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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND ORNIDAZOLE IN COMBINED DOSAGE FORM BY ABSORBANCE RATIO METHOD

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ABSTRACT

A new UV spectrophotometric absorbance ratio method was developed and validated for the simultaneous estimation of Levofloxacin Hemihydrate (levo) and Ornidazole (orni) in pure and pharmaceutical dosage form. Levo shows λ_{max} at 288 nm and orni at 317 nm and an isosbestic point at 305 nm in deionized water. The developed method was found to show linearity in concentration (conc.) range of 1-11 µg/ml for levo and 2-22 µg/ml for orni with the value of correlation coefficient (R^2) 0.9981 and 0.9969 respectively. The percent Relative Standard Deviation (% RSD) of inter and intra-day precision studies were found to be within limits of NMT 2 % concluding that the present method is precise as per ICH guidelines Q2 (R_1).[1] The developed method can be used for routine estimation of levo and orni in bulk and pharmaceutical dosage form.

KEYWORDS: UV spectrophotometry, Levofloxacin Hemihydrate, Ornidazole, Absorbance, Absorptivity.

INTRODUCTION

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 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]

The present work describes a simple, accurate, and precise absorbance ratio method for simultaneous determination of these two drugs in pharmaceutical dosage form.

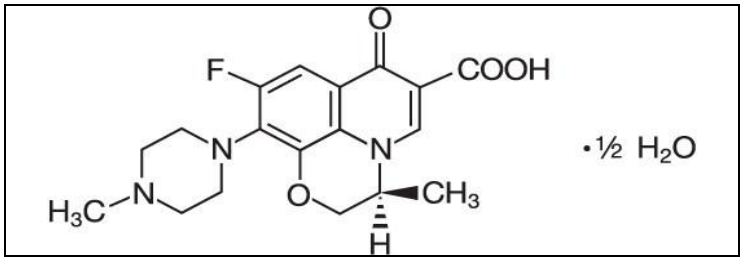

Fig. 1: Chemical structure of Levofloxacin hemihydrate

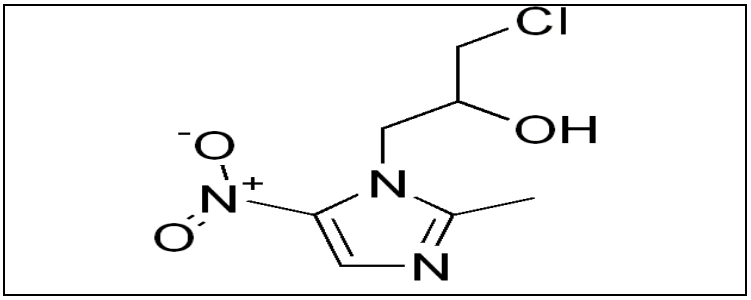

Fig. 2: Chemical structure of Ornidazole

MATERIALS AND METHODS

INSTRUMENTATION AND APPARATUS

UV analysis was carried out on LABINDIA® Analytical UV 3000+ UV/Visible Spectrophotometer using UVWIN5 software and all weight measurements were taken on WENSAR ELECTRONIC BALANCE MAB 220 at room temperature. Ultrapure deionized water processed through BIO-AGE water purification system was used for study.

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 [FYNAL® OZ brand containing Levofloxacin Hemihydrate 250 mg and Ornidazole 500 mg]. All the reagents used in this method were of analytical grade.

METHOD DEVELOPMENT

Preparation of standard stock solution

Standard stock solution (1000 µg/ml) of levo and orni were prepared separately by carefully dissolving weighed 25 mg of drug in 25 ml volumetric flask respectively and diluting up to the mark with deionized water. 10 ml of these solutions were diluted separately up to 100 ml with deionized water to get working stock solution (100 µg/ml). Then, these stock solutions were used to prepare further required concentrations.

Determination of Isoabsorptive Point and Wavelength of Maximum Absorbance (λ_{max})

Solutions of 10 µg/ml of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against deionized water as blank. The overlaying spectrum was also obtained to determine isoabsorptive point. Levo showed λ_{max} at 288 nm and orni at 317 nm. Isoabsorptive point was obtained at 305 nm.

Preparation of Sample Solution

A sample of the powdered tablets equivalent to 10 mg of orni and 5 mg of levo was transferred into a 100 ml volumetric flask. About 50 ml of the deionized water was added to the flask and shaken for 30 mins to disperse the material completely and then finally the volume was made up to the mark (100 ml) with the same solvent. This gave the solution having concentration of 100 µg/ml. The contents were then filtered through Whatmann filter paper (No.45). From this Stock Solution, sample solutions of various concentrations were prepared by diluting aliquots stock solutions appropriately.

