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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levofloxacin Hemihydrate and Ornidazole in Pharmaceutical Dosage Form

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ABSTRACT

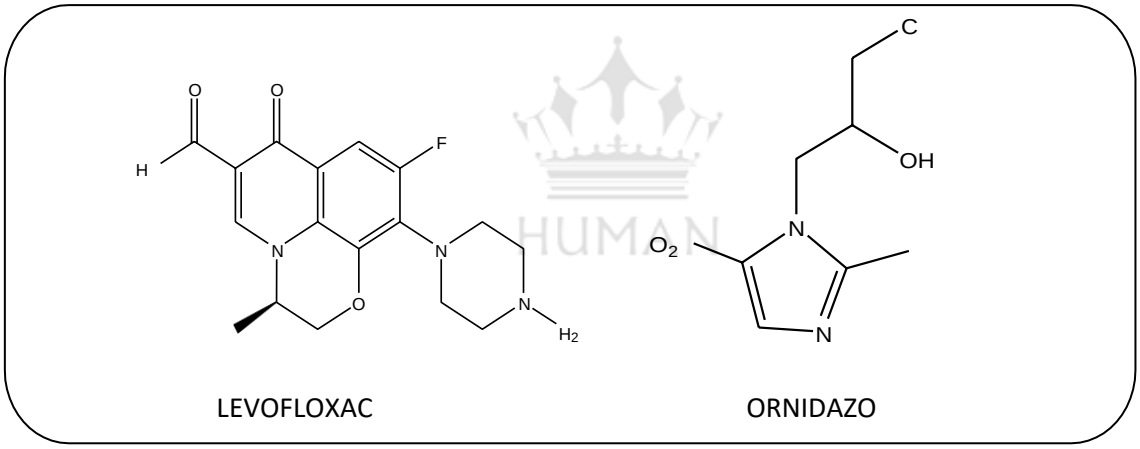
A simple, sensitive, rapid, and selective isocratic reversed-phase High-Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous estimation of Levofloxacin hemihydrate and Ornidazole from pharmaceutical dosage form using a mobile phase consisting of a mixture of Acetonitrile: Buffer (pH 3.15) (70:30v/v) Composition of buffer:(1ml triethylamine in 500ml HPLC water and pH adjusted to 3.15 using orthophosphoric acid)at the flow rate of 1.0 mL/min AKromasil C18 (250 cm x 4.6 mm, 5 µm) column was used as stationary phase. The retention time of LFX and ORN was 2.47 min and 5.65 min, respectively. The eluent was detected at 315 nm. Linearity was observed in the concentration range of 10-80ppm for Levofloxacin hemihydrate and Ornidazole. Percent recoveries obtained for Levofloxacin hemihydrate and Ornidazole were 99.95% and 99.38% respectively. The proposed method is precise, accurate, selective, and rapid for the simultaneous determination of Levofloxacin hemihydrate and ornidazole.

INTRODUCTION

Levofloxacin is chemically (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl piperazine-1-yl -7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid . It is used to treat Pneumonia and exacerbations of chronic bronchitis, sinusitis, enteric fevers, Pyelonephritis, and Skin/Softtissueinfections.[1]

Ornidazoleischemically1-chloro-3-(2-methyl-5-nitro-1H-imidazole-1-yl)propane-2-olandis official in the pharmacopoeia. Ornidazole is an imidazole derivative that is used to treat someprotozoaninfections.[1]

A Fixed-dose combination containing LFX (250mg) and ORN (500mg) is available in the market as tablets. This study aimed to develop a simple, specific, sensitive, precise, and accurate is ocratic reversed-phase HPLC method for simultaneous estimation of LFX and ORN in tablets.



MATERIALSANDMETHODS:

Drugs:

Levofloxacin Hemihydrate (LFX) and Ornidazole (ORN). Trade drug product (Relent)

Chemicals and Solvents:

Acetonitrile, Trietyleamine and O-phosphoric acid were issued from Kaytross ACG Life Sciences Pharmaceuticals (Nasik), India. The gift samples of the drug (LFX and ORN) were received from Aurochem Pharmaceuticals (Mumbai), India.

HPLC system:

The HPLC system consisted of a delivery pump, a reversed-phase analytical column C-18(250 × 4.6 mm), 5 µm (kromasil) a Rheodyne sample injector with a 20 µL loop volume and available wavelength (UV-Vis) detector.[4,6]

Chromatographic Conditions:

The following optimized parameters were used as a final method for the simultaneous estimation of Levofloxacin hemihydrate (LFX)and ornidazole (ORN).[4,5]

Table No.1 Chromatographic Conditions.

