protozoa and some anaerobic bacteria. Its selective toxicity to anaerobic microbes involves,

1. Drug enters the cell by diffusion
2. Nitro group of drugs is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules.
3. DNA helix destabilization and strand breakage has been observed.

Potentiates effect of coumarin type anti-coagulants. Prolongs the muscle-relaxant effect of vecuronium bromide.
Side effects: Nausea, vomiting, taste disturbances, liver impairment, skin interact ions like rash, itching and inflammation. Drowsiness, Dizziness, Headache, Epilepsy and temporary loss of consciousness and fainting.

MATERIALS AND METHODS
Materials
Chemicals: Levofloxacin (Levo) and Ornidazole (Orni) reference standards, Acetonitrile is of HPLC grade, Analytical reagent grade potassium dihydrogen orthophosphate and other chemicals of analytical grade, Triethylamine, Deionized and 0.45 μm membrane filter. The tablet formulation containing 250mg of Levofloxacin and 500 mg of Ornidazole per tablet.

Instrumentation: The HPLC system consisted of a Shimadzu (LC 2010 HT) equipped with LC 2010 HT solvent delivery system, photo diode array detector and auto sampler. Data acquisition was performed by using Chromeleon data software provided with the system.

Table 1: Details of Instruments Used

| Name of the Instruments	Manufactured	Model
Balance	Mettler Toledo	XP205
HPLC	Shimadzu	LC 2010 HT
Digital pH meter	Metrohm	867pH Module

METHOD DEVELOPMENT
Preparation of Standard Stock Solution: Accurately weigh and transfer 62 mg of Levofloxacin and 125 mg of Ornidazole

into 50 ml volumetric flask. Add about 30 ml of diluent and sonicate for about 10 mins with intermittent shaking to dissolve the contents and finally dilute to 50 ml with diluent, mix well. Further pipette and dilute 5 ml of resulting solution to 100 ml with volumetric flask, dilute to volume with mobile phase. This solution is used for recording chromatogram. For every trial, the standard stock solution was prepared on similar lines.

Development Trial:

Table 2: Chromatographic Conditions

Column	C-18 Luna Phenomenex (250 x 4.6 mm, 5 µ)
Elution mode	Isocratic
Mobile Phase (Ratio)	Phosphate buffer: Acetonitrile: Triethylamine (60:40:0.2mL)
Flow rate	1ml/min
Detection Wavelength	300nm
Injection volume	20µl
Run time	15 min
Column Temperature	36°C
Sample Temperature	25°C
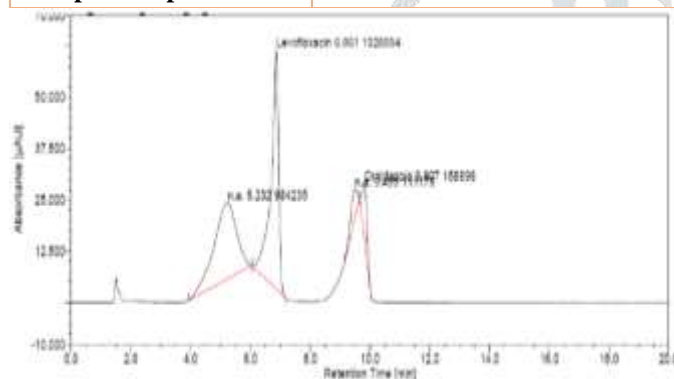
Fig.1 Chromatogram of Levo and Orni (trail)

SELECTION OF PARAMETERS

Mobile Phase Selection: On the basis of literature survey, previous experiences and several exploratory efforts, the chromatographic compatibility was achieved by using a combination of buffer, acetonitrile and triethylamine in the ratio of 85:15:0.2 as an isocratic elution. This gives the best results as a mobile phase.

Column Selection: Column selection is the most important part in the method development. Considering the column chemistry parameters, the most suitable selected column was Luna Phenomenex (250 x 4.6 mm, 5 µm).

Detection Wavelength Selection: After screening the standard solution over 200 nm to 400nm wavelength using the advantage of UV detector, based on the peak absorption maxima of analyte, the 300 nm was decided as the detection wavelength. This gives the maximum chromatographic compatibility to the method.

