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Simultaneous Estimation of Levofloxacin Hemihydrate and Ornidazole in Tablet Dosage Form by HPTLC

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A validated HPTLC method for simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablet formulations is described. Separation was achieved on precoated silica gel plate 60 F_{254} using *n*-butanol:water:glaceal acetic acid (60:20:20) as mobile phase. Quantitation was carried out by the use of densitometer in absorbance mode at 366 nm. Linearity of detector response for levofloxacin hemihydrate and ornidazole estimated in the average weight of the tablet were found to be 99.7 and 100.45 %, respectively. The proposed method is accurate, precise and reproducible and can be adopted for routine analysis of levofloxacin hemihydrate and ornidazole in tablet formulation.

Key Words: Levofloxacin hemihydrate, Ornidazole, HPTLC, Estimation.

INTRODUCTION

Levofloxacin (LVF) is chemically (-)-(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 (ORZ) is chemically 1-(3-chloro-2 hydroxy propyl)-2-methyl-5-nitro imidazole. Both drugs are available in combination as tablet, used for treating bacterial and protozoal infections. Literature survey reveals that levofloxacin[1-3] hemihydrate in tablet dosage form and in biological fluids are estimated by HPLC methods. Ornidazole[4-6] is estimated by HPLC and several other methods have been reported for estimation of ornidazole in biological fluids. No analytical method has been reported for analysis of these two drugs in combination. In present work, a successful attempt has been made to estimate both the drugs simultaenously by an accurate, precise, economical and less time consuming HPTLC[7].

EXPERIMENTAL

Instruments used

Application mode : CAMAG Linomat IV sample applicator
Scanner mode : CAMAG TLC Scanner III
Development mode : CAMAG Twin through chamber

Chromatograph conditions

Stationary phase :	Pre-coated silica gel plate 60 F_{254} pre washed with methanol
Mobile phase :	*n*-Butanol:water:glacial acetic acid (60:20:20 v/v/v)
Distance between bands :	15 mm
Injection volume :	10 µL
Sepration technique :	Ascending development
Scanning mode :	Absorbance
Lamp :	Deuterium
Wavelength :	366 nm

Label claim:

Levofloxacin - 250 mg; ornidazole - 500 mg

Preparation of standard stock solution: An accurately weighed quantity of 1250 mg of LVF (working standard) and 2500 mg of ORZ (working standard) were dissolved in methanol make upto 100 mL to obtain a stock solution containing 12,500 and 25,000 mcg/mL of LVF and ORZ, respectively.

Preparation of standard solution: Approximately 125 mg of LVF and 250 mg of ORZ were weighed and transferred to 100 mL volumetric flask and made up to volume with the diluent.

Preparatin of sample solution: 20 Tablets are weighed and powdered. The powder equivalent to 125 mg LVF and 250 mg of ORZ (620 mg of powdered drug that is average weight) was transferred to 100 m volumetric flask. The contents were dissolved in diluent and the volume is made up to the mark. The contents were mixed well using ultra-sonicator and filtered through Whatman filter paper number 42.

Estimation method: The sample was spotted on the chromplate with help of Linomat IV spotting system. The chromatograms were recorded and the peak area for levofloxacin and ornidazole were noted down. The amount of drug present was calculated by comparing the peak area values of sample with that of standard using the formula:

$$\text{Amount of drug in each tablet} = \frac{\text{Peak area of test} \times \text{conc. of std.} \times \text{std. purity} \times (100 - \text{LOD}) \times \text{average weight}}{\text{Peak area of std.} \times \text{conc. of test} \times 100 \times 100}$$

The results were tabulated in Table-1.

Validation: To validate the developed method parameters like linearity, range, system repeatability test, system suitability test, accuracy in terms of recovery, precision in terms of percentage and relative standard deviation were studied.

