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DEVELOPMENT AND VALIDATION OF UV- VISIBLE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE IN BULK AND MARKETED FORMULATION

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ABSTRACT

Introduction: The objective of this research was to develop and validate a simple, rapid, accurate and economical UV-visible Spectrophotometric method for determination of levofloxacin hemihydrate in bulk and marketed formulation. Methods: In chloroform, the λmax of the drug was found to be 257.4 nm. In the proposed method, levofloxacin hemihydrate follows linearity in the concentration range 5 – 30 µg/ml with a correlation coefficient of 0.993. Results: Assay results were in good agreement with label claim. The methods were validated statistically and by recovery studies. The relative standard deviation was found to be less than 2% with excellent precision and accuracy. Conclusion: It was concluded that the developed method is suitable for the quality control of levofloxacin in pharmaceuticals formulations.

INTRODUCTION

Quinolones are antimicrobials, structurally related to nalidixic acid, which were made available for clinical use in urinary infections, since 1960s. They are used in human and veterinary medicine, especially in animal breeding area. [1],[2] Considerable amounts of Quinolones are widely used under field conditions (in poultry, swine, and cattle production), both in the treatment of infections and as growth promoters.1 The bactericidal activity of levofloxacin is mediated by the inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, essential enzymes involved in bacterial DNA replication, transcription, repair and recombination. [3]

Levofloxacin [Figure 1] is pure (–)-(*S*)-enantiomer of the racemic drug substance ofloxacin, which was introduced in 1997. A third-generation fluoroquinolone with a wide spectrum of action against gram-positive and gram-negative bacteria, anaerobic microorganisms, and atypical pathogens. [4] Levofloxacin prepared as hemihydrate, whose molecular mass is 369.93 g mol^{-1}, is presented as white to light yellow needle like crystals, that melt at approximately 226 ºC. Its solubility is nearly constant from pH 0.6 to 5.8 (100.0 mg mL^{-1}). Above pH 5.8, solubility increases sharply, reaching a maximum of 272 mg mL^{-1} at pH 6.7, beyond which it decreases to a minimum of 50.0 mg mL^{-1}. [5] Levofloxacin is the quinolone of choice for airway infections, being active against several types of pathogens. [1], [2], [4], [6].[7],[8]

Various analytical methods have been reported in scientific literature for the analysis of levofloxacin in pharmaceutical formulation and/or biological fluids including high-performance liquid-chromatography with UV detection (HPLC-UV), vibrational spectroscopy, spectrofluorimetry (SF), colorimetric Spectrophotometry (CS), Spectrophotometry by ion-pair complex (CIPS), and UV Spectrophotometry (UVS). [9-14] Most Spectrophotometric methods in the literature for analysis of levofloxacin is based on the formation of ion-complexes, which use dye as Eriochrome black, bromophenol blue, bromocresol green, eosin, merbromin and chromogenic reagent such as Folin-Ciocalteau. The addition of these substances usually increases the cost of analysis and sample preparation is time consuming. Besides cost, toxicity of reagents and solvents used in the analysis should also be considered. Exposure to merbromin even at low concentrations and short exposure time can cause poisoning. The complexes formed normally need extraction with organic

solvents, for example, chloroform, [12], [13] which in addition to further increase the cost of analysis and require safe handling and proper disposal. Recently an UVS method was proposed with acetonitrile as solvent for the quantitative determination of levofloxacin in tablets and solution. [14] This solvent is more toxic and more expensive than methanol. Therefore, the proposed method is less toxic to the analyst when compared with the solvent acetonitrile and is more economical. In addition, there are no official methods for determination of this active substance. [15], [16] Thus, the aim of this study was to develop and validate a fast, simple and cost-effective UV-Spectrophotometric alternative method for determination of levofloxacin hemihydrate in bulk and marketed formulation.

MATERIAL AND METHODS

Solubility studies profile

Levofloxacin hemihydrate is water insoluble so a solvent require in which it get completely soluble in a very less quantity for the cheapness of the method that we want to develop for the estimation of Levofloxacin hemihydrate in pure & marketed formulations. [17]

Wavelength scanning

For the selection of wavelength we prepared a stock solution of 100µg/ml Levofloxacin hemihydrate in chloroform which was then filled in the cuvettes & then scanned it from 400nm-200nm wavelength for the selection of wavelength which shows maximum absorption. Levofloxacin presented maximum absorption at 257.4 nm.

Calibration curve

Calibration curve is the most important part of any method for the determination, through the calibration curve one can determine the concentration for which level it follows the Beer's-Lambert's Law. For the calibration curve different concentration of Levofloxacin hemihydrate were prepared with selected solvent i.e. Chloroform. A stock solution of 100µg/ml was prepared. From this stock solution different dilutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml were prepared & absorbance were recorded at λmax 257.4 & the plot was plotted between the concentration & absorbance.

