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DEVELOPMENT AND VALIDATION OF STABILITY INDIATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND ORNIDAZOLE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple, accurate, sensitive and robust reversed-phase high performance liquid chromatographic (RP-HPLC) coupled with degradation study for the simultaneous determination of Levofloxacin Hemihydrate and Ornidazole has been developed. The method was performed with a Thermo scientific Grace RP-C 18 column (250 mm x 4.6 i.d., 5µ particle size) and a UV detector, using Methanol: 0.02 mol/L Phosphate buffer pH 3.0 (50:50% v/v) as the mobile phase at flow rate of 1.0 mL/min and detection wavelength at 310 nm. The run time of the method was set at 10 mins. The retention time of Levofloxacin Hemihydrate and Ornidazole in the chromatogram was recorded at 4.42±0.5 min and Ornidazole at 5.57±0.5 min respectively. Calibration curve indicated linearity range from 5- 40 µg/mL for Levofloxacin Hemihydrate and 10 -80 µg/mL for Ornidazole respectively. The mean recovery was found to be in the range from 100.10-102.20% for Levofloxacin Hemihydrate and 98.54-102.54 % for Ornidazole. To establish the stability of the method and the drug solutions degradation study was carried out in acid, base, oxidation, thermal and photolytic study in UV chamber and the percent degradation was calculated. As this method could effectively separate the drug from its degradation products, it can be used as the stability indicating method.

KEYWORDS: Levofloxacin Hemihydrate, Ornidazole, RP-HPLC.

INTRODUCTION

Levofloxacin Hemihydrate

Levofloxacin Hemihydrate is a fluoroquinolone antibiotic. It has a broad-spectrum antibiotic activity against gram-positive and gram-negative bacteria and used in respiratory and urinary tract infections. It is the levo isomer of ofloxacin. Chemically it is (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6 carboxylic acid hemihydrate (Fig.1).[2,3] It inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. This results in strand breakage on a bacterial chromosome, supercoiling and resealing, Hence DNA replication and transcription is inhibited.[4,5]

Ornidazole

Ornidazole is a nitro imidazole which has broad spectrum cidal activity against protozoa and some anaerobic bacteria.[6] Chemically it is 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol (Fig.2).[7] It is used as an antiamoebic agent for amoebic dysentery. Its selective toxicity to anaerobic microbes involves:

1) Drug enters the cell by diffusion,
2) Nitro group of the drug is reduced by redox proteins present only in anaerobic organism to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical bio molecules resulting in DNA helix destabilization and strand breakage.[8]

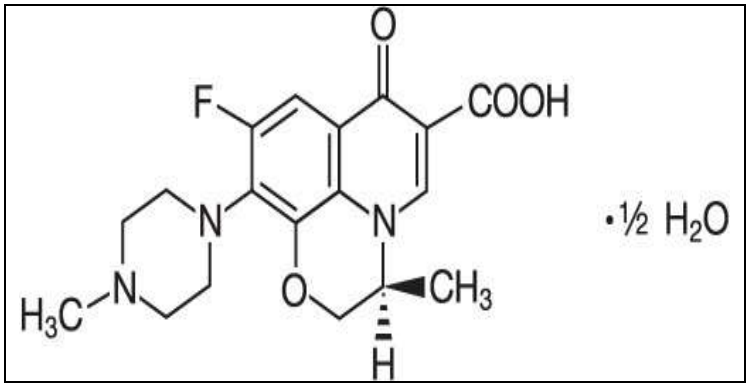

Fig. 1: Chemical structure of Levofloxacin Hemihydrate

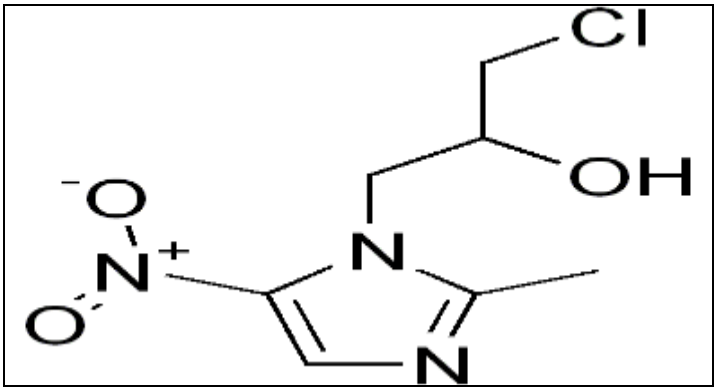

Fig. 2: Chemical structure of Ornidazole

EXPERIMENTAL

MATERIALS AND METHODS

INSTRUMENTATION AND APPARATUS

The analysis was carried out on Dionex Ultimate 3000^{+} HPLC system with reciprocating pump using Chromeleon software and variable wavelength UV detector. Column used was C18 GRACE Column having dimensions 250 mm×4.6 i.d., 5µ particle size, variable wavelength UV detector. All weight measurements were taken on WENSAR ELECTRONIC BALANCE MAB 220 at room temperature.

MATERIALS

Levofloxacin Hemihydrate was kindly gifted by Cipla Ltd., Verna Goa and Ornidazole by Cadila Healthcare Ltd, Kundaim, Goa. The tablet formulation was purchased from local market. All the reagents used in this method were of analytical grade.

CHROMATOGRAPHIC CONDITIONS

HPLC System	**Dionex Ultimate 3000^{+}**
Column	C18 GRACE column (4.6×250mm,5µm)
Mobile phase	Methanol : Phosphate buffer pH 3.0 (50:50) v/v (adjuste to pH 3.0 with o-phosphoric acid)
Flow rate	1ml/min
Injection volume	20µL
Total run time	10 min
Mode of separation	Isocratic
Detector	UV detector

SELECION OF DETECTION WAVELENGTH

As can be seen from the overlain absorption spectra (fig.3), the absorption maxima for Levofloxacin Hemihydrate and Ornidazole was found to be 294 nm and 317 nm. The

isosbestic point (wavelength where both the drug shows equal absorbance) was found to be 310 nm. Hence, 310 nm was chosen as the detection wavelength for the method.

