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SIMULTANEOUS RP – HPLC METHOD DEVELOPMENT AND VALIDATION OF LEVOFLOXACIN AND ORNIDAZOLE IN COMBINED PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise RP–HPLC method was developed for the estimation of levofloxacin and ornidazole in combined tablet formulation. The quantification was carried out using a Phenomenex C18 column 250 x 4.6 mm i.d, 5 µm particle size in isocratic mode, with mobile phase comprising of 0.1% v/v phosphate buffer pH 3.0 ± 0.05, acetonitrile, methanol in the ratio of 70 : 10 : 20 (v/v/v). The flow rate was 1 mL/min and the detection was carried out by UV detector at 295 nm. The retention times were 3.45 min and 6.67 min for levofloxacin and ornidazole, respectively. The method produced linear response in the concentration range of 40-60 µg/mL and 80-100 µg/mL for levofloxacin and ornidazole. The percentage recovery was found to be 98.79% and 99.47% for levofloxacin and ornidazole, respectively. The developed method was found to be accurate, precise, sensitive and rapid for the simultaneous estimation of levofloxacin and ornidazole in tablets.

Key words: RP-HPLC, Levofloxacin, Ornidazole.

INTRODUCTION

Levofloxacin is the L-isomer of the racemate, ofloxacin, a quinolone antimicrobial agent, which acts by inhibition of bacterial topoisomerase IV and DNA gyrase enzymes required for DNA replication, transcription, repair, and recombination[1]. Chemically, levofloxacin is (-)-(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 hemihydrate. Ornidazole is a 5-nitroimidazole derivative, which is active against protozoa and anaerobic bacteria. Ornidazole

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acts by damage of DNA strands or inhibition of their synthesis[2]. Chemically, ornidazole is 1-(3–chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole. Several methods such as HPLC[3-9], spectrophotometric[10], etc. have been reported in literature to estimate levofloxacin and ornidazole either alone or in combination with other drugs in biological fluids and market formulations. However, no HPLC method was reported for the simultaneous estimation of levofloxacin and ornidazole in combined tablet dosage form. The present work describes the development of a simple, precise and accurate RP-HPLC method for the simultaneous estimation of levofloxacin and ornidazole in combined tablet dosage form.

EXPERIMENTAL

Materials and methods

The drug samples, levofloxacin hemihydrate and ornidazole were obtained as gift samples from the Madras Pharmaceuticals, Chennai and the authenticity and purity of the samples were certified by the quality control laboratory of the Madras Pharmaceuticals. Tablets having combination of levofloxacin (250 mg) and ornidazole (500 mg) were purchased from local pharmacy. Water of HPLC grade was collected from Milli-Q-system. HPLC grade methanol and acetonitrile, and orthophosphoric acid (AR- grade), triethylamine (AR- grade), were purchased from E. Merck Co., Mumbai. A gradient high pressure chromatograph (Schimadzu HPLC class VP series) with two LC-10AT VP pumps, fitted with UV/visible detector and SPD-10 AVP, system controller (Schimadzu) and operating software Schimadzu Class VP version 6.12 SPS data system was used. The chromatographic column used was a reverse phase Phenomenex- C_{18} column (250 mm x 4.6 mm id, 5µ particle size). A mixture of 0.1%v/v phosphate buffer pH 3.0 ± 0.05, acetonitrile, and methanol (350 : 50 : 100) was used as the mobile phase and filtered through 0.45 µ cellulose nitrate filter. The flow rate of the mobile phase was maintained at 1 mL/min and the detection wavelength was set at 295 nm.

Preparation of standard stock solution

Mixed standard stock solution of levofloxacin and ornidazole was prepared by accurately weighing 50 mg of levofloxacin and 100 mg of ornidazole in a 100 mL standard flask by dissolving and the volume was made up with the mobile phase. Then further dilutions were made by using mobile phase to get a mixed concentration of 50 µg/mL of levofloxacin and 100 µg/mL of ornidazole.

Preparation of sample solution

Twenty tablets of Levoday-OZ (Zydus Recon Ltd.) each containing 250 mg of

levofloxacin and 500 mg of ornidazole were weighed and finely powdered. A quantity of powder equivalent to 50 mg of levofloxacin and 100 mg of ornidazole was accurately weighed and transferred in to a 100 mL standard flask, and sonicated with 15 mL of mobile phase for 5 min and the volume was made up with mobile phase. This solution was then filtered through 0.45 µ cellulose nitrate filter and diluted suitably to get a mixed concentration of 50 µg/mL of levofloxacin and 100 µg/mL of ornidazole. Twenty microlitres of the standard and sample solutions were injected separately and the chromatogram was recorded at a run time of 10 min.