Sample preparation

Levofloxacin tablets

Twenty tablets were weighed and pulverized. Amount of powder equivalent to 100mg of drug was taken and diluted with 100ml chloroform. The solution was diluted suitably to prepare a 100mcg/ml concentration. Finally solution was filtered through Whatman filter paper no.41; filtrate was suitably diluted to prepare 5 mcg/ml, 10 mcg/ml, 15 mcg/ml, 20 mcg/ml, 25 mcg/ml and 30mcg/ml concentration and analyzed using proposed method.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared form the stock solution (100µg/ml) ranging from 5- 30µg/ml. The samples were scanned in UV-VIS Spectrophotometer using chloroform as blank. It was found that the selected drug shows linearity between the 5-30µg/ml

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed Formulation (lamiwin-500) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery. The percentage of recovery (R) was calculated as indicated by Association of Official Analytical Chemists International. [18]

$R = [(C_F - C_U) / C_A] \times 100$

Where C_F represents the concentration of analyte measure in fortified test sample; C_U, the concentration of analyte measure in unfortified test sample; and, C_A, the concentration of analyte added to fortified test sample.

Precision

The precision of an analytical method or a test procedure is referred to as the degree of closeness of the result obtained by the analytical method or the test procedure to the true value. Precision of the method was demonstrated by intraday and interday variation studies.

In intraday variation study, 9 different solutions of same concentration that is 20µg/ml were prepared and analyzed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD In the interday variation study, solutions of same concentration 20µg/ml were prepared and analyzed three times for three consecutive days and the absorbances were noted. In order to be considered precise, the RSD of the method should be less than 2.0% & the result was indicated by % RSD.

Specificity

10mg of lamiwin (Levofloxacin hemihydrate) was spiked with 50% (5mg), 100% (10mg), and 150% (15mg) of excipients mix (Magnesium Stearate) and the sample was analyzed for % recovery of lamiwin (Levofloxacin hemihydrate).

Robustness

Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature and at 18°c. The respective absorbances were noted and the result was indicated by % RSD.

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD.

Limit of Detection (LOD)

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

Limit of Quantitation

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to International Conference on Harmonization guidelines. [19]

$LOD = 3.3 \cdot SD_b / a$

$LOQ = 10.0 \cdot SD_b / a$

Where SD_b represents the standard deviation of y-intercept and a is the slope of calibration curve.

Formula's used

1. Percentage purity = absorbance (test)/absorbance (stand.)*wt. of stand./dilution factor*dilution factor/wt. of test*avg. wt/1*100
2. Absorbance = εbc or A1%1cm bc
3. Molar absorbtivity(ε) = A1%1cm *molecular weight/10
4. %RSD = Standard deviation/ mean*100

RESULTS

Levofloxacin was soluble in acetone, acetonitrile and ethanol but it was freely soluble in chloroform [Table 1]. Levofloxacin was analyzed by proposed UV Spectrophotometric method in tablets. The calibration curve showed linearity over a concentration range from 5.0 to 30.0 µg mL^{-1} shown in Table 2.

Table 1: Solubilty profile of Levofloxacin hemihydrate.

Solvent	Quantity of drug	Volume used	Solubility
Acetone	1gm	Upto100ml	Soluble
Ethanol	1gm	Upto100ml	Soluble
Chloroform	1gm	Upto 10 ml	Freely soluble
Acetonitrile	1gm	Upto100ml	Soluble

Table 2: Calibration curve data of Levofloxacin hemihydrate.

Concentration (µg/ml)	Absorbance
5	0.035
10	0.083
15	0.132
20	0.172
25	0.206
30	0.241

The linearity can be defined by following equation A = 0.008C + 0.0009 [Figure 2], where A and C are levofloxacin absorbance and concentration respectively. The correlation coefficients of the curve obtained with linear regression method were 0.993.

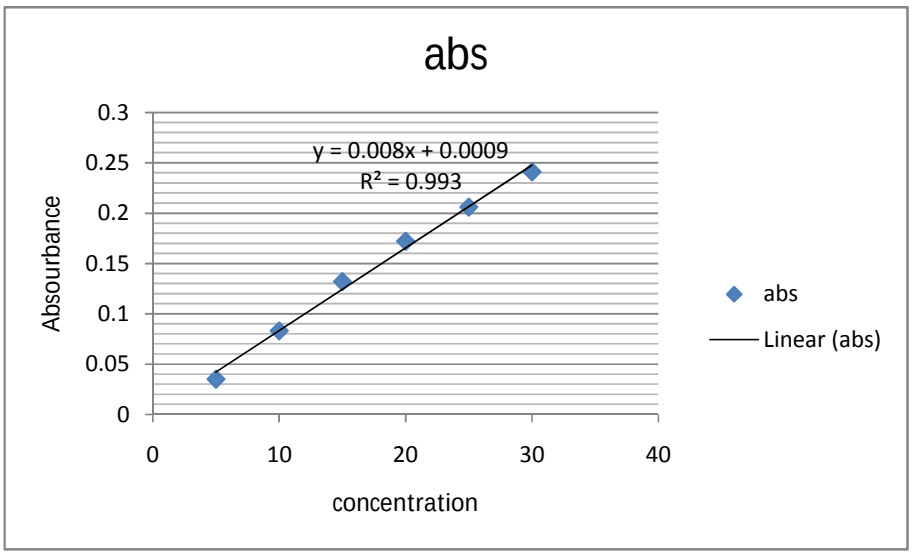

Figure 2: Calibration curve for Levofloxacin from standard solutions in the range 5.0 to 30.0 µg/ml

For accuracy, recovery studies were carried out and the percentage recovery was found to be in the range of 99.4-100.5%. The RSD amongst ten measurements for each sample found to be 0.578% tablets & percentage contents were 99.86 ± 0.01% for tablets. These results confirm accuracy of the proposed method. The percentage of recovery results are presented in Table 3.