Calibration Curve (Linearity)

A calibration curve was plotted over a concentration range of 1-11 µg/mL for levo and 2-22 µg/mL for orni. Accurately measured working stock solution of levo (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 and 1.1 ml) and working stock solution of orni (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 ml) were transferred separately to series of 10 ml volumetric flask

and diluted up to the mark with deionized water. The absorbance (Abs) of both solutions was taken at their respective absorbance maxima and at isoabsorptive point. The calibration curves were constructed by plotting concentration against absorbance where each reading was an average of three determinations.

Application of the Proposed Method for Estimation in Standard Laboratory Mixture

The absorptivity coefficient of both drugs was determined and the individual concentration of levo and orni was determined using the following equations

$$C_X = Q_M - Q_Y / Q_X - Q_Y * (A1/ ax_1) \quad \text{..... (1)}$$

$$C_Y = Q_M - Q_X / Q_Y - Q_X * (A1/ ay_1) \quad \text{..... (2)}$$

Where,

C_X: Concentration of orni in the mixture.

C_Y: Concentration of levo in the mixture.

Q_X: The ratio of absorptivity of orni at 288 nm and 305 nm.

Q_Y: The ratio of absorptivity of levo at 288 nm and 305 nm.

Q_M: The ratio of absorbance of mixture at 288 nm and 305 nm.

A_1: Absorbance of the mixture at the iso-absorptive point.

A_2: Absorbance of the mixture at the lambda max (λ_{max}) of one of the components.

ax_1: Absorptivity of orni at the iso-absorptive point, calculated using the formula A= abc (Beer –Lambert's law), A=Absorbance, b=1cm and c in g/100 ml.

ax_2: Absorptivity of orni at the lambda max (λ_{max}) of one of the selected components, calculated using the formula A= abc (Beer –Lambert's law), A=Absorbance, b=1 cm and c in g/100 ml.

ay_1: Absorptivity of levo at the iso-absorptive point, calculated using the formula A= abc (Beer –Lambert's law), A=Absorbance, b=1cm and c in g/100 ml.

ay_2: Absorptivity of levo at the lambda max of one of the selected components, calculated using the formula A= abc (Beer–Lambert's law), A=Absorbance, b=1cm and c in g/100 ml.[9]