Chromatograpy

The present work was aimed at developing a simple, precise and accurate HPLC method for the simultaneous estimation of levofloxacin and ornidazole in combined tablet dosage form. The column used was C_{18} Phenomenex column. The mobile phase was optimized with 0.1% v/v phosphate buffer pH 3.0 ± 0.05, acetonitrile, and methanol (350 : 50 : 100). With the above mentioned composition of mobile phase, a good resolution between levofloxacin and ornidazole was achieved with a reasonably short run time of 10 min. UV detection was carried out at 295 nm as both; levofloxacin and ornidazole showed good absorbance at this wavelength. The retention time of levofloxacin and ornidazole was found to be 3.45 min and 6.67 min, respectively. A typical chromatogram of the test solution is shown in Fig. 1.

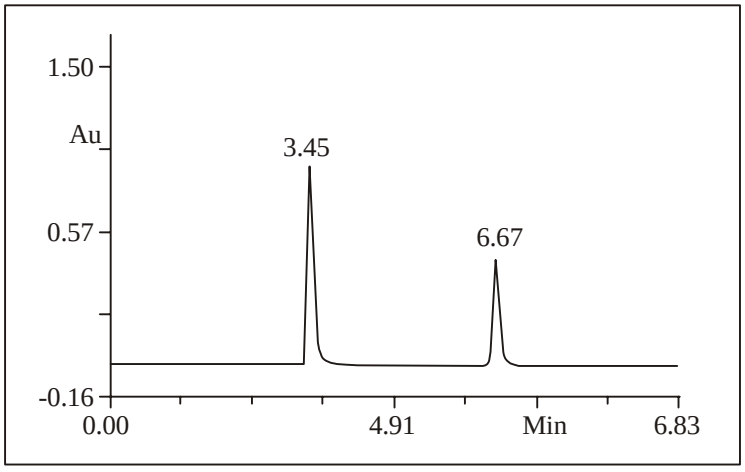

Fig. 1: Typical chromatogram of the sample solution

Chromatogram showing sample solution of levofloxacin and ornidazole at the approximate concentrations of 50 µg/mL and 100 µg/mL, respectively

Method validation

Linearity studies: Linearity experiments were performed for both; the components and the response was found to be linear in the range of 40-60 µg/mL for levofloxacin and 80-120 µg/mL for ornidazole. Linearity of levofloxacin and ornidazole was plotted by a graph of peak areas versus the concentration. The correlation coefficient 'r' values (n = 5) for both; levofloxacin and ornidazole were greater than 0.999. The linearity equation was y = 404340x + 98053 for levofloxacin and y = 242143x – 4609.1 for ornidazole. The linearity ranges and correlation coefficient obtained are presented in Table 1.

Table 1: Analytical performance parameters

Parameters	Levofloxacin	Ornidazole
Linear dynamic range (µg/mL)	40-60	80-120
Slope (m)	404340	242143.3
Intercept (c)	98053.4	- 4609.14
Correlation coefficient (r)	0.9999	0.9999

System suitability

The system suitability was performed by injecting five times, the mixed standard solutions having concentration of 50 µg/mL of levofloxacin and 100 µg/mL of ornidazole. The peak shapes of both the drugs were symmetrical and the asymmetry factor was less than 2.0. The system suitability data of levofloxacin and ornidazole are given in Table 2, and the respective calibration curves are shown in Figures 2 and 3.