Table 3: Recovery results of drug for determination of accuracy.

No. Of preparations	Concentration(µg/ml)		Percentage Recovery	Statistical results		
	Formulation	**Pure Drug**		**Mean**	**SD**	**%RSD**
S_1 : 80%	10	8	100.2			
S_2 : 80%	10	8	100.4	100.3	0.1527	0.152
S_3 : 80%	10	8	100.5			
S_4 : 100%	10	10	100.4			
S_5 : 100%	10	10	99.4	100.06	0.707	0.706
S_6 : 100%	10	10	100.4			
S_7 : 120%	10	12	100.2			
S_8 : 120%	10	12	100.4	100.3	0.115	0.114
S_9 : 120%	10	12	100.4			

Precision readings of ten observations are up to the mark & mean % RSD was found 0.578. The %RSD for intraday and interday was 0.502 and 0.27 respectively. The results are given in Tables 4 to 6.

Table 4: Precision readings showing repeatability of Levofloxacin hemihydrate.

Concentration(µg/ml)	Absorbance	Statistical Analysis
20	0.172	Mean=0.173
20	0.174	SD=0.001
20	0.173	%RSD=0.578
20	0.172	
20	0.173	
20	0.172	
20	0.175	
20	0.174	
20	0.173	
20	0.172	

Table 5: Intraday assay observations.

Concentration (µg/ml)	Absorbance			Average % RSD
	1	2	3	
20	0.172	0.173	0.172	
20	0.174	0.172	0.174	
20	0.173	0.174	0.173	
20	0.172	0.172	0.172	
20	0.173	0.173	0.175	
20	0.172	0.173	0.173	
20	0.175	0.174	0.172	
20	0.174	0.172	0.173	
20	0.173	0.173	0.172	
20	0.172	0.172	0.173	
% RSD	0.578	0.406	0.523	0.502

Table 6: Inter day assay precision.

Concentration (µg/ml)	% RSD			Average %RSD
	DAY1	DAY2	DAY3	
20	0.25	0.30	0.28	0.27

The results prove specificity of the proposed methods for inequivocal identification of analyte in the presence of matrix compounds (excipients). The excipients present in pharmaceutical dosage form (tablets) do not interfere in the analysis & recovery was 99.16% [Table 7].

Table 7: Test for specificity showing no effect of excipients.

Sample No.	Excipients conc.	Levofloxacin input(mg)	Levofloxacin recovered(mg)	% recovered	Mean	%RSD
1	50	10	9.89	98.9		
2	100	10	9.94	99.4	99.16	0.253
3	150	10	9.92	99.2		

The robustness at room temp. & at 18^{o}C was 0.289% & 0.465% RSD respectively. The ruggedness shown by analyst-1 & 2 was 0.520 % & o.289 % RSD respectively. [Table 8]

Table 8: Result showing the ruggedness & robustness of method for Levofloxacin hemihydrate.

Analyst-1			**Analyst-2**		
Conc.	**Abs**	**Statistical data**	**Conc.**	**Abs**	**Statistical data**
20	0.174	Mean=0.173	20	0.173	Mean=0.173
20	0.174	SD=0.0009	20	0.173	SD=0.0005
20	0.173	%RSD=0.520	20	0.174	%RSD=0.289
20	0.172		20	0.173	
Room temperature			**Temperature 18 ^{0}C**		
20	0.174	Mean=0.173	20	0.173	Mean=0.172
20	0.173	SD=0.0005	20	0.174	SD=0.0008
20	0.173	%RSD=0.289	20	0.173	%RSD=0.465
20	0.174		20	0.172	

The LOD and LOQ were 0.3µg/ml.and 0.78µg/ml, by using Equations 2 and 3 respectively. While comparing proposed analytical method for determination of levofloxacin in pharmaceutical formulations with those reported in literature, it can be observed that the developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2% .Good recoveries of the drug were obtained at each added concentration indicating that the method is accurate. The method is also specific indicated by the recoveries ranging from 98.4% to 99.4%. The LOD & LOQ were found to be in sub microgram level indicating the sensitivity of the method.

DISCUSSION

In this study, the developed and validated UV-Spectrophotometric alternative method for the determination of levofloxacin in pharmaceutical formulations has the advantage of being simple rapid, sensitive, cost-effective with high accuracy and precision. These advantages encourage the application of this method in routine analysis of levofloxacin.

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