Instrument	**Conditions**
Column	KromasilC18(250x4.6 mm,5 µm)
Mobile phase	Acetonitrile: Phosphate buffer (pH 3.15) (70:30v/v), Composition of buffer:(1ml triethylaminein500ml HPLC water and pH adjusted to 3.15usingorthophosphoric acid).
Flowrate	1.0mL/min
Wavelength	315nm
Injection volume	20µl
Runtime	10min
Temperature	Ambient
Mode of Operation	Isocratic elution

Standard solution and calibration curve:

A standard stock solution of LFX (125µg/ml), ORN (250µg/ml) [125 mg and 250 mg in100 mL respectively] was prepared in water for HPLC. Subsequent dilutions were made in the mobilephasetogivetheconcentrations10,20,40,60and80µg/mLforLFXandORNrespectively.
The calibration curve was obtained by plotting the ratio of peak area of drug versus concentration.

Assay:

Twenty tablets were weighed accurately and finely powdered. The powder (0.125g) equivalent to12 5.8 mg of LFX and 250.2 mg of ORN was weighed accurately and dissolved in100 mL water for HPLC. The solution was ultrasonicated for 10-15 min. and stirred magnetically for few minutes. Then it was filtered through 0.45 µm membrane filter paper. The solution was further diluted to get a solution having a concentration of 125µg/mL of LFX and 250µg/mL of ORN. 20 µL of this solution was injected in triplicate under the specified conditions. From the peak area of LFX and ORN, the number of drugs in samples was computed.[4,6,7]

Table No. 2 Assay of a marketed product

Drug	Label claim(mg)	Amount found (mg)	% Amount found	% label claim of formulation	SD	%R.S.D.
Levofloxacin hemihydrate	125	120.39	96.31	99.95	0.0033	0.45
Ornidazole	250	240.80	96.32	99.38	0.0035	0.31

Method Validation[4,7,9,10]

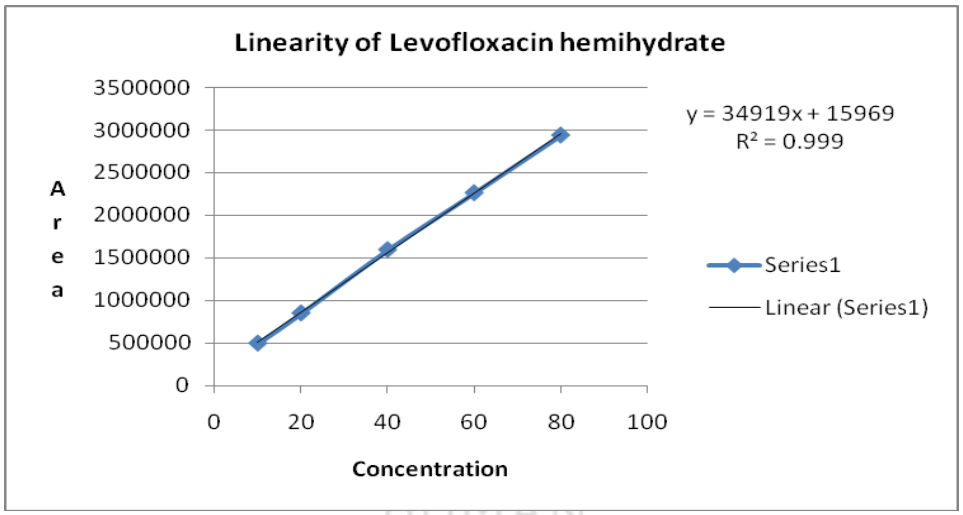
The method was validated in terms of linearity, range, specificity, accuracy, precision, the limit of detection (LOD), and the limit of quantitation (LOQ).

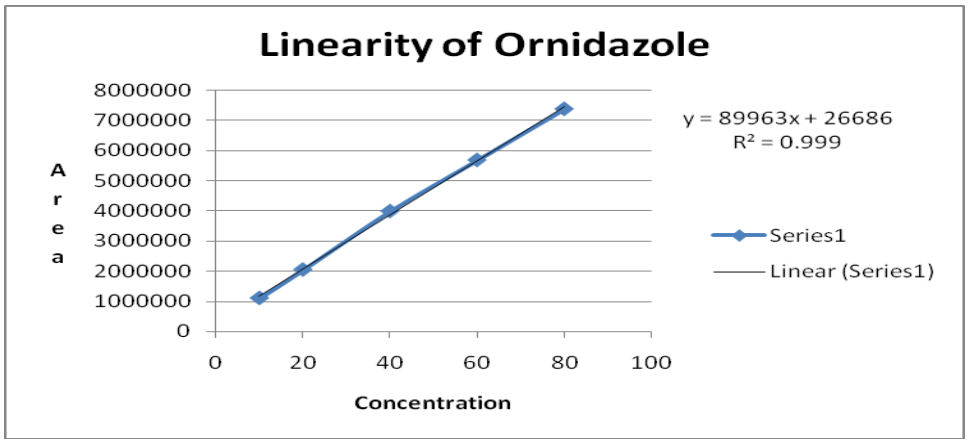
Linearity and Range

Five different concentrations (10, 20, 40,60, and 80 ppm) of LFX and ORN, were prepared for linearity studies. The responses were measured as peak areas. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration rangeof10-80 ppm for LFX and ORN.