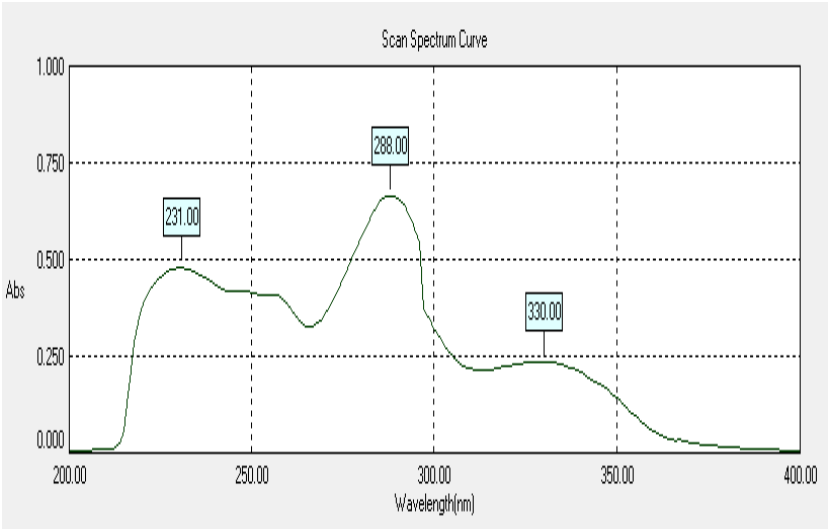

Fig. 3: UV absorption spectra of levo in water

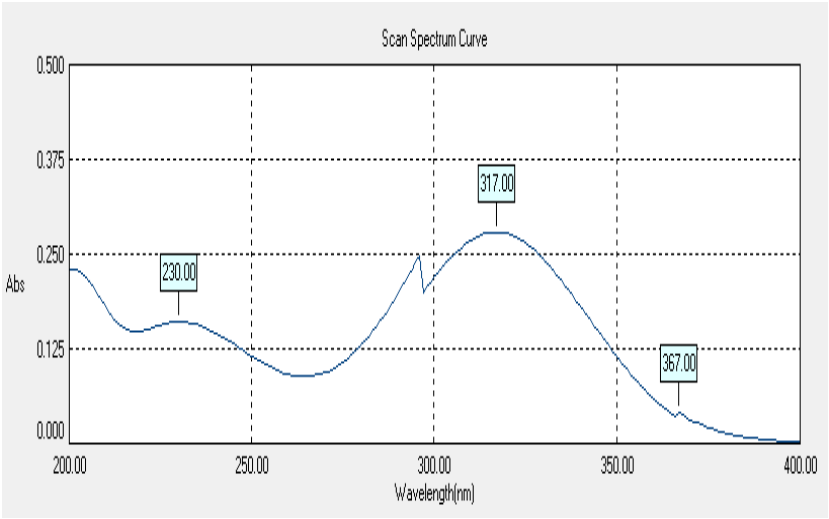

Fig. 4: UV absorption spectra of orni in water

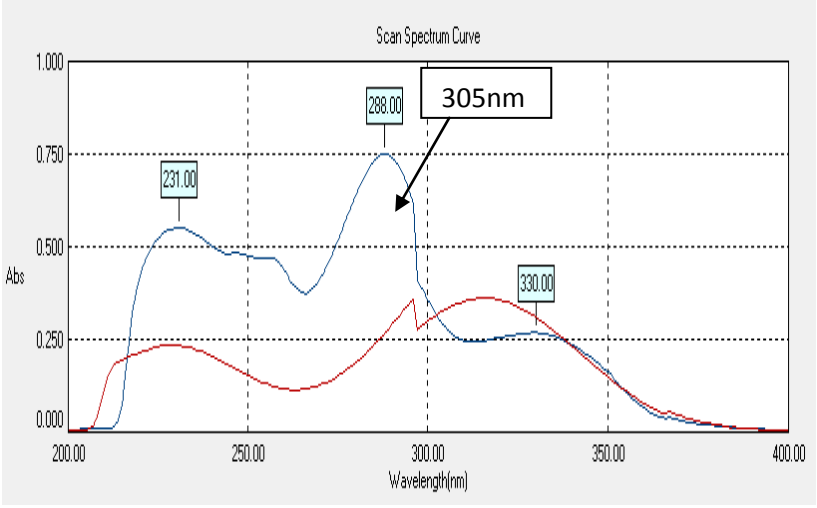

Fig. 5: Overlain of absorption spectra of levo and orni in deionized water

Determination of absorptivity value of Ornidazole and Levofloxacin Hemihydrate

The absorptivity values of the drugs were determined at the selected wavelengths. These absorptivity values are the mean of three determinations at each concentration level for orni and levo respectively as mentioned in the table 1 and 2.

Table 1: Data of Absorptivity values of Ornidazole

Conc. (µg/ml)	Abs at 305 nm	ax_1	Abs at 288 nm	ax_2	Abs at 317 nm	ax
2	0.0483	241.5	0.0325	162.5	0.0565	282.5
4	0.0946	236.5	0.0635	158.75	0.1101	275.25
6	0.1396	232.66	0.097	161.66	0.1616	269.33
8	0.1793	224.12	0.1243	155.37	0.2071	258.87
10	0.2176	217.6	0.1545	154.5	0.2495	249.5
12	0.2563	213.58	0.185	154.16	0.2916	243
14	0.2903	207.35	0.2168	154.85	0.3276	234
16	0.3275	204.68	0.2523	157.68	0.3666	229.12
18	0.3578	198.77	0.2813	156.27	0.3968	220.44
20	0.3953	197.65	0.3208	160.4	0.4340	217
22	0.4263	193.77	0.3556	161.63	0.4641	210.95

Table 2: Data of Absorptivity values of Levofloxacin Hemihydrate

Conc. (µg/ml)	Abs at 305 nm	ay_1	Abs at 288 nm	ay_2	Abs at 317 nm	ay
1	0.0188	188	0.0646	646	0.0168	168
2	0.0416	208	0.1283	641.5	0.0375	187.5
3	0.0641	213.66	0.1918	639.33	0.0591	197
4	0.084	210	0.2505	626.25	0.0781	195.25
5	0.1043	208.6	0.3105	621	0.0968	193.6
6	0.124	206.66	0.3696	616	0.1156	192.66
7	0.1405	200.71	0.423	604.28	0.1316	188
8	0.162	202.5	0.4785	598.12	0.1523	190.37
9	0.1808	200.88	0.5306	589.55	0.1695	188.33
10	0.2006	200.6	0.5786	578.6	0.1863	186.3
11	0.221	200.90	0.6288	571.63	0.2048	186.18