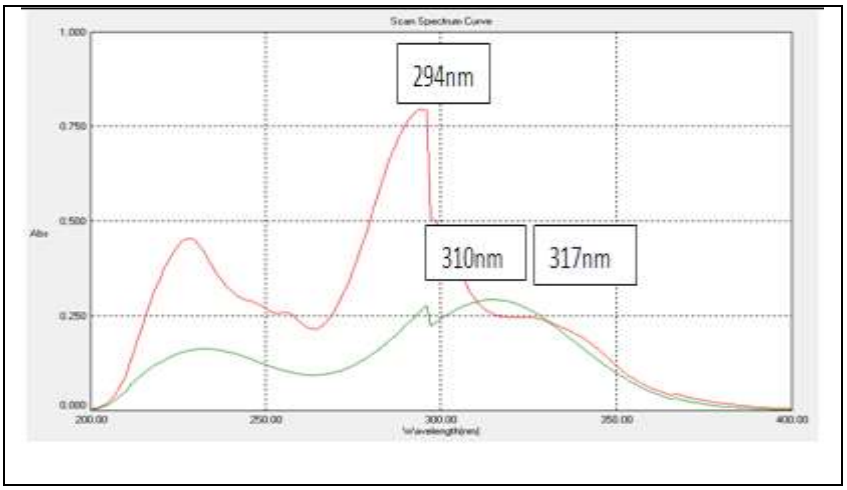

Fig. 3: Overlain spectrum 4of Levofloxacin Hemihydrate and Ornidazole in Methanol and Phosphate buffer pH 3 (50:50).

METHOD DEVELOPMENT

Preparation of standard stock solution

Accurately weigh and transfer 50mg of Levofloxacin Hemihydrate and Ornidazole into two separate 50ml volumetric flask add about 25ml of the mobile phase and shake till the drug dissolves completely and make up the volume with the same mobile phase to get 1000 µg/ml of standard stock solution of both Levofloxacin Hemihydrate and Ornidazole.

Preparation of working standard solutions

Transfer separately 5ml of each drug solution into two 50ml calibrated volumetric flasks to obtain the concentration of 100µg/ml of working standard solution.

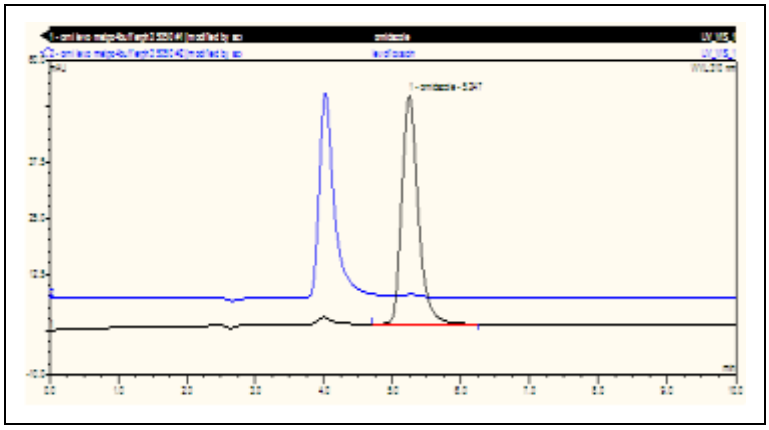

Fig. 4: Chromatogram of Standard Levofloxacin Hemihydrate and Ornidazole

Preparation of Calibration graph

The linearity of the method was determined in the concentration range of 10-80 µg/ml for Ornidazole and 5-40 µg/ml for Levofloxacin Hemihydrate. Each solution was injected six times. The peak area versus concentration data of both drugs was treated by **Least Squares Linear Regression Analysis (Fig. 5, 6).**

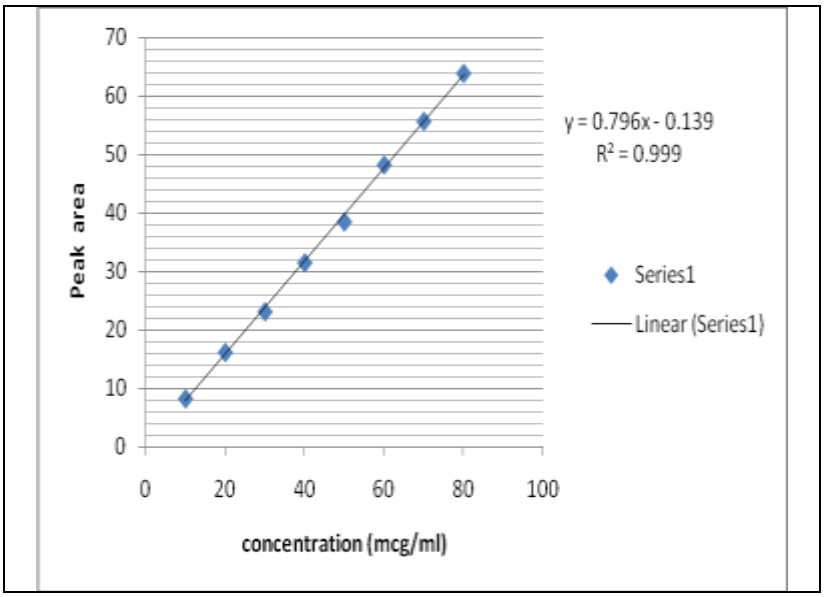

Fig. 5: Linearity graph of Ornidazole

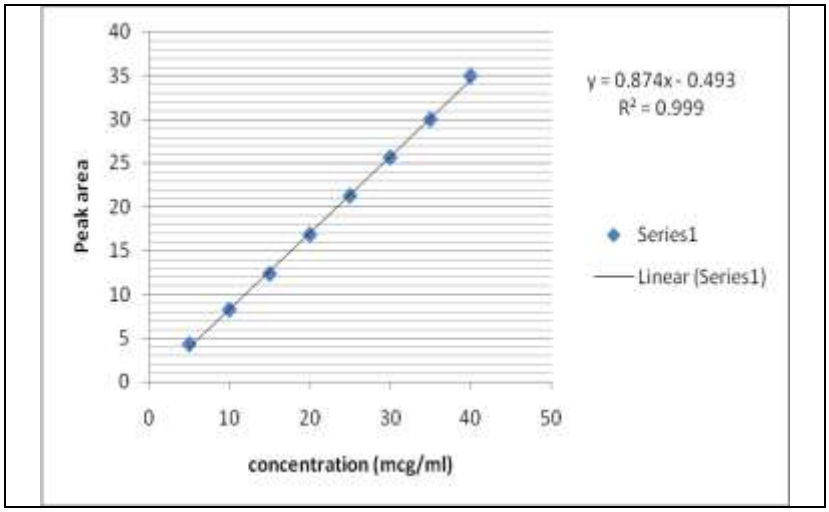

Fig. 6: Linearity graph of Levofloxacin Hemihydrate

Range

Range was calculated from linearity studies.

