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Development of a new method for the validation of levofloxacin by UV-Spectrophotometer

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ABSTRACT

The purpose of the study is to develop an efficient, fast, accurate and precise UV spectrophotometric method by using 0.1N HCl as a solvent, for the validation of levofloxacin in injection form. The λ_{max} for levofloxacin was found to be 293nm using 0.1N HCl as a blank. Calibration curve data proved that the proposed method was linear in the concentration range of 3 to 7µg/mL. The proposed method was validated to determine linearity, precision, accuracy, ruggedness and robustness. The results of above analytical parameters proved that the developed method could be used for the routine analysis of levofloxacin in injection dosage form. The proposed method is free from expensive solvents, chemicals and time consuming steps and can be used in quality assurance laboratories of pharmaceutical industries.

Keywords: Levofloxacin, ICH, Accuracy, Linearity, Precision, Robustness, Ruggedness

INTRODUCTION

Chemically levofloxacin or L-ofloxacin, is a 9-fluoro-2, 3-dihydro-3methyl-10- (4-methylpiperazine-1-yl) -7-oxo-7H-pyrido [1, 2, 3-de] -1, 4-benzoxazine-6-carboxylic acid hemihydrates (Fig.1). It is a wide range antimicrobial agent and *L*-isomer of the racemic mixture of fluoroquinolone of loxacin showing good bacteriological activity (Desai *et. al.,* 2011). Levofloxacin (fluoroquinolone) causes bacterial lysis by restraining the activity of gram-positive and gram-negative bacterial DNA gyrase and topoisomerase IV which are the major factors in the replication, transcription, repair, and recombination of DNA (William, 2001; Kohanski *et. al.,* 2010).

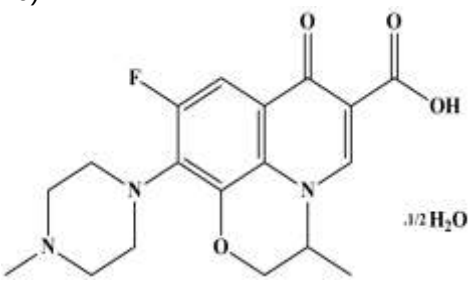

Fig. 1: Structure of levofloxacin hemihydrate

The bacteria showed reduction in susceptibility against fluoroquinolones due to mutation in bacterial genes that code for DNA topoisomerase IV and DNA gyrase (Hooper, 2001). Literature survey showed that a lot of analytical methods like HPLC (Okazaki *et. al.,* 1991; Böttcher *et. al.,* 2001), high performance thin layer chromatography (HPTLC) (Meyyanathan *et. al.,* 2003) and conductometry (Altiokka *et. al.,* 2002) have been described for the estimation of levofloxacin in bulk and various dosage forms. More recently some simple and accurate UV spectrophotometric methods were also reported for estimating levofloxacin with the help of different solvents such as distilled water, 100% methanol (Kassab *et. al.,* 2010; Ashour & Al-Khalil, 2005), acetonitrile (Shirkhedkar & Surana, 2009), water: methanol: acetonitrile with 9:0.5:0.5 ratio (Malequea *et. al.,* 2012). The analytical work on levofloxacin in injection form is limited and standard operating procedure (SOP) is not present in pharmacopoeia. In this study, efforts were made to develop and validate a simple, easy and economical UV-Spectrophotometric method by using 0.1N HCl. The proposed method was optimized and validated according to the guidelines of International Conference on Harmonization (Validation of

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analytical procedures: text and methodology, in: International Conference on Harmonization (ICH), Q2(R1), IFPMA, Geneva, Switzerland, 2005) and well explained linearity, precision and accuracy for levofloxacin.

MATERIALS AND METHODS

Instrument

An Ultrasonic Cleaner – Delmer and electronic balance (Electric Mettler Toledo balance, model AL 204 - Germany) were used. UV-VIS Double Beam Spectrophotometer, UV - 1800 Series, Shimadzu, Japan, having wavelength range of 200nm - 800 nm with two matched 1 cm matches quartz cell were used.

Chemicals and reagents

All the solvents, chemicals and reagents used were of analytical grade (Sigma Aldrich Ltd.) and used without further purification.

Sample injection

The present research work was performed on Injection Levofloxacin (Getz Pharma) that contained active ingredients levofloxacin. Each injection contained 500mg/100mL levofloxacin. The injection was pale yellow in colour and clear.

Preparation of standard stock solutions

Weighed accurately 100mg of levofloxacin hemihydrate into a 100mL volumetric flask, dissolved and sonicated for 10 minutes and diluted to volume with 0.1N HCl (1000µg/mL). Pipetted out 0.5mL of this solution into a 100mL volumetric flask and diluted to volume (100mL) with 0.1N HCl. The final concentration of working standard solution was made to 5µg/mL.

Selection of wavelength

The appropriate wavelength for the estimation of levofloxacin hemihydrate was selected from the UV spectrum. The 200 - 400nm was used to scan the standard solution of levofloxacin hemihydrate and the λ_{max} was found to be 293nm against 0.1N HCl.

Sample solution

20mL Levofloxacin injection was transferred into 100mL of volumetric flask and diluted with 0.1N HCl. Pipetted out 0.5mL of this solution into a 100mL volumetric flask and diluted to volume with 0.1N HCl. The final concentration of working standard sample solution was made to 5µg/mL.

Preparation of working standard solution for linearity

Suitable aliquots of 1000µg mL^{-1} of sample solution were diluted up to the mark with 0.1N HCl to get the concentration range of 3, 4, 5, 6 and 7µg mL^{-1}. The absorbance was measured at λ_{max} 293nm.