Preparation of Mobile Phase: Mix buffer, acetonitrile and triethylamine in the ratio of 85:15:0.2 v/v and mix well and sonicate to degas it.

Blank: Use mobile phase as blank.

Diluent: Mix acetonitrile and 0.5N HCl in the ratio of 1:1v/v and mix well.

METHOD VALIDATION

Developed method for the determination of LEVO and ORNI in bulk drug as well as in pharmaceutical dosage form is further validated as per ICH Q2R1 guidelines.

System Suitability: The system suitability test was assessed from six replicate injections.

Linearity: Standard solution was prepared by transferring 124mg of Levo and 250mg of Orni working standards into a 100 ml volumetric flask, 70ml of diluent was added and the mixture was sonicated for 10 min to dissolve and make up the volume with diluent. The standard solution was transferred using A-grade bulb pipette into 100 ml volumetric flask and made up to volume with mobile phase to get final concentrations of 29.6-93 µg/ml and 62.4-187.5 µg/ml for LEVO and ORNI, respectively. The solutions were then filtered through a 0.45 µ membrane filter. Each solution was injected three times and linearity was evaluated by linear-regression analysis.

Accuracy: It can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150%) by addition of known amounts of standard to placebo preparation.

Precision: The precision of the method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for ten determinations of peak areas of Levofloxacin and Ornidazole performed on the same day.

Robustness: Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate, change in wave length and column temperature.

Specificity:

Preparation of Standard Solution: Accurately weigh and transfer 62 mg of Levofloxacin and 125 mg of Ornidazole into 50 ml volumetric flask. Add about 30 ml of diluent and sonicate for about 10 mins with intermittent shaking to dissolve the contents and finally dilute to 50 ml with diluent, mix well. Further pipette and dilute 5 ml of resulting solution to 100 ml with volumetric flask, dilute to volume with mobile phase. This solution is used for recording chromatogram.

Preparation of Sample Preparation: For the preparation of sample solution, weigh and crush 20 tablets. Accurately weigh and transfer tablet powder equivalent to 250mg of Levofloxacin and 500mg of Ornidazole into a 200 ml volumetric flask, add 150 ml of diluent, sonicate for about 5 minutes with intermittent shaking to dissolve the contents, shake mechanically for 10 minutes finally dilute to 200 ml with diluent and mix well. Filter the solution using No.41 Whatman filter paper. Further pipette and dilute 5 ml of resulting solution to 100 ml volumetric flask and dilute to the volume with mobile phase.

Filter through 0.45 µ m nylon membrane filter and20 µl solution was injected in to the chromatographic system.

Assay: To determine the content of Levofloxacin and Ornidazole in tablets 250 mg Levofloxacin and 500 mg Ornidazole mg/tablet), twenty tablets were weighed and the content was finely powdered. The powder was weighed equivalent to 250mg of Levofloxacin and 500mg of Ornidazole, into a 200 ml volumetric flask, add about 150 ml of diluents, and sonicate for 5 min with intermittent shaking to dissolve the contents, shake mechanically for 10 minutes finally dilute to 200 ml with diluent and mix well. Filter the solution using 41 Whatman filter paper. Further pipette and dilute 5 ml of resulting solution to 100 ml volumetric flask and dilute to the volume with mobile phase to yield concentrations of Levo (62.5 µg/mL) and Orni (125µg/mL).

RESULTS AND DISCUSSION

The chromatographic conditions were optimized to develop assay method for Levofloxacin and Ornidazole in tablet dosage forms. The Phenomenex Luna column was used because of its advantages of high resolving capacity, better reproducibility, low-back pressure, and low tailing. Detection at 300nm resulted in good response and good linearity.