Sample analysis

10 tablets were weighed to get the average weight of each tablet. Tablets were placed in the mortar and finely powdered. Tablet powder equivalent to 10mg of Ornidazole was transferred into a 100ml volumetric flask. About 50ml of mobile phase was added to the flask and shaken for 15-20mins to disperse the material completely and then finally volume was made up to the mark (100ml) with the same mobile phase.

The contents were then filtered through Whatmann filter paper (No.45). From this sample stock solution, 2ml was then transferred into series of six 10ml volumetric flasks. The volume was made up to the mark with the mobile phase. The solutions prepared were then injected into the HPLC to obtain the % content of Ornidazole and Levofloxacin Hemihydrate in the tablets.

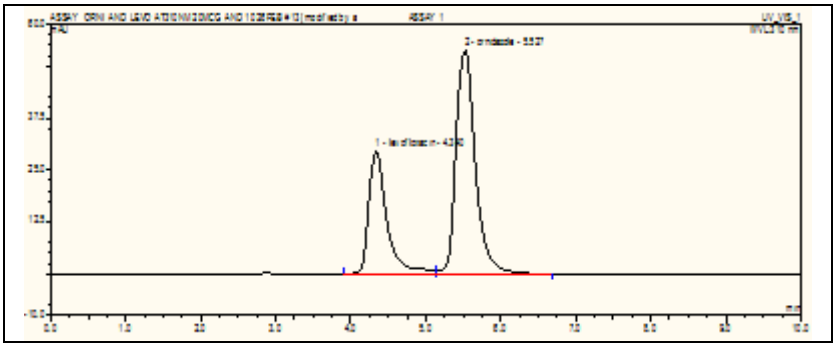

Fig. 7: Chromatogram of Injected Tablet mixture

Forced Degradation studies

Forced degradation studies were performed on Levofloxacin Hemihydrate and Ornidazole to prove the stability indicating property of the method. Both the drugs were subjected to the stress conditions which included acid hydrolysis (0.1N HCl), base hydrolysis (0.1 N NaOH), thermal (60^0C), oxidation (3% H_2O_2) and exposure to light (photolytic). The monitoring period for all the conditions was 8 hr at room temperature except in case of thermal (60^oC in an oven for 8 hr). After completion of degradation processes, the solutions were neutralized and diluted with mobile phase.

Acid Hydrolysis

An amount of 50mg of Levofloxacin Hemihydrate and Ornidazole were weighed separately and transferred separately to two 50ml volumetric flasks, to which 0.1N hydrochloric acid was added. The volume was made up to 50ml with 0.1N hydrochloric acid. These solutions were then kept standing at room temperature for 8hrs. After completion of 8 hours the

solutions were neutralized using 0.1N sodium hydroxide. The final volumes after neutralization were measured and in both cases it was found to be 90 ml. From these solutions an aliquot of 0.18ml (Levofloxacin Hemihydrate) and 0.36 ml (Ornidazole) were transferred separately to two 10ml volumetric flasks and diluted to 10ml using the mobile phase (Methanol:0.02 M phosphate buffer pH 3 in the ratio 50:50 v/v) (20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate). These solutions were then injected into the chromatograph. Fig. 8.

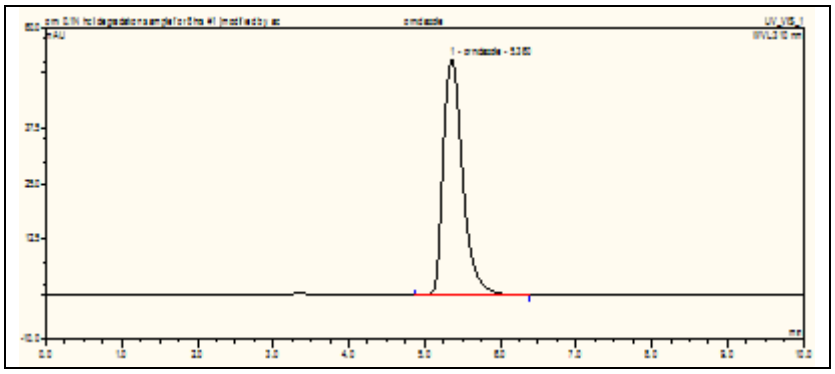

Fig. 8: Chromatogram for Ornidazole subjected to acid degradation

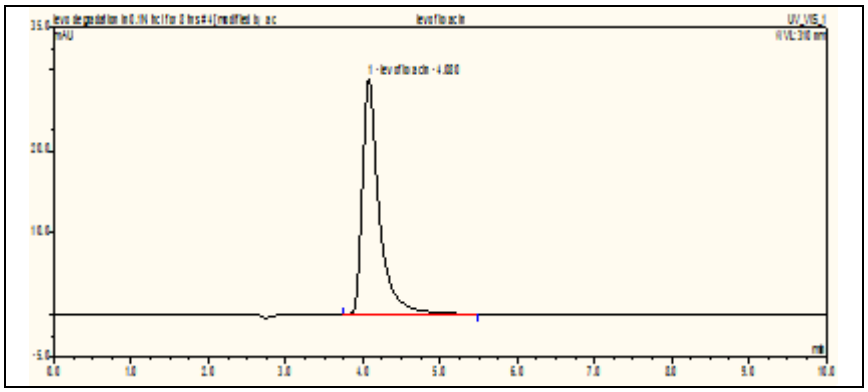

Fig. 9: Chromatogram for Levofloxacin Hemihydrate subjected to acid degradation

Base Hydrolysis

An amount of 50mg of Ornidazole and Levofloxacin Hemihydrate were weighed separately and transferred separately to two 50ml volumetric flasks, to which 0.1N sodium hydroxide was added. The volume was made up to 50ml with 0.1N sodium hydroxide. These solutions were then kept standing at room temperature for 8hrs. After completion of 8 hours the solutions were neutralized using 0.1N hydrochloric acid. The final volumes after neutralization were measured and it was found to be 84 ml (Ornidazole) and 80ml (Levofloxacin Hemihydrate). From these solutions an aliquot of 0.33 ml (Ornidazole) and 0.16ml (Levofloxacin Hemihydrate) were transferred separately to two 10ml volumetric

flasks and diluted to 10ml using the mobile phase (Methanol:0.02 M phosphate buffer pH 3 in the ratio 50:50v/v) (20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate). These solutions were then injected into the chromatograph.