Linearity and range: The linearity of the method was assessed by performing single measurement at several analyte concentrations. A minimum of 5 concentrations were recommended for linearity studies. Varying quantities of standard stock solution was diluted with diluent to give a concentration of 1050-1460 µg/mL of LVF and 2100-2900 µg/mL of ORZ.

A calibration curve was constructed for each sample by plotting peak areas against concentration.

There exist a linear relationship in the range of 1050-1450 µg/mL 2100-2900 µg/mL for LVF and ORZ, respectively. From the constructed curve co-efficient of variance, slope and intercept were calculated. The results were tabulated in Table-3.

System repeatability: Three replicate applications to the HPTLC plate were performed for the standard solution containing 1250 µg/mL LVF and 2500 µg/mL of it should be ORZ. The results obtained by repeating the estimation procedure five times were observed to be good. Precision of the method is expressed in terms of percentage relative standard deviation of the data obtained, here in this case the peak area obtained. The relative standard deviation for these areas was calculated. The results were tabulated in Table-4.

System suitability parameters is like resolution of peaks, tailing factor were also determined and tabulated in Table-5.

The accuracy of the developed method can be assessed by performing recovery studies. To ensure the reliability of the method, recovery studies were carried out by mixing a known quanity of standard drug with the pre-analyzed formulation and the contents were reanalyzed by the proposed method. The results were tabulated in Table-6.

RESULTS AND DISCUSSION

The solvent system of the mobile phase having *n*-butanol:water:glacial acetic acid (60:20:20 v/v/v) offered maximum resolution for the two drugs at the wavelength of 366 mm at which both the drugs had optimum absorption.

The assayed values in terms of percentage label were found to be within the standard acceptable limits (Table-1) and so the method can be adopted for simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablet formulation. The statistical validation was also done for the assay values (Table-2).

Linearity studies were carried out and there exists linearity in the concentration range of 1050-1450 and 2100-2900 µg/mL for LVF and ORZ, respectively (Table-3).

Lower percentage relative standard deviation of measurement indicates the precision of the developed method (Table-4).

TABLE-1
QUANTITATIVE ESTIMATION

Sample	Label claim* (mg)	Label claim (%)	Deviation (%)
Levofloxacin hemihydrate	250	97.26	-2.74
Ornidazole	500	98.00	-2.00

TABLE-2
STATISTICAL DATA

Sample	Label claim (mg)	SD	RSD (%)	SE
Levofloxacin hemihydrate	250	0.087	0.035	0.048
Ornidazole	500	0.450	0.093	0.025

SD = Standard deviation, RSD = Relative standard deviation,
SE = Standard error

TABLE-3
LINEARITY

Parameter	Levofloxacin hemihydrate	Ornidazole
Linearity range	1.05-1.45 mg/mL	2.1-2.9 mg/mL
Regression (r)	0.999	0.9999
Slope (m)	20.129	6.3000
Intercept (c)	-33.240	0.6000

TABLE-4
SYSTEM REPEATABILITY AND PRECISION

Parameter	Levofloxacin hemihydrate	Ornidazole
Concentration (µg/mL)	1250	2500
Peak area*	25013.2	15774.1
Standard deviation	10.4068	7.9563
Relative standard deviation (%)	0.0416	0.05604

*Mean of five individual readings.

Resolution factor and tailing factor are ensuring proper functioning of HPTLC system (Table-5) thus making the method suitable. The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for estimation of drug in tablet formulation (Table-6).

Thus the developed method was found to be accurate, precise, suitable and cost effective for the simultaneious estimation of levofloxacin hemihydrate and ornidazole in tablet formulation.

TABLE-5
SYSTEM SUITABILITY

Parameter	Levofloxacin hemihydrate	Ornidazole
Resolution	–	5.65
Tailing factor	0.67	0.86

TABLE-6
RECOVERY STUDIES

Sample	Amount of drug added (mg)	Amount of drug recovered*	Recovery* (%)
Levofloxacin hemihydrate	25.10	23.52	99.70
Ornidazole	50.10	51.45	100.45

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