From the Table 1 and 2, following data was obtained

$ax_{1=}$ 215.2920 ax_2= 157.9823 ax=244.54

$ay_{1=}$ 203.683 ay_2= 612.024 ay=188.47

Absorptivity value of Levofloxacin Hemihydrate in deionized water at 317 nm is less as compared to absorptivity at 288 nm. Hence 288 nm was selected as λ max

VALIDATION PROCEDURE

The objective of present validation study is to demonstrate whether the developed method is suitable for intended use. The current validation of the analytical procedure has been conducted for parameters like linearity, range, precision and accuracy with respect to ICH guidelines $Q_2(R_1)$.

Linearity and Range: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. [1] The linearity was determined by using working standard solutions between 1-11 µg/ml for levo and 2-22 µg/ml for orni. Calibration curve for linearity range was developed and simple linear regression was performed (Table No. 3 and 4). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

Table 3: Data of Linearity range for levo

Conc. (µg/ml)	Abs (305 nm)	Abs (288 nm)
1	0.0188	0.0646
2	0.0416	0.1283
3	0.0641	0.1918
4	0.084	0.2505
5	0.1043	0.3105
6	0.124	0.3696
7	0.1405	0.423
8	0.162	0.4785
9	0.1808	0.5306
10	0.2006	0.5786
11	0.221	0.6288

Table 4: Data of Linearity range for Orni

Conc. (µg/ml)	Abs (305 nm)	Abs (288 nm)	Abs (317 nm)
2	0.0483	0.0325	0.0565
4	0.0946	0.0635	0.1101
6	0.1396	0.097	0.1616
8	0.1793	0.1243	0.2071
10	0.2176	0.1545	0.2495
12	0.2563	0.185	0.2916
14	0.2903	0.2168	0.3276
16	0.3275	0.2523	0.3666
18	0.3578	0.2813	0.3968
20	0.3953	0.3208	0.4340
22	0.4263	0.3556	0.4641