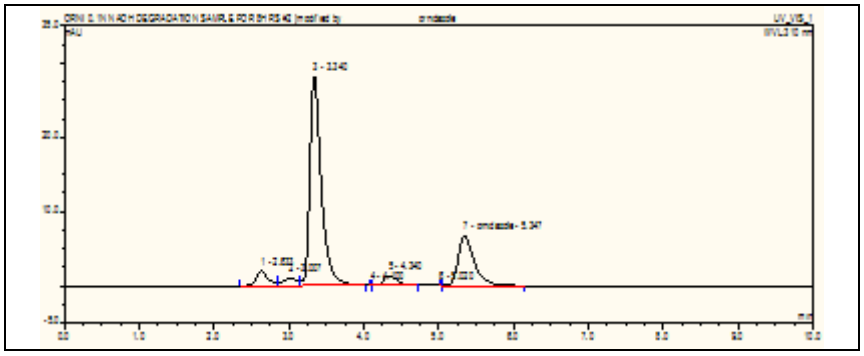

Fig. 10: Chromatogram for Ornidazole subjected to base degradation

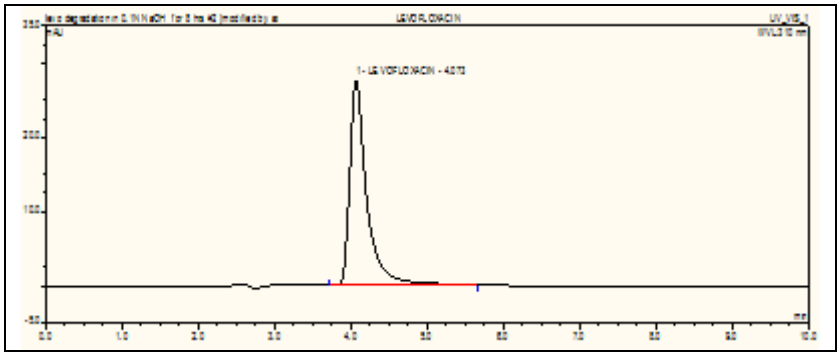

Fig. 11: Chromatogram for Levofloxacin Hemihydrate subjected to base degradation

Thermal Degradation

An amount of 50mg of Ornidazole and Levofloxacin Hemihydrate were weighed separately and transferred separately to two 50ml volumetric flasks. The volumetric flasks were placed in an oven and the temperature was set to 60^0C. After 8 hours the sample was removed and dissolved in mobile phase (Methanol: 0.02 M phosphate buffer pH 3 in the ratio 50:50v/v) (20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate). The volume was made up to 50ml with mobile phase. An aliquot of 0.2ml Ornidazole solution and 0.1ml of Levofloxacin Hemihydrate solution were transferred separately to two 10ml volumetric flasks and diluted to 10 ml using the mobile phase, to obtain the concentration of 20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate. These solutions were then injected into the chromatograph.

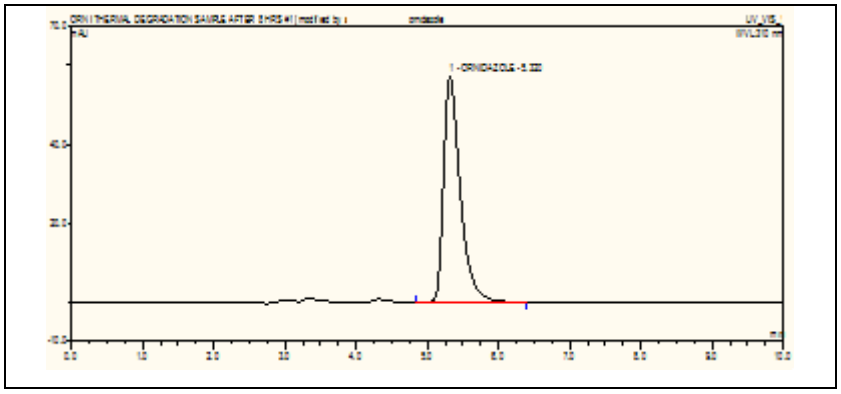

Fig. 12: Chromatogram for Ornidazole subjected to thermal degradation study

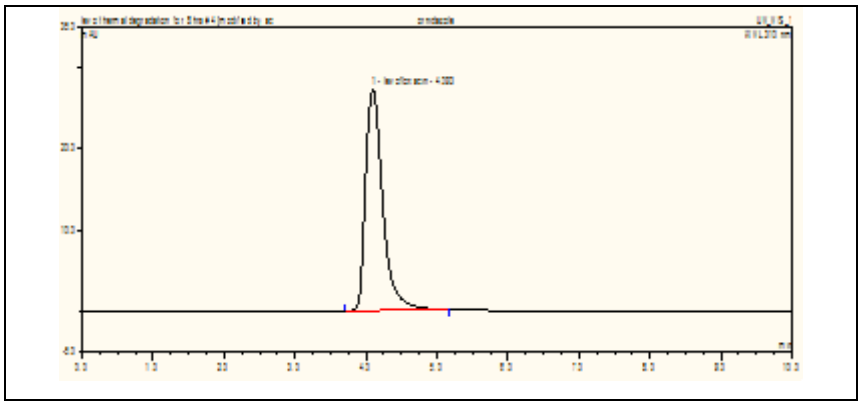

Fig.13: Chromatogram for Levofloxacin Hemihydrate subjected to thermal degradation study

Oxidative Hydrolysis

An amount of 50mg of Ornidazole and Levofloxacin Hemihydrate were weighed separately and transferred separately to two 50ml volumetric flasks, to which 3% hydrogen peroxide was added. The volume was made up to 50ml with 3% Hydrogen Peroxide. These solutions were then kept standing at room temperature for 8hrs. After completion of 8 hours 0.2ml of Ornidazole solution and 0.1ml of Levofloxacin Hemihydrate solution were transferred separately to two 10ml volumetric flasks and diluted to 10ml using the mobile phase (Methanol: 0.02 M phosphate buffer pH 3 in the ratio 50:50v/v) (to get concentration of 20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate). These solutions were then injected into the chromatograph.

