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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Levofloxacin hemihydrate and ornidazole in Tablets

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ABSTRACT: A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablet formulation. The mobile phase was triethylamine (0.5%v/v adjusted to pH 3 using orthophosphoric acid), Acetonitrile and Methanol (40:30:30) at a flow rate of 0.5 ml/min. Detection was carried out at 310nm. The stationary phase was Phenomenex Luna C_{18} column (5µ, 150x4.6mm I.D).Retention time was 3.42 and 5.65 min for levofloxacin hemihydrate and ornidazole, respectively. Linearity was established in the range of 10-50 µg/ml and 20-80 µg/ml for the assay of levofloxacin hemihydrate and ornidazole.Mean recovery obtained for levofloxacin hemihaydrate and ornidazole were 100.58% and 99.68%, respectively.Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablets.
Key words: RP-HPLC, Simultaneous determination, Levofloxacin hemihydrate, Ornidazole.

INTRODUCTION

Levofloxacin(LFH),(-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl]-7-oxo-7H-Pyridol[1,2,3-di]-1,4-benzoxazine-6-carboxylic acid hemihydrate (LFH),is chemically, a chiral fluorinated carboxy quinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin [1].It is used mainly as an antibacterial agent. Ornidazole (ORN), [1-chloro-3-(2-methyl-5-nitroimidazole-1-yl) 2-propanol] is 5-nitroimidazole derivative[2] with antiprotozoal properties against anaerobic bacteria. Fixed dose combination containing LFH (250mg) and ORN (500mg) is available in market as tablets.

Literature survey revealed few methods have been reported for the spectrophotometric methods for the estimation of Levofloxacin hemihydarte, alone or in combination with other drugs in pharmaceutical formulation[3, 4]. Ornidazole, alone or in combination with other drugs, is reported to be estimated by spectrophotometry[4-9] and HPLC[10, 11]in biological fluids or pharmaceutical formulations.

However, no HPLC method for the simultaneous estimation of Levofloxacin hemihydrate and Ornidazole in combined dosage forms has been reported so far. The present work describes the development of simple, precise and accurate isocratic reverse phase HPLC method for simultaneous estimation of LFH and ORN in tablets.

MATERIALS AND METHODS

The standard bulk drug sample, LFH and ORN were obtained as gift samples from the Glenmark research center, Mumbai. All the solvents and chemicals were of analytical grade and supplied by Qualigens, India (Pvt) Ltd.

A gradient high pressure liquid chromatograph (shimadzu HPLC class Vp series) with two LC- 10ATVp pumps, variable wavelength programmable UV/VIS detector SPD-10AVp, SCL-10AVp system controller (shimadzu) and winchrome software used. The chromatography column used was a reverse phase Luna C18 column (150 x 4.6mm.I.D. particle sizes 5µ).A mixture of methanol, triethylamine (0.5%v/v adjusted to pH 3 using orthophosphoric acid), acetonitrile and methanol (40:30:30)was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of the mobile phase was maintained at 0.5ml/min.detection was carried out at 310nm at 25^0.

Standard stock solution

Standard stock solution of LFH and ORN (1000µg/ml) was prepared in HPLC grade water as diluents separately. The standard solutions were further diluted to contain a mixture of 10µg/ml of LFH and ORN.

Analysis of formulation

Twenty tablets of LOXOZ (Glenmark research centre, Mumbai) containing 25mg of LFH and 500mg of ORN were weighed and finely powdered separately. Powder equivalent to 100mg of LFH and ORN was weighed and transferred to a sintered glass crucible and drug was extracted with three 20 ml quantities of mixture of diluents. The combined extracts were made up to 100ml and further dilutions were made to get a concentration of 10µg/ml of LFH and 10 µg/ml of ORN. The contents were mixed thoroughly and filtered through a 0.45µ filter. Twenty micro liters of the test and standard solutions were injected separately and chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in **Figure-1**.The peak area ratios of each of the drugs were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in [**Table-1**].The stability of the sample in mobile phase was analyzed after 24hrs; it was found no change in analytical parameters.

