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Statistical Assurance of Process Validation By Analytical Method Development and Validation for Levofloxacin IR Tablets and Blend

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Abstract: A new simple, rapid and reliable UV Spectrophotometry method was developed and validated for the estimation of Levofloxacin Hemihydrate in blend & tablets formulations. The method was based on simple UV estimation in cost effective manner for regular laboratory analysis. The instrument used was Perkin Elmer, UV Spectrophotometer (Lambda 25) and using 0.1 N HCl as solvent system. Sample were analysed using UV Win Lab 5.2.0 Software and matched quartz cells 1 cm and was monitored at 293.7 nm. Levofloxacin was used as an internal standard. Linearity was obtained in the concentration range of 2 - 10 µg mL^{-1} for Levofloxacin hemihydrate. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. Spectrophotometric interferences from the tablet excipients were not found. Stastical tools of ANNOVA and Boneforri's multiple tests were implemented on results of blend uniformity and content uniformity, done on process validation batches samples.

Key Words: UV Spectrophotometer, Levofloxacin Hemihydrate, Process Validation, Tablet Formulations, Quantitative analysis

INTRODUCTION

Levofloxacin, (-) S-9- fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinly)-7-oxo-7H-pyrido [1, 2, 3,-de]-1, 4-benzoxazine-6-carboxylic acid hemihydrate. Levofloxacin is a chiral fluorinated carboxyquinolone, a Racemate of Ofloxacin. It is S-(-) isomer of the fluoroquinolone antibacterial Ofloxacin and have broad-spectrum antimicrobial[1, 2] activity and penetrates well into cerebrospinal fluid (CSF), bone tissue, bronchial mucosa, and subcutaneous adipose tissues. Levofloxacin is given as the hemihydrate, but doses are expressed in terms of the base; Levofloxacin Hemihydrate 256 mg is equivalent to about 250 mg of the base. Levofloxacin is given by mouth or intravenously to treat susceptible infections[3].

Literature survey revealed spectrophotometric[4] and chromatographic[5, 6] methods for analysis of Levofloxacin. So far, no analytical methods are reported for analysis which is looking to pharmacokinetic characteristics of drug i.e. having Tmax of 1 – 2 hour. The objective of this investigation is to develop, two simple, accurate and economical UV-spectrophotometric[7] methods for estimation[8] of Levofloxacin using 0.1 N HCl in which drug have good solubility. Dissolution is also performed in 0.1 N HCl, looking to its Pharmacokinetic[9] and Immediate Release dosage form and so desired method is appropriate for analysis.

Process validation samples (blend and tablets) are withdrawn at all stages and for all three validation batches for which analysis was performed using developed method.

EXPERIMENTAL

Instrument

For method, Perkin Elmer UV-Vis spectrophotometer (Lambda 25, spectral bandwidth 1nm) with 10 mm matched quartz cells; Shimadzu, Electronic Weighing Balance (AUX – 220), Oscar Ultrasonic Cleaner, Sonicator (Micro Clean 103) were used.

Reagent

Concentrated Hydrochloric Acid (A.R.)

Procedure

Method of analysis

Standard stock solution of Levofloxacin was prepared by dissolving 55 mg drug in 100 mL 0.1 N HCl (i.e.550 µg/mL). Aliquot of these solutions were further diluted to obtain concentrations of 5.5 µg /mL for Levofloxacin and scanned in the UV-range. From the spectra, wavelength 293.7 nm (λ_{max} of Levofloxacin) was selected. As reported in **Figure1**. The linearity was observed in the concentration range of 2- 10 µg/mL for Levofloxacin. The absorptivity coefficient of drug at desired wavelengths was determined and the results are presented in **Table1**. The spectral data from this scan was used to determine the concentration of drug in blend and tablet sample solutions.

Analyses of Process validation samples (Blend and Tablet formulation)

Twenty tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 60 mg Levofloxacin was transferred to a 100 mL calibrated volumetric flask, extracted with 0.1 N HCl by shaking mechanically (for Content Uniformity). Similarly blend equivalent to 60 mg Levofloxacin was transferred to a 100 mL calibrated volumetric flask, extracted with 0.1 N HCl by shaking mechanically (for Blend Uniformity). The solution was diluted to mark with the same solvent and filtered through Whatmann filter paper. (no. 41). Aliquot portion of this solution was diluted to get concentration of 6 µg/mL of Levofloxacin. Absorbance of the sample solutions were recorded, at 293.7nm respectively (Perkin Elmer, Lambda 25). And, the concentrations of drug in samples were determined, by using calibration curve. The concentration of each drug was determined by analysis of spectral data of the sample solutions with reference standards. The results are reported in **Table 2**.

Recovery Studies

The recovery studies were carried out at three different level i.e. 80,100 and 120%. It was performed by adding known amount of standard drug solutions of Levofloxacin to preanalysed tablet solutions. The resulting solutions were then reanalyzed by proposed methods. The results of recovery studies are shown in **Table 3**.