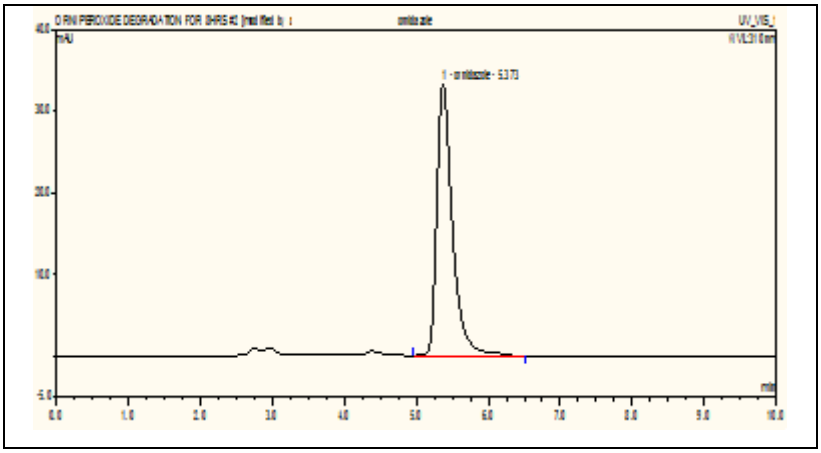

Fig. 14: Chromatogram of Ornidazole of oxidative hydrolysis study

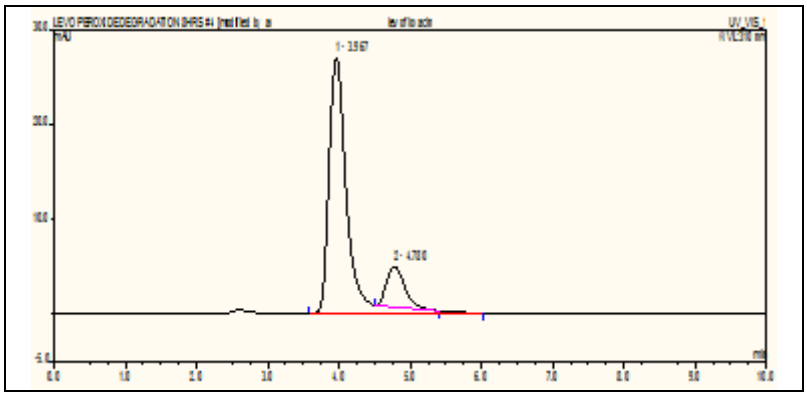

Fig. 15: Chromatogram of Levofloxacin Hemihydrate of oxidative hydrolysis study

Photolytic Degradation

An amount of 50mg of Ornidazole and Levofloxacin Hemihydrate were weighed separately and transferred separately to two 50ml volumetric flasks. The volumetric were placed in an UV chamber. After 8 hours the sample was removed and dissolved in mobile phase (Methanol: 0.02 M phosphate buffer pH 3 in the ratio 50:50v/v) (20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate). The volume was made up to 50ml with mobile phase. An aliquot of 0.2ml Ornidazole sample solution and 0.1ml of Levofloxacin Hemihydrate sample solution were transferred separately to two 10ml volumetric flasks and diluted to 10 ml using the mobile phase, to obtain the concentration of 20µg/ml of Ornidazole and 10µg/ml of Levofloxacin Hemihydrate. These solutions were then injected into the chromatograph.

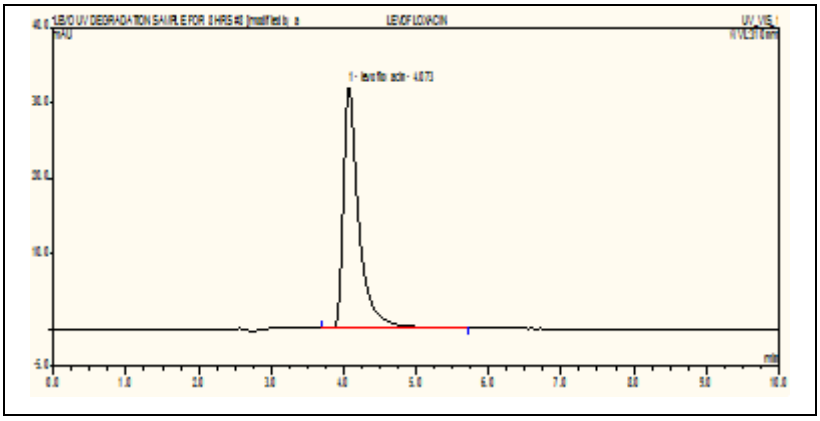

Fig. 16: Chromatogram of Levofloxacin Hemihydrate of photolytic degradation study

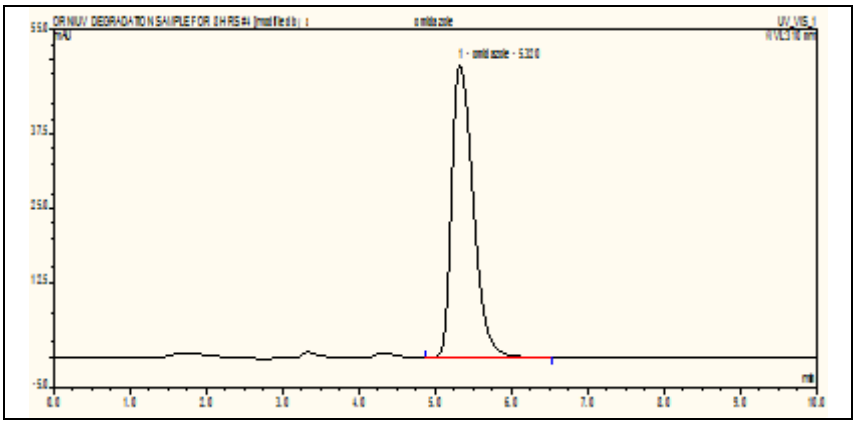

Fig. 17: Chromatogram of Ornidazole of photolytic degradation study

Table 1: Stability profile for Ornidazole (8hrs)

