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

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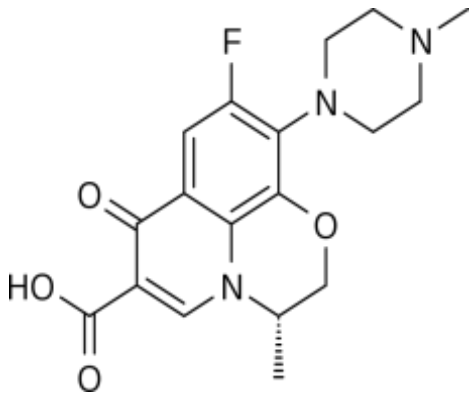
Abstract:
High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. A simple, specific and accurate reverse phase HPLC method was developed for the simultaneous determination of Levofloxacin and Ornidazole in bulk and table dosage forms. Waters C18 Column (250×4.6mm. 5µm) with mobile phase Methanol-0.1% Ortho phosphoric acid buffer (50:50) was used. The flow rate was 1 ml/min and effluent was monitored at 288 nm. The retention time of Levofloxacin and Ornidazole was 1.96min and 3.18 min, respectively. The method was validated for specificity, linearity, accuracy, and precision, robustness limit of detection and limit of quantitation. Linearity, accuracy, and precision were acceptable in the ranges. The linearity range for Levofloxacin and Ornidazole was in the range of 10-50 µg/ml,, 10.04-50.19 µg/ml, respectively. The proposed method was also validated and successfully applied to the estimation Levofloxacin and Ornidazole in both bulk and tablet formulations.
Keywords: Levofloxacin, Ornidazole, Methanol, Ortho phosphoric acid.

I.INTRODUCTION

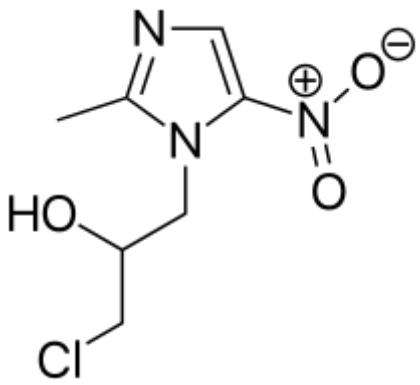
According to the literature survey it was found that few analytical methods on simultaneous estimation of Levofloxacin and Ornidazole by using HPLC were reported. The objective of the proposed method is to develope simple and accurate method for the simultaneous estimation of Levofloxacin and Ornidazole in pharmaceutical dosage forms by HPLC.

LEVOFLOXACIN



Molecular Formula – $C_{18}H_{20}FN_3O_4$
Molecular Weight – 361.373 g/mol
Solubility: Freely soluble in glacial acetic acid, chloroform; sparingly soluble in water
Uses - Levofloxacin is used to treat infections including: respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, endocarditis, meningitis, pelvic inflammatory disease, traveler's diarrhea, tuberculosis, and plague and is available by mouth, intravenously, and in eye drop form.

ORNIDAZOLE



Molecular Formula – $C_7H_{10}ClN_3O_3$
Molecular Weight – 219.63 g/mol
Solubility: Soluble in ethanol
Use - Ornidazole is an effective medicine to treat infections caused by protozoa and certain strains of anaerobic bacteria. It is used to treat infections of the stomach, intestine, urinary tract and genital area. It is also used to prevent possible infections during a surgical procedure.

II. EXPERIMENTAL WORK

A simple, specific and accurate reverse phase HPLC method was developed for the simultaneous determination of Levofloxacin and Ornidazole in bulk and table dosage forms. Waters C18 Column (250×4.6mm. 5µm) with mobile phase Methanol: 0.1% Ortho phosphoric acid buffer (50:50) was used. The flow rate was 1 ml/min and effluent was monitored at 288 nm. The retention time of Levofloxacin and Ornidazole was 1.96min and 3.18 min, respectively.