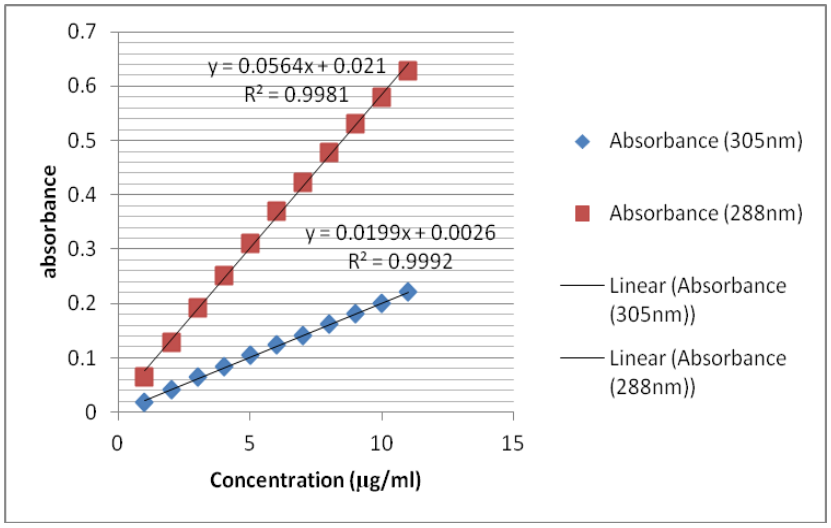

Fig. 6: Calibration Curve of Levo

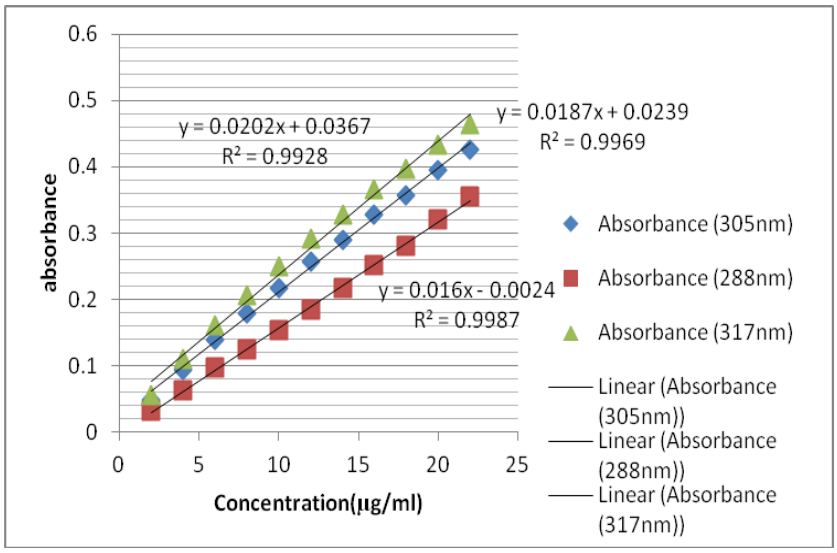

Fig. 7: Calibration Curve of orni

Table No. 5: Data of Accuracy studies of levo

Conc. of sample added (µg/ml)	Level of addition (%)	Amount of pure drug spiked (µg/ml)	Total Conc. (µg/ml)	Abs (305 nm)	Abs (288 nm)	Total content found (µg/ml)	Amount of standard drug recovered (µg/ml)	Mean recovery % (n=3)
3.9204	80	3.2	7.2	0.338	0.576	7.0904	3.1699	99.66
		3.2		0.337	0.577	7.1279	3.2074	
		3.2		0.334	0.574	7.1106	3.1901	
	100	4	8	0.358	0.632	7.9837	4.0633	101.433
		4		0.357	0.631	7.9780	4.0575	
		4		0.356	0.630	7.9722	4.0518	
	120	4.8	8.8	0.371	0.672	8.6423	4.7218	98.47
		4.8		0.369	0.671	8.6524	4.7319	
		4.8		0.368	0.670	8.6466	4.7262	

Accuracy

The recovery experiments were carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards (80 %, 100 % & 120 %). 4 µg/ml and 8 µg/ml of levo and orni were used as nominal 100 % sample concentration. The % recovery of the same pure drugs were then determined. Results were expressed in terms of % recoveries. The results are tabulated in Table No. 5 and 6.

Table No. 6: Data of Accuracy studies of Orni

Conc. of sample added (µg/ml)	Level of addition (%)	Amount of pure drug spiked (µg/ml)	Total Conc. (µg/ml)	Abs (305 nm)	Abs (288 nm)	Total content found (µg/ml)	Amount of standard drug recovered (µg/ml)	Mean recovery % (n=3)
8.2746	80	6.4	14.4	0.410	0.509	14.7867	6.5121	101.85
		6.4		0.408	0.503	14.7864	6.5119	
		6.4		0.409	0.505	14.8071	6.5324	
	100	8	16	0.442	0.535	16.2216	7.94697	99.50
		8		0.442	0.536	16.2011	7.9265	
		8		0.444	0.538	16.2831	8.0085	
	120	9.6	17.6	0.483	0.584	17.7391	9.46451	98.94
		9.6		0.483	0.583	17.7596	9.48496	
		9.6		0.484	0.583	17.8210	9.5464	

SAMPLE SOLUTION STABILITY

Stability of the solution was studied by storing the tablet sample solution (8 µg/ml of orni and 4 µg/ml of levo) at room temperature for 3 hours and then analyzed by measuring the absorbance of the solution at 305 nm and 288 nm. % RSD was calculated. The results obtained were compared with the results of the freshly prepared solution.

Table 7: Data of Stability Analysis of the sample solution

	Absorbance of the Sample Solution	
	305 nm	288 nm
Freshly prepared	0.255	0.366
After 1 hour	0.256	0.366
After 2 hours	0.256	0.366
After 3 hours	0.257	0.368
Mean	0.256	0.3665
SD	0.000816	0.001
% RSD	0.3187	0.2728

Method Precision

a) Repeatability study

Aliquots of 0.8 ml each of the working sample solution were transferred to six 10 ml volumetric flask which were then diluted to 10 ml using deionized water (8 µg/ml of orni and 4 µg/ml of levo). The absorbances for each of these solutions were recorded at 305 nm and 288 nm against reagent blank. The data of repeatability study is shown in Table No. 8.