Stress Conditions	Peak Area	Concentration by graph	Content	% Content	% Degradation	Average
Acid Hydrolysis	15.6199	19.7976	49.494	98.988	1.012	1.1499
	15.5372	19.6937	49.234	98.4685	1.5315	
	15.6367	19.8187	49.5467	99.0935	0.9064	
Base Hydrolysis	1.6656	2.2670	5.6675	11.335	88.665	88.6258
	1.6707	2.2734	5.6835	11.367	88.633	
	1.6792	2.2841	5.7102	11.4205	88.5795	
Thermal	15.4517	19.5863	48.9657	97.9314	2.0686	1.3662
	15.5827	19.7508	49.377	98.754	1.246	
	15.6562	19.8432	49.608	99.216	0.784	
Oxidation	15.1253	19.1762	47.9405	95.881	4.119	3.9275
	15.1543	19.2126	48.0315	96.063	3.937	
	15.1878	19.2547	48.1367	96.2735	3.7265	
UV Light(254nm)	15.7767	19.9945	49.9862	99.9725	0.0275	0.2169
	15.7067	19.9066	49.7665	99.533	0.467	
	15.7561	19.9687	49.9217	99.8435	0.1564	

Table 2: Stability profile for Levofloxacin Hemihydrate (8hrs)

Stress Conditions	Peak Area	Concentration by graph	Content	% Content	% Degradation	Average
Acid Hydrolysis	7.2518	8.8624	44.312	88.624	11.376	10.479
	7.3394	8.9627	44.8135	89.627	10.373	
	7.3993	9.0312	45.156	90.312	9.688	
Base Hydrolysis	7.1503	8.7463	43.7315	87.463	12.537	34.608
	7.2584	8.870	44.35	88.7	11.3	
	7.3047	8.9229	44.6145	89.229	10.771	
Thermal	7.6894	9.3631	46.8155	93.631	6.369	6.2239
	7.7039	9.3797	46.8985	93.797	6.203	
	7.7132	9.3903	46.9515	93.903	6.099	
Oxidation	7.9728	9.6874	48.437	96.874	3.126	2.8133
	7.9881	9.7049	48.524	97.049	2.951	
	8.0395	9.7637	48.818	97.637	2.363	
UV Light(254nm)	7.9160	9.6224	48.112	96.224	3.776	3.0833
	8.0394	9.7636	48.818	97.636	2.364	
	7.9742	9.6890	48.445	96.89	3.11	

Validation of the method

The analytical method was validated with respect to parameters such as linearity, range, precision, accuracy, selectivity and robustness.

Linearity

The linearity of the method was determined in the concentration range of 10-80 µg/ml for Ornidazole and 5-40 µg/ml for Levofloxacin Hemihydrate. Each solution was injected six times. The peak area versus concentration data of both drugs was treated by **Least Squares Linear Regression Analysis** shown in Fig. 5 and 6. The correlation coefficientfor both the drugs was found to be 0.999 and the regression equation were y = 0.796x-0.139 (Ornidazole) and y = 0.874x-0.493 (Levofloxacin Hemihydrate), respectively.

Repeatability

Repeatability studies were carried out by injecting six replicate injections of the standard drug mixture. The results were calculated in terms of % RSD. The results are shown in Table No. 3.

Table 3: Data of repeatability study

	Levofloxacin Hemihydrate		Ornidazole	
Sr. No.	Peak Area	Retention Time (min)	Peak Area	Retention Time (min)
1	8.1634	4.24	16.1498	5.407

2	8.1025	4.24	16.2842	5.40
3	8.2299	4.20	16.2372	5.373
4	8.2975	4.24	16.3299	5.407
5	8.1699	4.34	16.0840	5.487
6	8.2533	4.25	16.2872	5.40
Average	8.2027		16.2287	
Standard Deviation	0.0706		0.09378	
% RSD	0.8610 %		0.57790 %	

Precision

Precision studies were performed by injecting six replicate injections of the standard drug mixture on two different days. The results were calculated in terms of % RSD. The results are shown in Table No. 4.

Table 4: Data of precision study

	Levofloxacin Hemihydrate				Ornidazole			
	Day 1		Day 2		Day 1		Day 2	
Sr. No	Peak Area	RT	Peak Area	RT	Peak Area	RT	Peak Area	RT
1	8.1634	4.24	8.1551	4.24	16.1498	5.407	16.1037	5.407
2	8.1025	4.24	8.1567	4.26	16.2842	5.40	16.2242	5.427
3	8.2299	4.20	8.2463	4.26	16.2372	5.373	16.2573	5.427
4	8.2975	4.24	8.2960	4.167	16.3299	5.407	16.1923	5.340
5	8.1699	4.34	8.3573	4.213	16.0840	5.487	16.0932	5.380
6	8.2533	4.25	8.2079	4.213	16.2872	5.40	16.2495	5.380
Avg	8.2027		8.2365		16.2287		16.1867	
SD	0.0706		0.08005		0.09378		0.07210	
% RSD	0.8610		0.9718		0.57790		0.4454	

Accuracy

Accuracy (% Recovery) was evaluated in triplicate, at three different concentrations equivalent to 80,100 and 120% of the target concentration of active ingredient, by adding a known amount of each of the standard to a sample of known concentration of both drugs and calculating the recovery, % RSD for each concentration.

Table 5: Data of Recovery study of Levofloxacin Hemihydrate

Amount Present In sample (µg/ml)	Level of addition (std) (%)	Amount of Standard added (µg/ml)	Peak area of Levofloxacin Hemihydrate	Net area	Amount of Levofloxacin Hemihydrate Recovered (µg/ml)	% Recovery	Mean
9.89 of Levofloxacin Hemihydrate	80	0.8	14.4961	6.3444	7.8242	97.80	100.10
		0.8	14.9472	6.7955	8.3403	104.25	
		0.8	14.5289	6.3772	7.8617	98.27	

	100	1	16.5143	8.3626	10.1334	101.33	101.90
		1	16.5887	8.4370	10.2185	102.18	
		1	16.5905	8.4388	10.2205	102.20	
	120	1.2	18.1580	10.0063	12.0140	100.11	102.20
		1.2	18.4508	10.2991	12.3490	102.90	
		1.2	18.5226	10.3709	12.4312	103.59	

Table 6: Data of Recovery study of Ornidazole

Amount Present in sample (µg/ml)	Level of addition (std) (%)	Amount of Standard added (µg/ml)	Peak Area of Ornidazole	Net area	Amount of Ornidazole Recovered (µg/ml)	% Recovery	Mean
20.27 of Ornidazole	80	1.6	28.4595	12.0626	15.3286	95.80	98.54
		1.6	28.6039	12.607	16.0125	100.07	
		1.6	28.5606	12.5637	15.9581	99.73	
	100	2	32.4675	16.4706	20.8663	104.33	102.54
		2	31.8207	15.8238	20.0537	100.26	
		2	32.26	16.2631	20.6056	103.02	
	120	2.4	35.2450	19.2481	24.3556	101.48	101.29
		2.4	34.6550	18.6581	23.6144	98.39	
		2.4	35.73	19.7331	24.9649	104.02	

Specificity

Selectivity was demonstrated by resolution of two compounds, Levofloxacin Hemihydrate and Ornidazole. Sample matrix did not show any interference with the analyte peaks. Retention time for Levofloxacin Hemihydrate and Ornidazole were 4.425 and 5.573 mins respectively. The degradation products formed during forced degradation studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating.

