QUANTITATIVE DETERMINATION OF LEVOFLOXACIN HEMIHYDRATE IN BULK AND TABLETS BY UV-SPECTROPHOTOMETRY AND FIRST ORDER DERIVATIVE METHODS

SHIRKHEDKAR AA* AND SURANA SJ

RC Patel College of Pharmacy, Karwand Naka, Shirpur, Dist: Dhule (MS), 425 405, India

ABSTRACT

Two Simple, rapid, accurate and economical 'UV Spectrophotometry' and 'First Order Derivative' methods have been developed for determination of levofloxacin hemihydrate in bulk and tablets. In (10% *v/v*) acetonitrile, the λmax of the drug was found to be 288 nm. The same spectrum was derivatised into first order derivative, using UV probe software of instrument (Shimadzu-2450), at Δλ = 4. The amplitude of the trough was recorded at 297 nm. In both the proposed methods, levofloxacin hemihydrate follows linearity in the concentration range 2 – 12 µg/ml with a correlation coefficient of 0.9999. Assay results were in good agreement with label claim. The methods were validated statistically and by recovery studies. The relative standard deviation were found to be less than 2% with excellent precision and accuracy.

Keywords: Levofloxacin hemihydrate; UV-spectrophotometry; first order derivative.

INTRODUCTION

Levofloxacin hemihydrate, (-)-(S)-9-fluro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperzinyl)-7-oxo—7H pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-caboxylic acid hemihydrate is broad spectrum fluorinated quinolone antibacterial (The Merck Index, 2001). It acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication and thus causes bacterial lyses (Goodman and Gillman, 2001). In literature , various analytical methods such as HPLC (Bottcher *et al*., 2001; Hairui *et al*., 2002; Wong *et al*., 2001), HPTLC (Meyyanathan *et al*., 2003) and conductometry (Altioka and Atkosar, 2002) have been reported for estimation of levofloxacin.

The objective of the present work is to develop simple, rapid and economical 'UV-spectrophotometry' and 'First Order Derivative' methods for determination of levofloxacin hemihydrates in bulk and finished products**.**

MATERIAL AND METHODS

Reagents
Acetonitrile (AR grade); Double reverse osmosis (RO) water.

Preparation of standard stock solution and study of calibration curves
Standard stock solution containing 100 µg/ml of levofloxacin hemihydrate was prepared in (10% *v/v*) acetonitrile. Different aliquots were taken from the stock, diluted to 10 ml mark with the same solvent to obtain a series of concentrations. The solutions were scanned on UV- visible spectrophotometer (Shimadzu-2450 with UV probe 2.21 software in the UV range 200 - 400 nm.

Corresponding author: e-mail: atul_shirkhedkar@yahoo.com

levofloxacin hemihydrate showed absorbance maxima at 288 nm. The same spectra were derivatised into first order derivative, using UV probe software of instrument, where Δλ = 4 (fig.). The amplitudes of the corresponding troughs were measured at 297 nm. In both the methods, levofloxacin hemihydrate follows linearity in the concentration range 2 - 12 µg/ml.
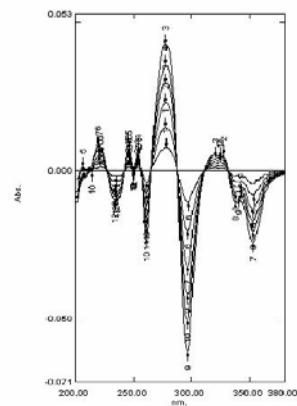
Fig. An overlain first order derivative spectra of levofloxacin hemihydrate in (10% v/v) acetonitrile.

Preparation of sample solution
For analysis of commercial formulation; twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 100 mg of levofloxacin was transferred into 100 ml volumetric flask containing 25 ml acetonitrile (10% *v/v*), shaken manually for 10 min, volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 41. After appropriate dilution absorbance was recorded at 288 nm and amplitude of the trough (first order derivative) was recorded at 297 nm. The results are shown in table 1.

Table 1: Results of assay

Drug	UV-Spectrophotometry		First order derivative	
	Amount found (%) [n = 6]	% RSD	Amount found (%) [n = 6]	% RSD
Levofloxacin (250mg/Tablet)	99.39	0.419	100.56	0.453

Table 2: Summary of validation parameters

Parameters	UV-Spectrophotometry	First order derivative
% Recovery (n = 9)	99.98	100.06
% RSD	0.25	0.737
Precision [%RSD]		
Intra-day (n = 3)	0.989 -1.133	0.624 -1.767
Inter-day (n = 3)	0.330 - 1.347	0.485 - 0.769
Repeatability(%RSD,n = 6)	0.651	0.253
Ruggedness [%RSD]		
Analyst-I (n = 6)	1.116	0.331
Analyst –II (n = 6)	1.005	0.564

Recovery studies

The recovery studies were carried out at three different levels i.e. 80%, 100% and 120% level. To the preanalyzed sample solution, a known amount of standard drug solution was added and reanalysed by the proposed methods. The results are shown in table 2.

RESULTS AND DISSCUSSION

Levofloxacin hemihydrate in acetonitrile (10%v/v) showed absorbance maximum at 288 nm. In first order derivative spectrum, the amplitude of the trough was recorded at 297 nm. In both the methods, levofloxacin hemihydrate follows linearity in the concentration range of 2 - 12 µg/ml with linear regression equations $Y = 0.0953 X + 0.0032$ in UV-spectrophotometry method($r^2 = 0.9999$) and $Y = 0.005 X + 0.0008$ for first order derivative method ($r^2 = 0.9999$).

Amount of drug determined by the proposed methods was in good agreement with the label claimed. The methods were validated for accuracy, precision and ruggedness as per USP (USP, 2005). The results are as shown in table 2. The results of validation parameters demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%). Both these proposed methods are simple, economical, rapid and can suitably be used for the determination of levofloxacin in tablet formulation.

REFERENCES

Altiokka G, Atkosar Z and Can NO (2002). The determination of levofloxacin by flow injection analysis using UV detection potentiometry and conductometry in pharmaceutical preparations. *J. Pharm. Biomed. Anal.*, **30**: 881.

Bottcher S, Baum HV, Hoppe-Tichy T, Benz C and Sonntag HG (2001). An HPLC and microbiological assay to detertmine levofloxacin in soft tissue, bone and serum. *J. Pharm. Biomed. Anal.*, **25**: 197.

Hairui L, Michel BK and Kevin MS (2002). Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin, and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. *J. Chromat.*, **60**: 771-775.

Meyyanathan SN, Ramasarma GVS and Suresh B (2003). Analysis of levofloxacin in pharmaceutical preparations by high performance thin layer chromatography. *J. Seperation Sci.,* **26**: 1698-1700.

The Merck Index (2001). An Encyclopedia of Chemicals Drugs and Biological, 13th edn., USA: Merck and Compony, Inc., **67**: 1213.

United State Pharmacopeia (2005). United State Pharmacoepoeial Convention, 28th ed., Inc., Rockville, MD, p.2749.

William AP (2001). Antimicrobial agents. *In*: Gilman AG, Goodman LS. The Pharmacological asis of Therapeutics; 10th ed, Mcgrew- Hill; New York, p.1183.

Wong FA, Juzwin SJ and Flor SC (2004). Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine. *J. Pharm. Biomed. Anal.*, **15**: 765.