Table No.3 Linearity study

Levofloxacin Hemihydrate (LFX)		Ornidazole (ORN)	
Concentration (ppm)	**Peak area (µV)**	**Concentration(ppm)**	**Peak area (µV)**
10	495018.91	10	1102727.26
20	848882.17	20	2046076.23
40	15920216.57	40	3992962.40
60	2259752.63	60	5992864.34
80	2935732.17	80	7391979.71





Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of standard drugs and drugs from the sample solutions, it was observed that peaks for levofloxacin Hemihydrate (LFX)and ornidazole (ORN) were obtained at retention times 2.47 min. and 5.65 min. respectively. Retention times of standard drugs and drugs from the sample solutions were the

same so the method was specific. The specificity of the method was evaluated by preparing a placebo. The solution was prepared by the procedure described in the preparation of sample solutions and injected three times. Moreover, it was used as the chromatographic peak purity tool, which is another way to verify the specificity of the method. Studies proved the absence of interference since none of the peaks of and ORN appeared at the same retention time. The interaction study in standard solution was also carried out by comparing the peak of each drug individually and in the drug mixture.

Precision

From the standard stock solutions, mixed standards containing LF and ORN were prepared. Standard solutions (n=6) were injected using a universal rheodyne injector with an injection volume of20µL. The intraday and interday precisions were determined.

Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard LFX and ORN corresponding to 115%, 125%, and 150% of the label claim was added to the pre-analyzed sample of tablet dosage form separately. The recovery studies were carried out at sixth times, at each level of recovery.

Table No. 4 Accuracy and Recovery data

Drug	% Simulated Dosage Nominal	Amount added (mg)	Mg of amount Found	% Recovery	±SD
LFX	115	143.75	142.61	99.23	0.9871
ORN	115	287.5	285.57	98.06	1.321
LFX	125	156.25	152.2	97.39	0.9567
ORN	125	312.5	300.77	96.24	1.235
LFX	150	187.5	185.3	98.93	0.9645
ORN	150	375.0	366.89	98.91	1.332

Label claim for LFX is 125mg/tab and ORN is 250mg/tab.

Table No.5 System Suitability Parameter:

Parameter	Levofloxacin Hemihydrate	Ornidazole
Rt	1.98	4.41
Rs	2.1	2.6
N	2137	2450
Tf	1.7	1.5
K'	2.4	2.7

Rt-Retentiontime, Rs-Resolution, N-Theroticalplate, Tf-Tailingfactor, K'–Capacityfactor.[3,4,5]

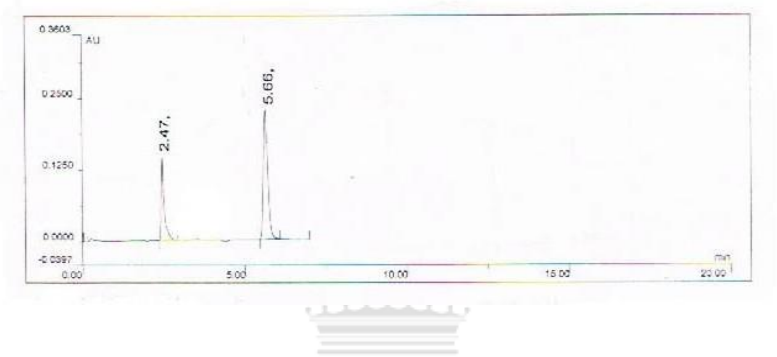


Figure 1: Typical Chromatogram of LFX and ORN.

RESULT AND DISCUSSION

Several mobile phase compositions were tried to resolve the peaks of LFX and ORN. The optimum mobile phase containing Acetonitrile: Buffer (70:30 v/v) was selected because it could resolve the peaks of LFX (Rt = 2.47) and ORN (Rt = 5.65) with a resolution factor of 7.0. The pH was adjusted to 3.15 with orthophosphoric acid. Quantification was achieved with UV detection at 315 nm based on peak area.[4,5]. A typical HPLC chromatogram obtained during the simultaneous determination of LFX and ORN is given in linear regression data showed a good relationship over a concentration range of 10-80 PPM for LFX and ORN. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.21mcg/ml and 0.65mcg/ml for LFX and 0.22 mcg/ml and 0.67mcg/ml for ORN respectively. The values indicate that the method is sensitive. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The %RSD was found to be 0.32 and 0.54 for LFX and ORN respectively. The lower values of %RSD indicate that the method is precise.[8,9,10]

Analysis of marketed tablets (Relent) was carried out using an optimized mobile phase. The % drug content of tablets obtained by the proposed method was found to be between 99.95% and 99.38%, which showed that the estimation of dosage forms was accurate within the acceptancelevelof95%to105%. The results are given in Table2.[2,3]

CONCLUSION

The method gives a a good resolution for both the drugs with a short analysis time. Percentage recovery shows that the method is free from the interference of the excipient used in the formulation. The proposed study describes an HPLC method for the estimation of LFX and ORN combinations. The validated isocratic reversed-phase method employed here proved to be simple, sensitive, fast, accurate, precise, and robust. Therefore, the proposed method can be used for routine analysis of Levofloxacin Hemihydrate and Ornidazole in the combined dosage form.

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