Robustness

To evaluate the robustness of the method the chromatographic conditions were deliberately varied and degree of reproducibility was evaluated. Robustness was carried out on standard drug solution and formulation. Robustness of the proposed method was assessed with respect to:1) Change in mobile phase composition (50:50 ± 2), 2) Change in flow rate(1ml/min ± 0.2ml/min) 3) Change in pHof the mobile phase (3.0 ± 0.2).

Table 7: Data of Robustness Study

1) Ornidazole: i) Change in Mobile phase composition (Methanol: Phosphate buffer pH 3.0)

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
48:52	16.2525	16.1749	5.90	5.84	101.87
48:52	16.2953	16.1480	5.88	5.87	101.70
48:52	16.2776	16.2611	5.84	5.85	102.41
Average	16.2751	16.1946	5.87333	5.8533	
Standard deviation	0.02150	0.05908	0.03055	0.0152	
% RSD	0.1321	0.36483	0.52016	0.2609	
50:50	16.1143	16.0617	5.52	5.49	101.16
50:50	16.1119	16.1054	5.50	5.48	101.43
50:50	16.3279	16.0251	5.50	5.51	100.93
Average	16.1847	16.0640	5.50666	5.4933	
Standard deviation	0.12402	0.04020	0.01154	0.0152	
% RSD	0.76628	0.25026	0.20969	0.278	
52:48	16.2693	16.0934	5.15	5.10	101.36
52:48	16.2380	16.1123	5.14	5.10	101.48
52:48	16.2013	16.0768	5.10	5.10	101.25
Average	16.1990	16.1025	6.84333	6.84	
Standard deviation	0.06047	0.072936	0.00577	0.02	
% RSD	0.3733	0.45294	0.08437	0.2923	

ii) Change in flow rate

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
0.8	16.1684	16.1864	6.84	6.84	101.94
0.8	16.16	16.0542	6.85	6.82	101.11
0.8	16.2687	16.0669	6.84	6.86	101.19
Average	16.1847	16.06407	5.50666	5.4933	
Standard deviation	0.12402	0.040202	0.01154	0.0152	
% RSD	0.76628	0.25026	0.20969	0.2780	
1	16.1143	16.0617	5.52	5.49	101.19
1	16.1119	16.1054	5.50	5.48	101.43
1	16.3279	16.0251	5.50	5.51	100.93
Average	16.1847	16.06407	5.50666	5.4933	
Standard deviation	0.12402	0.040202	0.01154	0.0152	
% RSD	0.76628	0.25026	0.20969	0.2780	
1.2	16.1831	16.0247	4.56	4.55	100.93
1.2	16.2305	15.9966	4.57	4.52	100.75
1.2	16.1735	16.1617	4.54	4.51	101.78
Average	16.1957	16.061	4.55666	4.5266	
Standard deviation	0.03051	0.088333	0.01527	0.0208	
% RSD	0.18843	0.5499	0.33522	0.4598	

iii) Change in pH of the Mobile Phase

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
2.8	16.2016	16.0863	5.46	5.47	101.31
2.8	16.2885	16.1368	5.46	5.45	101.63
2.8	16.1460	16.0322	5.47	5.39	100.98
Average	16.2120	16.0851	5.46333	5.4366	
Standard deviation	0.07182	0.05231	0.00577	0.0416	
% RSD	0.44301	0.3252	0.10568	0.7657	
3.0	16.1143	16.0617	5.52	5.49	101.16
3.0	16.1119	16.1054	5.50	5.48	101.43
3.0	16.3279	16.0251	5.50	5.51	100.93
Average	16.1847	16.06407	5.50666	5.4933	
Standard deviation	0.12402	0.040202	0.01154	0.0152	
% RSD	0.76628	0.25026	0.20969	0.2780	
3.2	16.2720	16.0436	5.52	5.54	101.05
3.2	16.3856	16.0622	5.52	5.55	101.16
3.2	16.2134	16.0669	5.52	5.54	101.19
Average	16.2903	16.05757	5.52	5.5433	
Standard deviation	0.08755	0.012322	0	0.0057	
% RSD	0.53744	0.07673	0	0.1041	

2) Levofloxacin: i) Change in Mobile phase composition (Methanol: Phosphate buffer pH 3.0)

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
48:52	8.2202	8.1444	4.66	4.59	98.25
48:52	8.1559	8.2169	4.64	4.62	99.08
48:52	8.2855	8.1398	4.60	4.60	98.20
Average	8.2205	8.1670	4.6333	4.6033	
Standard deviation	0.0648	0.0432	0.0305	0.0152	
% RSD	0.7882	0.5294	0.6593	0.3318	
50:50	8.2661	8.1229	4.34	4.31	98.01
50:50	8.2580	8.1056	4.32	4.29	97.81
50:50	8.2032	8.1517	4.32	4.32	98.33
Average	8.2424	8.1267	4.3266	4.3066	
Standard deviation	0.0342	0.0232	0.0115	0.0152	
% RSD	0.4151	0.2865	0.2668	0.3546	
52:48	8.2964	8.1983	3.96	3.91	98.86
52:48	8.2182	8.1452	3.95	3.90	98.26
52:48	8.2009	8.2660	3.91	3.91	99.63
Average	8.2385	8.2031	3.94	3.9066	
Standard deviation	0.0508	0.0605	0.0264	0.0057	
% RSD	0.6176	0.7380	0.6715	0.1477	