Table 3: Optimized Chromatographic Conditions

Parameters	Conditions
Column	Phenomenex Luna (250 × 4.6 mm, 5µm)
Mobile Phase	Phosphate Buffer: Acetonitrile: Triethylamine (85:15:0.2)
pH	2.5
Flow Rate	1.5 ml/min
Wavelength	300 nm
Injection Volume	20 µl
Column Oven Temp.	36°C
Sample Temp.	25°C
Run Time	15 minutes
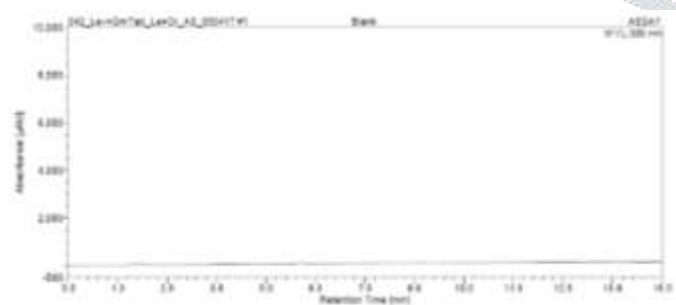
Figure 2: Blank Chromatogram of the proposed method
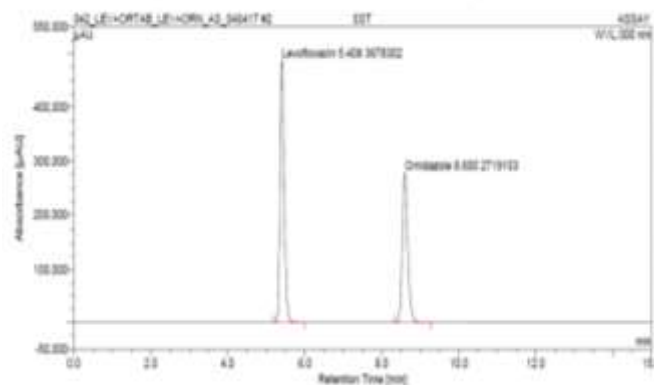
Figure 3: Optimized Chromatogram of the Proposed Method

System Suitability: The system performance parameters obtained from system suitability test were found to be within limits for Levofloxacin and Ornidazole indicating that the developed method is suitable for its intended usage.

Table 4: System suitability parameters of the proposed method

Parameters	Levo	Orni
Peak area	3678302	2719103
Retention time	5.408	8.60
Tailing Factor	1.25	1.20
Theoretical plate number	11957	18783
Resolution	14.22	

* Six replicate injections

Linearity: The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient. The data of regression analysis and calibration curve were shown in Table 5 & 6, Figure 4 & 5. Correlation coefficient of the linearity study were found to R2 = 0.996 and 0.999 with linear regression equations y=59069x+19820and y=21860x+43688 for Levo and Orni respectively, which proves that the method is highly linear over the working range of 29.6-93 µg/ml and 62.4-187.5 µ g/ml for Levo and Orni, respectively**.**
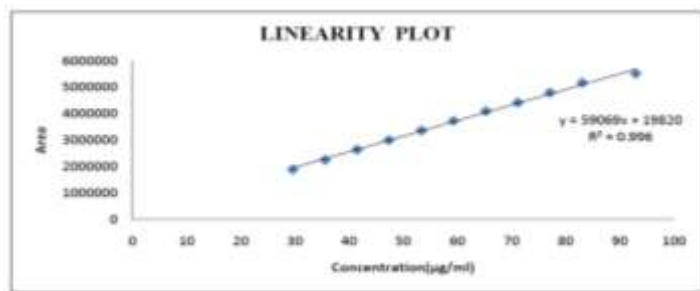
Table 5: Linearity Data of Levofloxacin

Analyte	Concentration (µg/ml)	Peak area*	Linear regression Equation
Levofloxacin	29.6	1895350	y = 59069x +19820 R² = 0.996
	35.6	2258515	
	41.5	2643516	
	47.4	3008018	
	53.4	3374865	
	59.3	3738104	
	65.3	4105033	
	71.2	4428269	
	77.1	4801871	
	83.1	5180212	
	93	5525380	

* Six replicate injections

Table 6: Linearity Data of Ornidazole

Analyte	Concentration (µg/ml)	Peak area*	Linear regression Equation
Ornidazole	**62.4**	139977	
	74.9	167155	
	87.4	195841	
	99.9	223164	
	112.3	250810	**y = 21860x + 43688**
	124.8	278026	**R² = 0.999**
	137.3	305825	
	149.8	330393	
	162.3	358444	
	174.8	387129	
	187.5	413516	