Table No.8: Data of repeatability study

Conc. (µg/ml)	Abs (305 nm)	Abs (288 nm)	Conc. Obtained (µg/ml)	Statistical Analysis		
				orni	levo	
8.2746 of orni + 3.9204 of levo	0.255	0.369	8.1243, 3.9320	97.93	100.89	Mean
	0.257	0.370	8.2268, 3.9219			
	0.256	0.371	8.1449, 3.959	1.1399	0.9659	SD
	0.253	0.370	7.9809, 3.985			
	0.254	0.372	8.0015, 4.0127	1.1639	0.9573	% RSD
	0.255	0.368	8.1448, 3.9104			

b) Intermediate Precision

The intra-day and inter-day precision of the proposed method was determined by measuring the absorbance of the solutions 6 times on the same day and on two different days. The results were reported in terms of percentage relative standard deviation (% RSD). Aliquots of 0.8 ml each of the working sample solution were transferred to six 10 ml volumetric flask which were then diluted to 10 ml using deionized water ((8 µg/ml of orni and 4 µg/ml of levo). The absorbance was recorded at 305 nm and 288 nm against the reagent blank. The data of inter-day precision study is shown in Table No.9.

Table No. 9: Data showing intermediate Precision

Conc. (µg/ml)	Abs Day 1		Abs Day 2		Abs Day 3		Average of Abs		%RSD		Conc. found (µg/ml)	
	λ_1(305 nm)	λ_2 (288 nm)	λ_1(305 nm)	λ_2 (288 nm)	λ_1(305 nm)	λ_2 (288 nm)	λ_1(305 nm)	λ_2(288 nm)	orni	levo	orni	levo
8.2746 orni + 3.9204 levo	0.255	0.366	0.256	0.367	0.254	0.368	0.25	0.36	0.32	0.29	8.20	3.90
	0.256	0.367	0.257	0.368	0.255	0.369						
	0.257	0.369	0.258	0.370	0.257	0.371						

RESULTS AND DISCUSSION

RESULT TABLE

Table No. 10: Results of validation of parameters of levo and orni in deionized water

Parameters	Results	
	Levofloxacin Hemihydrate	Ornidazole
Range (µg/ml)	1-11	2-22
Linearity (µg/ml)	1-11	2-22
Regression Coefficient (R^2 Value)	0.9981	0.9969
Assay (%)	98.005%	103.4260%
Recovery (%)	98.47–101.433 %	98.94– 101.85%
Interday Precision (Intermediate)	0.29%	0.32%
Intraday Precision (Repeatability)	0.9573%	1.1639%

The literature survey reveals that there are several validated spectrophotometric, RP-HPLC, HPTLC and other numerous methods available for estimation of levo and orni in combination as well as individually (as a single component). The attempt was made to develop sensitive, precise & accurate UV spectrophotometric absorbance ratio method for estimation of levo and orni. The method was developed using deionized water as the solvent. It involves formation of Q-absorbance equation at 305 nm (isosbestic point) and λ_{max} 288 nm (maximum wavelength of absorption of levo). The absorptivity values (A 1%, 1cm) were calculated at all the three wavelengths for levo and orni i.e. at 305 nm (iso-absorptive point), 288 nm (λ max) and 317 nm (λ max of orni). It was found that absorptivity values for levo at 317 nm was less as compared to absorptivity at 288 nm. Hence 288 nm was selected as the λmax

The concentration of each component was determined by using the following Q-absorbance equation.

$$C_x = Q_M - Q_Y / Q_X - Q_Y * (A1/ ax_1) \quad \text{..... (1)}$$

$$C_y = Q_M - Q_X / Q_Y - Q_X * (A1/ ay_1) \quad \text{..... (2)}$$

The linearity range was found to be 1-11 µg/ml for levo and 2-22 µg/ml for orni respectivel. The regression coefficient was 0.9981 and 0.9969 for levo and orni respectively. The method showed mean absolute recovery ranging from 98.47–101.433 % for levo and 98.94– 101.85 % for orni. Method showed insignificant variation in results, which demonstrated that the method was repeatable with

a) % RSDs (intraday precision): 0.9573 % and 1.1639 % for levo and orni respectively.

b) % RSDs (interday precision): 0.29 % and 0.32 % for levo and orni respectively.

CONCLUSIONS

The developed UV spectrophotometric - absorbance ratio method for estimation of Levofloxacin Hemihydrate and Ornidazole is simple, sensitive, precise and accurate. The method could be applied successfully and economically for the simultaneous estimation of Levofloxacin Hemihydrate and Ornidazole in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

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