ii) Change in flow rate

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
0.8	8.2121	8.1643	5.40	5.40	98.48
0.8	8.0584	8.1071	5.41	5.38	97.83
0.8	8.1312	8.2428	5.40	5.42	99.37
Average	8.1339	8.1714	5.4033	5.4	
Standard deviation	0.0768	0.0681	0.0057	0.02	
% RSD	0.9452	0.8337	0.1068	0.3703	
1	8.2661	8.1229	4.34	4.31	98.01
1	8.2580	8.1056	4.32	4.29	97.81
1	8.2032	8.1517	4.32	4.32	96.75
Average	8.2424	8.1267	4.3266	4.3066	
Standard deviation	0.0342	0.0232	0.0115	0.0152	
% RSD	0.4151	0.2865	0.2668	0.3546	
1.2	8.266	8.2704	3.61	3.59	99.68
1.2	8.296	8.1083	3.62	3.56	97.84
1.2	8.1848	8.1146	3.59	3.55	97.91
Average	8.2489	8.1644	3.6066	3.5666	
Standard deviation	0.0575	0.0918	0.0152	0.0208	
% RSD	0.6974	1.1246	0.4235	0.5836	

iii) Change in pH of the Mobile Phase

Variation	Peak Area		Retention Time		% Assay
	Standard	Formulation	Standard	Formulation	
2.8	8.2874	8.1348	4.42	4.42	98.14
2.8	8.1182	8.1548	4.41	4.40	98.37
2.8	8.2974	8.0459	4.43	4.32	97.13
Average	8.2343	8.1118	4.42	4.38	
Standard deviation	0.1006	0.0579	0.01	0.0529	
% RSD	1.2229	0.7146	0.2262	1.2081	
3.0	8.2661	8.1229	4.34	4.31	98.01
3.0	8.2580	8.1056	4.32	4.29	97.81
3.0	8.2032	8.1517	4.32	4.32	98.33
Average	8.2424	8.1267	4.3266	4.3066	
Standard deviation	0.0342	0.0232	0.0115	0.0152	
% RSD	0.4151	0.2865	0.2668	0.3546	
3.2	8.2653	8.1191	4.34	4.36	97.96
3.2	8.1831	8.14	4.35	4.37	98.20
3.2	8.2945	8.2578	4.34	4.36	99.54
Average	8.2476	8.1723	4.3433	4.3633	
Standard deviation	0.0577	0.0747	0.0057	0.0057	
% RSD	0.7003	0.9150	0.1329	0.1323	

Table 8: Data of Parameters of System Suitability Tests

Sr No	Parameters	Ornidazole	Levofloxacin Hemihydrate
1	Range	10-80 µg/ml	5-40µg/ml
2	Detection wavelength	310 nm	310 nm
3	Theoretical Plates	3292.833	2600.66
4	Tailing factor	1.4033	1.54833
5	Retention Time	5.57333	4.4255
6	Resolution	3.125	

Assay: The method was applied for the determination of Levofloxacin Hemihydrate and Ornidazole in commercially available tablet formulation.

OBSERVATION

Weight of 10 tablets = 12.178g

Average weight per tablet= 1.2178g

Weight of powder equivalent to 10 mg of Ornidazole = 0.024356g (24.356 mg).

Table 9: Data of Assay of formulation

Drug	Label claim	Standard Peak Area	Concentration Found (µg/ml)	Amount Present In Tablet	% Assay	% RSD
Ornidazole	500mg	15.9969	20.2713	503.80	100.76	1.6452
Levofloxacin Hemihydrate	250mg	8.1517	9.8921	245.84	98.33	1.8464

(*Average of 6 readings).

RESULTS AND DISCUSSION

- A simple, accurate, sensitive and robust reversed-phase high performance liquid chromatographic method for simultaneous determination of Levofloxacin Hemihydrate and Ornidazole has been developed.
- The optimized method was performed on a Dionex Ultimate 3000^{+} with reciprocating pump using Chromeleon software; C18 GRACE COLUMN having dimensions 250mm×4.6 i.d., 5µ particle size, using the isocratic mode of elution. The detector employed for detection was variable wavelength UV detector. A mixture of 0.02 mol/L potassium dihydrogen orthophosphate buffer pH 3.0 adjusted with trifluoroacetic acid: Methanol (50:50) was used as a mobile phase; at a flow rate of 1.0 ml/min.
- UV Spectras of individual drugs in the optimized mobile phase were overlaid. At 310nm both the drugs showed significant absorbance, so this wavelength was used for detection in the experiment.

- The run time of the method was set for 10min. The retention time of Levofloxacin Hemihydrate in the chromatogram was recorded at 4.42±0.5min and Ornidazole at 5.57±0.5min.

The separation of Levofloxacin Hemihydrate and Ornidazole was highly satisfactory with good peak shape and resolution.

The system suitability parameters were set and were checked before starting with every analysis. System suitability was performed by six replicate injections of standard solutions of Levofloxacin Hemihydrate and Ornidazole.

The linearity range was found to be 5-40µg/ml for Levofloxacin Hemihydrate and 10-80µg/ml for Ornidazole with the trend line equation of y=0.874x - 0.493 and y=0.796x - 0.139 for Levofloxacin Hemihydrate and for Ornidazole respectively. The regression coefficient was 0.999 for both the drugs.

The assay performed on formulation of Levofloxacin Hemihydrate and Ornidazole showed the % content of 98.33 and 100.76% for Levofloxacin Hemihydrate and Ornidazole respectively.

The method was found to be accurate as the percent recovery for Levofloxacin Hemihydrate and Ornidazole was found to be 100.10-102.20% and 98.54-102.54% which was within the acceptable range of 95-105%.

Repeatability studies carried out showed that the method was found to be precise as observed from the % RSD values for Levofloxacin Hemihydrate and Ornidazole, 0.8610% and 0.57790% respectively which were within the acceptable limit of NMT 2%.

Table 10: Data of Degradation study

Sr no.	Stress conditions	% Degradation	
		Levofloxacin Hemihydrate	Ornidazole
1	Acid hydrolysis	10.479	1.1499
2	Base hydrolysis	34.608	88.6258
3	Thermal	6.2239	1.3662
4	Oxidation	2.8133	3.9275
5	UV light (254 nm)	3.0833	0.2169

CONCLUSION

A simple, accurate, precise and reliable stability indicating HPLC analytical method has been developed and validated for the routine analysis of Levofloxacin Hemihydrate and Ornidazole in tablet dosage forms. The results of force degradation studies reveal that the proposed method has the ability to separate the analyte from their degradation products and excipients found in tablet dosage forms.

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