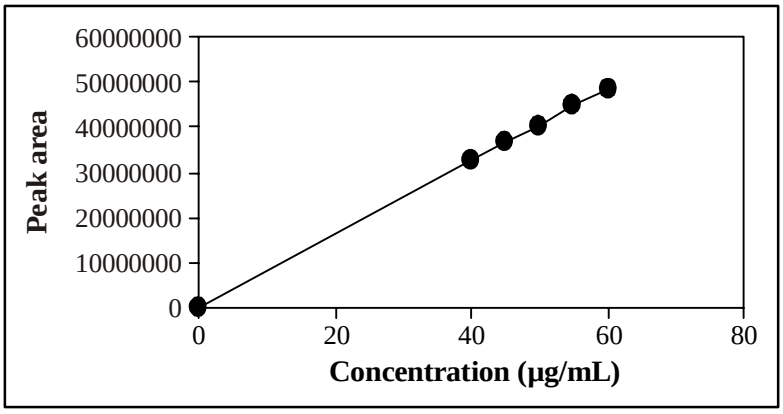

Fig. 2: Calibration curve of levofloxacin

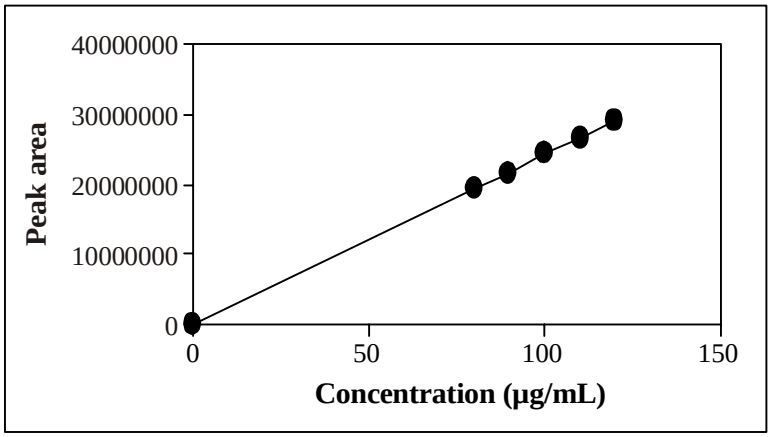

Fig. 3: Calibration curve of ornidazol

Table 2: System suitability data

Parameter	Levofloxacin	Ornidazole
Resolution	10.0	
Tailing factor	1.50	1.04
Number of theoretical plates	5017	13236

Quantitative estimation

Quantitative estimation was carried out using the marketed formulation having concentration of 50 µg/mL of levofloxacin and 100 µg/mL of ornidazole and the data are summarized in Table 3.

Table 3: Quantitative estimation

Tablet sample	*Label claim (mg / tablet)	*Peak area	*Assay value (mg)	*Percentage label claim (% w/w)	Percentage deviation
Levofloxacin	250	40983816	49.54	99.09	-0.90
Ornidazole	500	24250230	99.97	99.97	-0.26

*Each value is mean of five readings

Precision

The precision was determined by preparing the sample of a single batch of levofloxacin and ornidazole tablet formulation six times and analyzing as per the proposed method.

Accuracy

Accuracy of the method was calculated by recovery studies (n = 3) at three levels. Standard drug solutions containing drugs in the range of 80-120% of the target concentration was added to previously analyzed test solution. Amount of the drug recovered at each level was determined. The mean percentage recovery at each level was calculated and the data are shown in Table 4.

Table 4: Recovery studies

Drug	Amount added (µg/mL)	Amount recovered (µg/mL)	Recovery (%)	Average recovery (%)
Levofloxacin	40	39.61	99.13	98.79
	50	49.25	98.51	
	60	59.24	98.75	
Ornidazole	80	79.12	99.24	99.47
	100	99.23	99.23	
	120	119.34	99.95	

RESULTS AND DISCUSSION

In the proposed RP-HPLC method, linearity was obeyed in the range of 40-60 µg/mL for levofloxacin and 80-120 µg/mL for ornidazole. From the chromatogram, the retention time of levofloxacin and ornidazole were found to be 3.45 min and 6.67 min, respectively. The limit of detection and limit of quanititation were 0.96 µg/mL and 3.01 µg/mL for levofloxacin and 0.89 µg/mL and 2.70 µg/mL for ornidazole, respectively. The mean percentage purity values were 99.09% and 99.97% for levofloxacin and ornidazole, respectively. The system suitability parameters indicate that the developed method has acceptable accuracy and precision. The percentage relative standard deviation was below 1, which indicates that the proposed method has good accuracy and precision. The mean percentage recoveries obtained for levofloxacin and ornidazole were 98.79% and 99.47%,

respectively. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of the excipients used in the formulation.

CONCLUSION

The method was simple and had short run time of 10 min, which makes this method rapid. The results of the study indicate that the proposed RP-HPLC method for the simultaneous estimation of levofloxacin and ornidazole is simple, rapid, accurate and precise and highly suitable for routine laboratory analysis.

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