Equipment and Apparatus used:

- Electronic balance
- HPLC Schimadzu Seperation Module LC- 10AT Liquid chromatograph.
- Waters C18 Column 250×4.6mm. 5µm
- Vacuum filter pump
- Mobile phase reservoir
- Ultra Sonicator , Membrane filter(0.45 and 0.2microns)

Reagents:

- Metanol HPLC grade
- Water (HPLC)
- Ortho phosphoric acid buffer

The HPLC system was equipped with Empower2 software for data processing. Chromatographic Condition: The mobile phase containing Buffer Ortho phosphoric acid (50:50) was found to resolve Levofloxacin and Ornidazole. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to1.0ml/min. The drug shows good absorbance at 288nm, which was selected as wavelength for further analysis.

Buffer Preparation: Prepared 0.1% Ortho phosphoric acid by dissolving some amount of Ortho phosphoric acid in 1000ml of water.

Preparation of Mobile Phase: Preparedly filtered and degassed mixture of buffer and methanol in the ratio of 50:50 v/v Diluent solutions: HPLC grade water was used as diluent.

Preparation of Standard solution: Accurately weighed and transferred about 25mg of Levofloxacin and 50mg of Ornidazole working standard into a 50ml volumetric flask add 30 ml of diluent, sonicated for 15 minutes and makeup to the mark with diluent (this is standard stock solution). Transferred 5.0 ml of the above solution in to 10ml volumetric flask made up to the mark with diluent. Filter the solution through 0.45µm nylon filter.

Preparation of Sample solution: Crush 20 tablets and transferred accurately weighed powder equivalent to 25mg of Levofloxacin and 50mg of Ornidazole into 50ml volumetric flask add 30ml of diluent sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45µm nylon filter. Transfer 5ml of above solution into 10ml volumetric flask and make up to the volume with diluent.

III. METHOD DEVELOPEMENT

1) System Suitability Mixed working standard solutions were injected and chromatograms were recorded. The system suitability studies were carried out as specified in USP. These parameters include Column efficiency, Resolution, Capacity factor, Theorotical plates and Tailing factor.
2) System Precision: To assess the system precise for conducting validation inject six replicates of standard preparations of Levofloxacin & Ornidazole and expressed as %RSD of peak area.
3) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system.
4) Method Precision: Method Precision was measured in terms of repeatability of application and measurement. Repeatability of sample application was carried out using six replicates of the same sample concentration.
5) Linearity: Standard solutions of different concentrations were injected separately and chromatograms were recorded. Peak areas were recorded for each injected concentrations of drugs and the calibration curves concentration vs peak area were constructed for the drugs.
6) Accuracy (%Recovery) : %Recovery studies were carried out at three different levels of 50%, 100% and 150% of standard solution (i.e. Levofloxacin and Ornidazole API spiked to the placebo) in triplicate in each level.
7) Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, buffer composition and column temperature which may differ but the responses were still within the specified limits.

IV. RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for Levofloxacin and Ornidazole. The Mobile phase Methanol: buffer (phosphate buffer, 50:50) was found to be satisfactory and gave symmetric and well resolved peak for Levofloxacin and Ornidazole. Results were summarized in Table.1. The percentage relative standard deviation for peak area of Levofloxacin and Ornidazole in system precision was found to be 0.84 and 1.01 which indicate that the test method meets the acceptance criteria. Results were summarized in Table.2. Good resolution obtained between the analytes Levofloxacin and Ornidazole peaks and no interference of blank and placebo observed at the retention times of Levofloxacin and Ornidazole. Precision was determined & the results are represented in the form of %RSD for assay of Levofloxacin and Ornidazole were found to be below 2% & shows that the test method was highly precise. The correlation coefficient for Levofloxacin and Ornidazole was found to be 0.997 & 0.996 and shows good linearity. The data of the calibration curve was given in Table.3.The % mean recovery for Levofloxacin and Ornidazole were found to be in the range of about 100.28%-105.43% and 98.43%-104.16%.The results were summarized in Table.4. As part of the robustness, deliberate changes in the flow, column temp & buffer composition was made to impact on the method. Results were summarized in Table.5