RESULTS AND DISCUSSION

The proposed methods are simple, sensitive, accurate, precise, reproducible, economic and rapid for simultaneous analysis of Levofloxacin in tablets. Accuracy of the method was evaluated by carrying out recovery studies. Low values of %RSD are indicative of high precision of the methods. The repeatability and ruggedness study signifies the reproducibility of the method as shown in **Table 4**.

Based on the validation study data, it can be concluded that the proposed method is accurate and precise for the analysis of drug. No interference was found from excipients used in tablet formulation and hence the methods is suitable for analysis of blend and tablet formulation.

Process validation samples, blend uniformity was found to be good within and between all three validation batches as shown in **Table 5**. Compression of tablets, sample for content uniformity were collected at three stages (initial, mid, end) for all three validation batches, results for which show that there is uniformity in dosage units within batch and similarity between batches as shown in **Table 6**.

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Table 1: Absorptivity A (1%, 1Cm) Values of Levofloxacin at 293.7 nm

Concentration µg/ml	Absorbance	A(1%,1cm) Mean ± S.D	Molar Absorptivity Mean ± S.D
6	0.5874	979.11 ± 0.720	36234.93 ± 11.87

*mean of Six Estimations

Table 2: Result of Assay

Label Claim (mg/tab)	% Label Claim*	± SD	%RSD	SE
500 mg	100.75	0.88	0.83	0.14

*mean of Five Estimation

Table 3: Results of Recovery Studies

Sr. No.	Amount of Drug Added (µg/mL)	%Recovery* ± SD	% R.S.D
1	3.2	99.58 ± 0.41	0.43
2	4.0	99.1 ± 0.60	0.64
3	4.8	98.8 ± 0.88	0.89

*mean of three estimations at each level

Table 4: Results of Repeatability and Ruggedness studies

Parameters	% RSD
Precision %RSD	
Intra-day (n = 3)	0.40 - 1.46
Inter-day (n = 3)	0.72 - 1.39
Repeatability(n=6) %RSD	0.89
Ruggedness (n=5)	
Analyst I	0.65
Analyst II	0.74

Table 5: Blend Uniformity* (% Assay for Each Sample)

	Batch 1	Batch 2	Batch 3
Mean	100	98.4	102.1
Min.	98.6	98.1	101.5
Max.	100.8	98.8	102.8
%RSD	0.77	0.3	0.53

* Final blend analysed for 6 locations from Rapid Mixing Granulator

Table 6: Content Uniformity* (% Assay for Each Sample)

Sr. No.	Batch 1 Stage			Batch 2 Stage			Batch 3 Stage		
	1	2	3	1	2	3	1	2	3
Mean	99.9	100.2	100.1	99	100.3	101.3	102.7	103	103.2
Min.	99.9	99.5	99.6	97.4	98.4	100.2	102	102.6	102.7
Max.	98.9	100.6	100.6	100.2	102.6	102.3	103.8	104.1	103.9
%RSD	0.50	0.4	0.3	1.1	1.2	0.7	0.7	0.5	0.4

* Ten units individual assay was analysed for each stage of all the batches

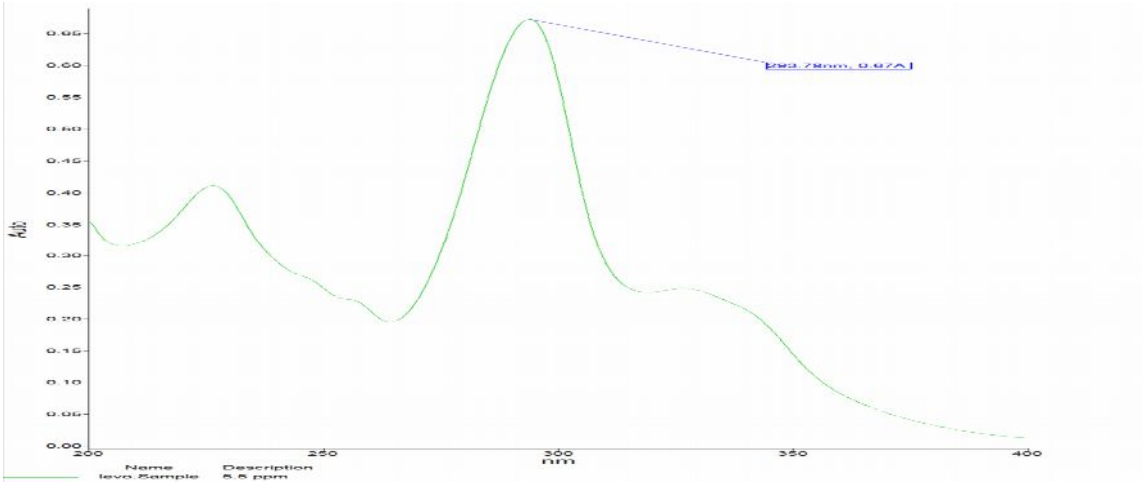

Figure 1 Spectra of Levofloxacin API

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