* *Six replicate injections*

Figure 4: Calibration Curve for Levofloxacin
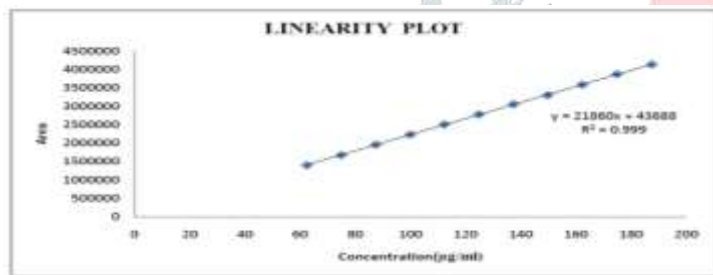
Figure 5: Calibration Curve for Ornidazole

Table 7: Linearity Data of Levofloxacin and Ornidazole

Parameters	Levofloxacin	Ornidazole
Slope	59069	21860
Intercept	19820	43688
Correlation coefficient (R²)	0.9960	0.999

Accuracy: Commonly used formulation excipients were subjected to chromatographic analysis and it was observed that there was no interfering peak at the retention time of Levofloxacin and Ornidazole.

**Table 8: Recovery Studies of Levofloxacin**

Spiked level	Sample taken (mg)	Peak area*	Sample Recovery (mg)	Recovery	
				% Recovery	%RSD
50	119.14	185153	119.7		
	119.59	186424	120.5	100.6	0.2
	119.62	186016	120.3		
100	239.19	374800	242.4		
	239.4	372186	240.7	100.9	0.4
	238.96	372157	240.7		

* *Six replicate injections*
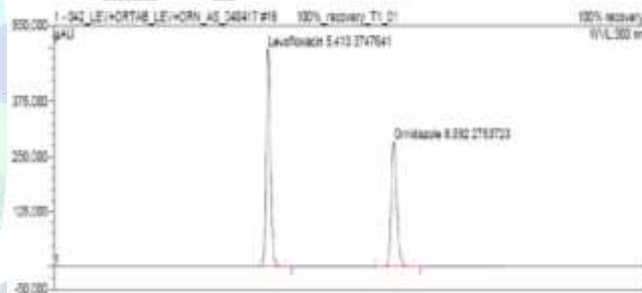
Table 9: Recovery Studies of Ornidazole

Spiked level	Sample taken (mg)	Peak area*	Sample Recovery (mg)	Recovery	
				% Recovery	%RSD
50	251.06	13687	251.8		
	249.94	13683	251.7	100.5	0.2
	249.99	13660	251.3		
100	499.64	27643	508.5		
	499.44	27489	505.7	101.5	0.3
	499.02	27502	505.9		

* *Six replicate injections*
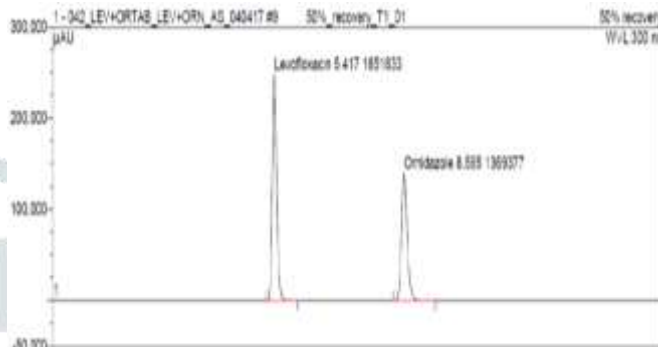
Figure 6: Accuracy Chromatogram of Levofloxacin and Ornidazole at 50%

Figure 7: Accuracy Chromatogram of Levofloxacin and Ornidazole at 100%

From the above data it has been proven that the percentage recovery is within the limits of 98 to 102 % and % RSD value is below 2 %. Hence this suggests that proposed method is highly accurate. The average percent recoveries obtained as 99.8 - 101.5%, indicating that the method was accurate.

Precision: Injection repeatability values (%RSD) of Levo and Orni were found to be 0.99 and 0.77, respectively. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzed the samples by using same instrument and same laboratory. There were no significant difference between %RSD values, which indicates that the proposed method was reproducible.