Recovery studies

To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Results of recovery studies were found to be satisfactory and are reported in **[Table-1].**

Table 1: Analysis of formulation and recovery studies

Dugs	Labelled amount (mg)	*Amount found (mg)	%label claim	*%Recovery	*Precision# (%RSD)	
					Interday	Intraday
Levofloxacin hemihydarte	250	249.86±1.140	99.94	100.58±0.896	0.511	0.565
Ornidazole	500	499.19±0.679	99.84	99.68±0.652	0.297	0.289

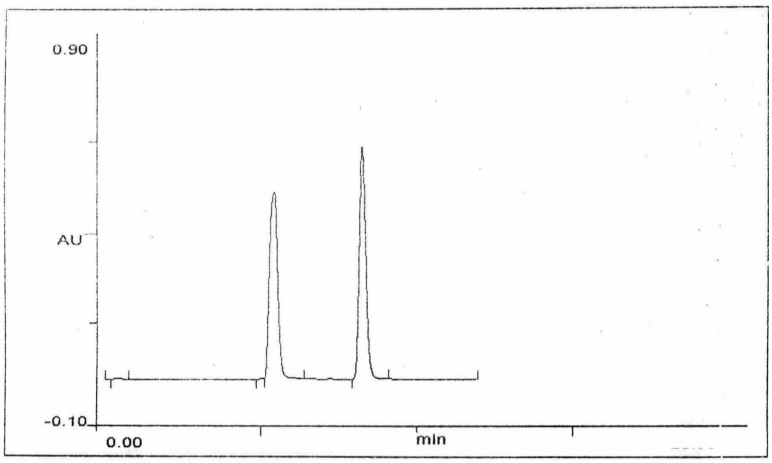
*Each value is a mean of six observations.

Table 2: Optical and Regression Characteristics for Both Drugs

Validation parameters	310 nm	
	LFH	**OR**
Linearity (µg/ml)	10-50	10-50
Molar Absorptivity	4.16	2.28
Regression Equation:Slope	1108286	1037705
Intercept	147304	15538.71
r	0.999941	0.999966
LOD(ng/ml)	6	8
LOQ(ng/ml)	10	20
Standard error of mean	248372.9	169901.9
Intraday (%RSD)*	0.586	0.784
Interday (%RSD)*	0.484	0.287
Repeatability(%RSD)*	0.321	0.425
Peak purity index	1.0000	1.0000
Asymmetry factor(A_S)	0.9	1.48
No. of theoretical plates(N)	8238	7716
Tailing factor	1.01	1.28

*Each value is a mean of six observations.

Figure- 1 HPLC Chromatogram for formulation



No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	3.42	135.91	1.01	0.90	8238	0.00	0.00
2	5.65	514.43	1.28	1.48	7716	0.00	0.00

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REFERENCES

1. Budavari S., Eds., In; The Merck index, 13th Edn.,merck & Co.,Inc.,white house station,NJ, 1998,3124.
2. Budavari S., Eds., In; The merck index, 12th Edn.,merck & co.,Inc.,white house station,NJ 1996,1178.
3. Nagori B.P., Shrivastava B.,Sharma V and Rajput A.S., Spectrophotometric Method for Simultaneous Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form, Indian drugs,2006,43(10),676-678..
4. Somashekar M., Vidyasager J., Narsaiah N., Anand kumar R and Krishna D.R., Validated HPLC Method for the Determination of Ornidazole in Human Serum and Urine, Indian J.Pharm.Sci.,2005,67(3),302-306.
5. Groppi A.,Papa P.,Montagna M and Carosi G., Determination of Ornidazole in Human Plasma and RBCs Using HPLC, J.Chromatogr.,1986,380(2),42-437.
6. Kale U.N., Naidu K.R and Shingare M.S., Spectrophotometric Determination of Ornidazole and Norfloxacin in Tablets, Indian J. Pharm.Sci., 2003, 65(5), 439-556.
7. Abu., Zuhri A.Z., Voelter W., Al-Kahalil S and Salahat., Extractional Spectrophotometric Determination of Febendazole and Ornidazole in Pharmaceutical Formulations, J.Pharm.Sci.,,2000,68(1),109-122.
8. Kasture V.S., Bhagat A.D., Pure N.C., More P.S and Bandari N.K., Spectrophotmetric Method for Simultaneous Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form, Indian drugs ., 2004,41(1),51-53.
9. Lakshmi Sivasubramaniam., Kasi Sankar V., Sivaraman V., Senthil Kumar V., Senthil Kumar K., Muthukumaran A., and Raja T.K, Visible Spectrophotometric Determination of Levofloxacin in Tablet Dosage Forms, Indian J.Pharm.Sci.2004,779-802.
10. Patel U.N., Suhagia B.N., Patel M.M., Gayathri C., Patel and Geetha M. Patel, Simultaneous Spectrophotometric Estimation of Gatifloxacin and Ornidazole in Mixture, Indian J.Pharm.sci, 2005, 67(3), 356-357.
11. Chepurwar S.B., Shirkedkar A.A., Bari S.B and Surana S.J., Spectrophotometric Method for Simultaneous Estimation of Levofloxacin and Ornidazole in Tablet Dosage forms, Indian Drugs,2006,43(10),803-806.
12. ICH, Guidelines for Validation of Analytical Methods, centre for drug evaluation and research, Procedures, 1996.

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