Table-1 System suitability parameters of Levofloxacin and Ornidazole

Parameters	Levofloxacin	Ornidazole
Area	1043490	283212
Retention time	1.96	3.18
Theorotical plates	9631	22492
Tailing factor	1.46	1.18
Resolution	0.000	6.85

Table-2.1 observation on Precision of Levofloxacin

LEVOFLOXACIN		
Sample ID	Peak Retention Time	Peak Area
1	1.96	1043490
2	1.96	1023946
3	1.95	1039015
4	1.95	1044846
5	1.95	1026583
6	1.96	1034080
MEAN	**1.955**	**1035326.667**
STDEV	**0.0055**	**8699.2756**
%CV	**0.28**	**0.84**

Table-2.2 observation on Precision of Ornidazole

ORNIDAZOLE		
Sample ID	Peak Retention Time	Peak Area
1	3.18	283212
2	3.17	277620
3	3.17	281768
4	3.17	283316
5	3.16	277808
6	3.22	277620
MEAN	**3.178**	**280224.000**
	0.0214	**2838.0004**
%CV	**0.67**	**1.01**

Table-3.1 Shows Linearity observation of Levofloxacin

Concentration (Microgram/mL)	Retention Time	Peak Area	Back Calc Concentration	% Accuracy
10.00	1.95	406263	10.22	102.16
20.00	1.94	804093	20.47	102.36
30.00	1.93	1146536	29.30	97.67
40.00	1.93	1527213	39.12	97.79
50.00	1.93	1984065	50.89	101.79

Table-3.2 Shows Linearity observation of Ornidazole

Concentration (Microgram/mL)	Retention Time	Peak Area	Back Calc Concentration	% Accuracy
10.04	3.16	103579	10.26	102.14
20.08	3.15	198331	19.51	97.18
30.11	3.14	304679	29.91	99.33
40.15	3.14	392019	38.44	95.75
50.19	3.14	520270	50.98	101.57

Table-4.1Shows observation of Accuracy of Levofloxacin

SR NO	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	LQC 1	12.50	505947	1.940	12.79	101.09
2	LQC 2	12.50	512219	1.960	12.95	101.28
3	LQC 3	12.50	511818	1.950	12.24	100.70
MEAN					**12.89**	**101.12**
STDEV					**0.09**	**0.72**
% CV					**0.70**	**0.70**

SR NO	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	MQC 1	25.00	1054528	1.940	26.93	107.72
2	MQC 2	25.00	1017383	1.950	25.97	103.89
3	MQC 3	25.00	1025058	1.940	26.17	104.68
MEAN					**26.36**	**105.43**
STDEV					**0.51**	**2.02**
% CV					**1.92**	**1.92**

SR NO	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	HQC 1	37.50	1493497	1.950	38.25	101.99
2	HQC 2	37.50	1450364	1.950	37.13	99.03
3	HQC 3	37.50	1462158	1.950	37.44	99.84
MEAN					**37.61**	**100.28**
STDEV					**0.57**	**1.53**
% CV					**1.53**	**1.53**

Table-4.2Shows observation of Accuracy of Ornidazole

SR NO	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	LQC 1	13.00	135752	3.150	13.40	103.07
2	LQC 2	13.00	138466	3.180	13.66	105.11
3	LQC 3	13.00	137393	3.180	13.56	104.30
MEAN					**13.54**	**104.16**
STDEV					**0.13**	**1.03**
% CV					**0.99**	**0.99**

SR NO	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	MQC 1	26.00	275180	3.180	27.02	103.94
2	MQC 2	26.00	266486	3.170	26.17	100.67
3	MQC 3	26.00	267826	3.170	26.31	101.18
MEAN					**26.50**	**101.93**
STDEV					**0.46**	**1.76**
% CV					**1.73**	**1.73**

S.no	SAMPLE ID	Conc (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			PEAK AREA	RETENTION TIME		
1	HQC 1	39.00	398467	3.180	39.07	100.19
2	HQC 2	39.00	384269	3.180	37.69	96.63
3	HQC 3	39.00	391680	3.180	38.41	98.49
MEAN					**38.39**	**98.43**
STDEV					**0.69**	**1.78**
% CV					**1.81**	**1.81**