**Table 10: Precision Data of the Proposed Method for Levofloxacin**

| Concentration	Injection number	Standard Area	Sample Area
Levofloxacin (60μg/mL)	1	3905276	4119417
	2	3909826	4180593
	3	3910329	4211544
	4	3906614	4221404
	5	3908064	4219590
	6	3909017	4231076
Mean		3908188	4197271
Standard deviation		1945.6	41872.3
%RSD		0.04	0.99

*%RSD Values

Table 11: Precision Data of the Proposed Method for Ornidazole

Concentration	Injection number	Standard Area	Sample Area
Levofloxacin (60μg/mL)	1	2762498	2721540
	2	2765275	2722650
	3	2767056	2721679
	4	2764371	2762320
	5	2766874	2760926
	6	2767043	2758776
Mean		2765520	2741315
Standard deviation		1845.26	21240
%RSD		0.06	0.77

*%RSD Values
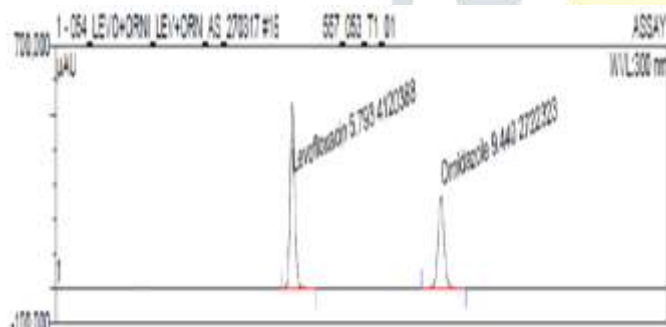
**Figure 8: Precision Chromatogram of Levofloxacin and Ornidazole at Injection 1**
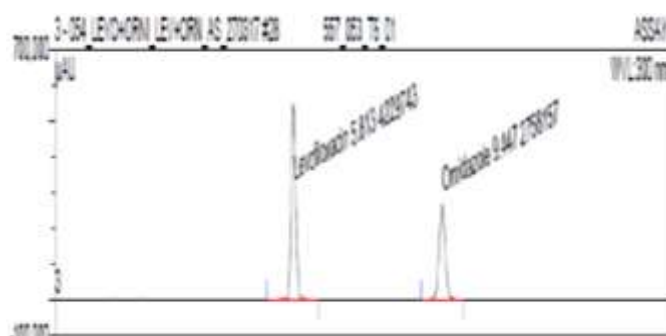
Figure 9: Precision Chromatogram of Levofloxacin and Ornidazole at Injection 6

Robustness: The robustness of the method was assessed by assaying the test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition, the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value. System suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions. The results (Table 12 and 13) obtained for selected factors remained unaffected by small variations of these parameters indicated that the method was robust.

Table 12: Robustness Data of Developed Method – Levofloxacin

Parameter	Changes in the Chromatographic conditions	Area 1	Area 2	Mean	Std. Dev	%RS
Flow rate	1.3 ml (Low)	4562 911	4549 780	4556 346	928 5.02	0.2
	1.5 ml (Original)	3952 674	3952 252	3952 463	298. 399	0.00
	1.7 ml (High)	3507 943	3486 988	3497 466	148 17.4	0.42
Column Temperature	34°C (Low)	4015 999	3948 751	3982 375	475 51.5	1.19
	36°C (Original)	3952 674	3952 252	3952 463	298. 399	0.00
	38°C (High)	4032 859	3951 320	3992 089	576 56.8	1.44
Wavelength	225 nm (Low)	4427 812	4382 746	4405 279	318 66.5	0.72
	230 nm (Original)	3952 674	3952 252	3952 463	298. 399	0.00
	235 nm (High)	3592 169	3543 608	3567 888	343 37.8	1.01

Six replicate injections
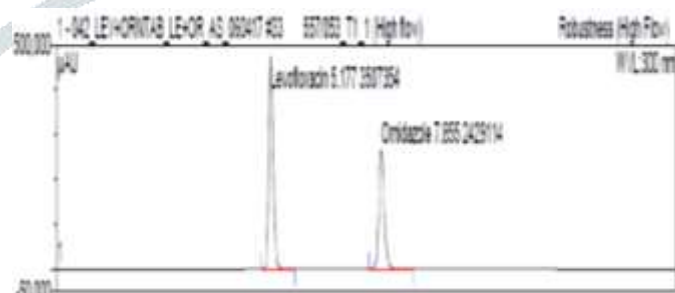
Figure 10: Chromatogram Showing Variation of Flow Rate (High)

Table 13: Robustness Data of Developed Method – Ornidazole

| Parameter	Changes in the Chromatographic conditions	Area 1	Area 2	Mean	Std. Dev	%RSD
Flow rate	1.3 ml (Low)	3161973	3149820	3155897	8593.47	0.27
	1.5 ml (Original)	2737166	2736749	2736958	294.864	0.01
	1.7 ml (High)	2429333	2412099	2420716	12186.3	0.50
Column Temperature	34°C (Low)	2771720	2721327	2746523	35633.2	1.29
	36°C (Original)	2737166	2736749	2736958	294.864	0.01
	38°C (High)	2789745	2730511	2760128	41884.8	1.51
Wavelength	298 nm (Low)	2619107	2590721	2604914	20071.9	0.77
	300nm (Original)	2737166	2736749	2736958	294.864	0.01
	302 nm (High)	2876571	2834195	2855383	29964.4	1.04

* Six replicate injections
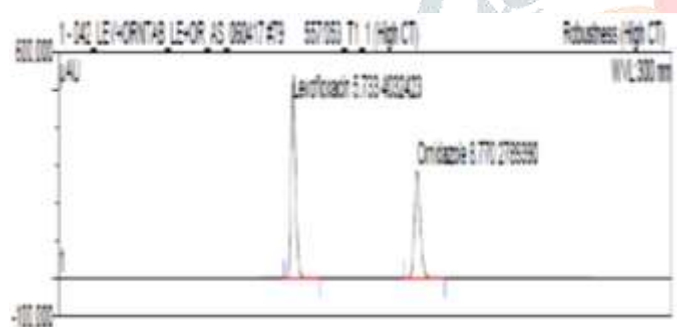
Figure 11: Chromatogram Showing Variation of Column Temperature (high)
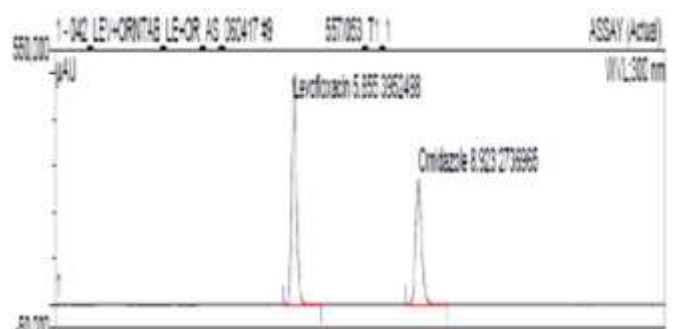

Figure 12: Chromatogram Showing Variation of Wavelength (High)

LOD & LOQ: Signal to noise ratios of 3:1 and 10:1 was obtained for the LOD and LOQ respectively.

Table 14: LOD and LOQ Values

Sr. No.	LEVOFLOXACIN	ORNIDAZOLE
LOD	2.339μg/mL	7.088μg/mL
LOQ	3.206μg/mL	9.716μg/mL

Assay: The % assay can be calculated by using the formula = AT/AS*WS/DS*DT/WT*P/LC*AVG.WT/100*100

The proposed method was applied to the analysis of marketed formulations and the results obtained were given in Table 15. The results were indicated that the method is suitable for routine analysis of Levofloxacin and Ornidazole in pharmaceutical dosage forms.

Table 15: Assay results for optimized method

Tablet Formulation	Label Claimer Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)
Levofloxacin 250mg + Ornidazole 500mg	Levo-250	101.3	0.04
	Orni-500	99.0	0.03

SUMMARY

This research describes Reversed-phase high-performance liquid chromatographic method which has been developed and validated for simultaneous estimation of Levofloxacin and Ornidazole in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Phenomenex Luna (250 x 4.6 mm,5μm) with potassium di-hydrogen ortho phosphate buffer: Acetonitrile: triethylamine (850:150:0.2), pH 2.5 (adjusted with ortho - phosphoric acid) and detection at 300nm. The flow rate was kept at 1.5mL/min and injection volume 20μl. The separation was performed at 360C for column and 250C for sample. Retention time of Levofloxacin and Ornidazole was found to be 5.408 and 8.600 minutes respectively. Linearity of the method was found to be for Levofloxacin 29.6-93μg/ml and for Ornidazole 62.4-187.5μg/ml respectively. The correlation coefficient of Levofloxacin was found to be 0.996 and Ornidazole is 0.999. Accuracy of the method was determined at spiking levels of 50%, 100%, 150%and was found to be 99.8%-100.9% for Levofloxacin and 100.5%-101.5% for Ornidazole respectively and precision of the method was demonstrated that % RSD is less than 2%. The system suitability parameters such as theoretical plates and tailing factor were found to be 11957 & 1.25 and 18783 & 1.20 respectively for Levofloxacin and Ornidazole.