Table-5.1 Robustness of Levofloxacin

Proposed variations		Asymmetry factor	Acceptance Criteria
Variation in Flow Rate	0.8ml	1.43	In between 0.5 and 2.0
	1.2ml	1.44	
Variation in mobilephase composition	45:55	1.43	
	55:45	0.99	

Table-5.2 Robustness of Ornidazole

Proposed variations		Asymmetry factor	Acceptance Criteria
Variation in Flow Rate	0.8ml	1.16	In between 0.5 and 2.0
	1.2ml	1.20	
Variation in mobilephase composition	45:55	1.21	
	55:45	1.15	

Fig 1.Chromatogram of Levofloxacin and Ornidazole sample:

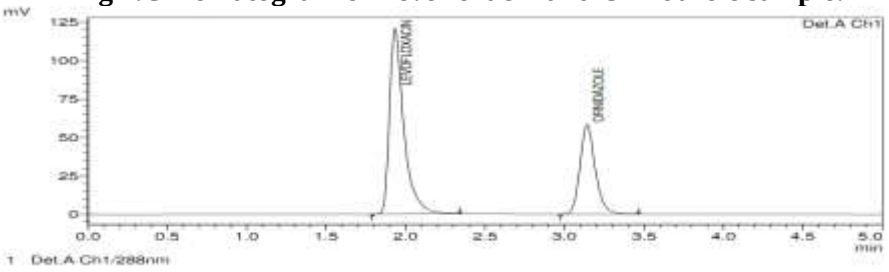


Fig 2: Chromatogram of LOQ of Levofloxacin

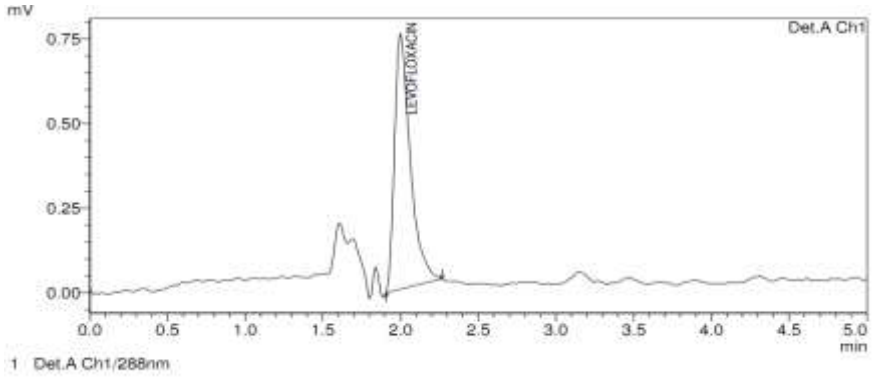
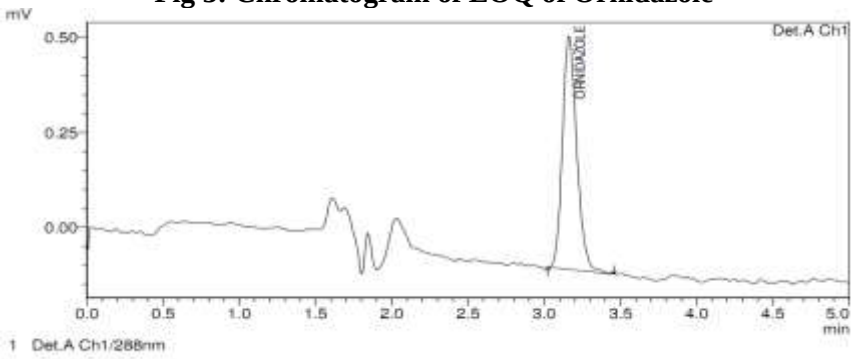


Fig 3: Chromatogram of LOQ of Ornidazole



V. CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Levofloxacin and Ornidazole in bulk and tablet dosage form with less tailing and also economical. The proposed method was validated according to ICH guidelines and correlating the obtained values with the standard values, satisfactory results were obtained.

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