CONCLUSION

The linearity studies were performed for the standard and found to be linear. The precision was checked and found to be within limits, hence the method is precise. From accuracy studies, % recovery was calculated and found to be within limits. The ruggedness of the method was checked on different systems and by different columns and standard was able to give same results

which indicate that the method is rugged. The robustness of the method was checked by changing flow rate and temperature, and standard was able to give system suitability parameters within limit, which indicates that the method is robust. The developed method for the simultaneous estimation of Levofloxacin and Ornidazole has the advantages of sensitivity, accuracy, precision and low cost. The non-interference of tablet excipients makes the method suitable for the determination of these drugs in tablets, and hence can be used for routine quality control of Levofloxacin and Ornidazole in this dosage form.

REFERENCES

1. Nagavalli D, Rekha Rajeev Kumar. RP-HPLC Method Development and validation for the Simultaneous Estimation of Levofloxacin hemihydrates and Ornidazole on tablets. International Journal of Pharma-tech Research, Oct-Dec 2009; 1(4): 1161-1163.

2. Shafrose Syed, Haritha Pavani. Validated Simultaneous Estimation and Development of Levofloxacin and Ornidazole by RP-HPLC Method. International Journal of Pharmaceutical and Clinical Research 2012; 4(4): 52-55.

3. Sevak Manan, Patel Nirav. Stability indicating method development & validation on RPUPLC for simultaneous estimation of Levofloxacin and Ornidazole in their combine dosage form. IOSR Journal of Applied Chemistry, Sep 2014, Volume 7, 27-31.

4. Surendra Kumar Jain, Meena Singh. Spectrophotometric & RP_HPLC Method Development and Validation for Simultaneous Estimation of Levofloxacin & Ornidazole. International Journal of Pharmaceutical Sciences and Research, 2014; 0975-8232.

5. Surendra Kumar Jain, Meena Singh. Spectrophotometric & RP_HPLC Method Development and Validation for Simultaneous Estimation of Levofloxacin & Ornidazole. International Journal of Pharmaceutical Sciences and Research, 2014; 0975-8232.

6. Alfred Goodman Gilman, Louis S. Goodman, Theodore W. Rall, Ferid Murad. Goodman and Gilman's the pharmacological basis of therapeutic" 7th edition, 1985. Macmillan publishing company.

7. Ambrose PG, Owens RC, Quintiliani R, Nightingale CH. New generations of quinolones: with particular attension to levofloxacin". Conn Med 1997; 61:269-72.

8. Garey KW, Amsden GW. "Trovafloxacin: an overview". Pharmacotherapy 1999; 19:21- 34.

9. Stein, GE. Pharmacokinetics and pharmacodynamics of newer fluoroquinolones. Clin Infect Dis. 1996; 23(1): S19-24.

10. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch. Practical HPLC method development, 2nd ed.; Wiley India Pvt. Ltd.: New Delhi, India 2011.

11. Rashmi. M An introduction to analytical method development for pharmaceutical formulations. Pharm Rev 2008; 6: 1-10.

12. Michael W. Dong. A three –pronged template approach for rapid HPLC method development. LCGC Europe 2013; 26(8): 455-60.

13. Parag Gadkari. Challenges in analytical method development for drug products. Pharma Times 2012; 44(1): 22-4.

14. Chatwal R. G, Anand K. S, High performance liquid chromatography, Instrumental methods of chemical analysis, 5th Ed., Himalaya Publishers, Mumbai, 2010. pp 2.570- 2.629.

15. Sharma B. K. High performance liquid chromatography, Instrumental methods of chemical analysis, 24th Ed., Goel Publishing House, Meerut, 2005. pp 295- 300.