Method development

The standard stock solution of 1000 µg mL^{-1} of levofloxacin was prepared by weighing accurately 100mg of standard levofloxacin hemihydrate, taken in 100mL of the flask and diluted with 0.1N HCl. The sample stock solution of levofloxacin injection was prepared by accurately measuring 20mL from 500mg/100mL bottle, taken in 100mL of flask and diluted with distilled 0.1N HCl. By appropriate dilution of standard and sample stock solutions, different concentrations (3 – 7µg mL^{-1}) of standard solution and sample solution with 0.1N HCl were prepared, these different solutions of standard and sample levofloxacin were scanned in the range of 200 to 400nm to determine the wavelength of maximum absorbance, using the 0.1N HCl as a blank. Levofloxacin showed maximum absorption at 293nm in 0.1N HCl.

Amount of levofloxacin in each injection was calculated by measuring absorbance of standard and sample solutions was measured at 293 nm using 0.1N HCl as blank. The %age of levofloxacin was calculated by using the following formula:

$$\text{Contents of levofloxacin (\% assay)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$
$$= 0.51033/0.510 \times 100$$
$$= 100.06\%$$

Method validation

The method was developed and validated according to the analytical procedure as per the ICH guidelines for validation of analytical procedures in order to determine linearity, accuracy precision, ruggedness, and robustness for the analyte.

Linearity

Different concentrations of the sample solution of levofloxacin were used to determine the linearity. The concentration range for Beer-Lambert's law was found to be 3 to 7µg mL^{-1}.

Accuracy (% Recovery)

To study the accuracy, the standard addition method (Bhinge & Malipatil, 2016) was performed. The accuracy of developed method was

performed by preparing nine sample solutions of 500mg/100mL concentration. Added a known amount of active drug (10 – 30mg) to each sample solution and scanned at 293nm to measure the % and mean % recoveries.

System precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same concentrations. The system precision is a measure of the method variability. The precision was performed using five samples of 5µg/mL concentration of levofloxacin.

Intraday precision (repeatability) and intraday precision (intermediate precision)

Five different solutions containing same concentration of levofloxacin (5 µg mL^{-1}) were used to measure the repeatability.

Robustness

Robustness of developed method is its tendency to remain unaffected by small but deliberate variation in method parameters for estimation of levofloxacin. For this purpose, sample and standard solutions were scanned at λ_{293} and $\lambda_{max\pm1}$.

Ruggedness

Ruggedness was determined by analyzing the sample solution by two different analysts or at two different days by changing environmental conditions. The % RSD was calculated (table 6), ruggedness parameters were checked by the sample containing the active drug.

RESULTS AND DISCUSSION

Method development

The methodology described in the present study provides an expedient and efficient way for the analysis of levofloxacin injection by UV spectrophotometry method. The selected wavelength for analysis was 293 nm (Fig. 2).

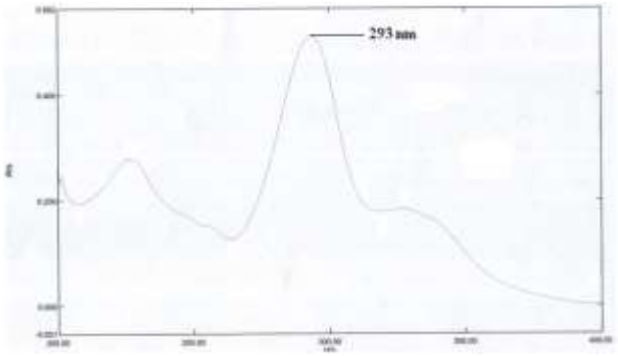

Fig. 2: UV spectrum of levofloxacin

The absorbance range 0.305 to 0.713 was found to be for levofloxacin (table 3). The concentration range of 3-7 µg mL^{-1} was taken for developed method to establish linearity. The results of different physical and validation aspects for determination of levofloxacin are described as follow:

Physical parameters

General tests

The appearance of sample injection leflox 100mL containing levofloxacin 500mg/100mL was observed for following parameters (table 1 and 2).

Table I: The general tests of sample levofloxacin injection

Sr. No.	Tests	Specification	Results
1	Dosage Form	Injection	Injection
2	Colour	Light Yellow	Light Yellow Liquid
3	Clarity	Clear from any visible Contamination	Clear from any visible Contamination
4	pH	4.20 to 5.20 at $30^{o}C$	5.15
5	Average Volume/bottle	100mL	105mL
6	Leakage	Leak Proof	Leak Proof

Table II: Identification of levofloxacin in injection

Sr. No.	Test	Obser-vation	Confirmation
1	5mL of sample was taken in the test tube, added 10mL of distilled water, 1mL of ferric chloride solution	Red color appeared	Levofloxacin confirmed

Linearity

The calibration curve for different concentrations of levofloxacin between absorbance and concentration was plotted for the determination of linearity as shown in table 3 and Fig. 3.

Table III: Calibration data for linearity at 293nm

Linearity (Working standard)				
Standard concentration (µg/mL)	**No. of samples**	**Absorbance**	**Mean**	**%Assay**
5	1	0.510	0.510	99.80
	2	0.509		
	3	0.511		
Linearity (Sample solution)				
Sample concentrations (µg/mL)	**No of samples**	**Absorbance**	**Mean**	**%Assay**
3	1	0.305	0.30566	59.93
	2	0.306		
	3	0.303		
4	1	0.410	0.40933	80.19
	2	0.409		
	3	0.409		
5	1	0.510	0.51033	100.06
	2	0.509		
	3	0.512		
6	1	0.610	0.61066	119.73
	2	0.611		
	3	0.611		
7	1	0.712	0.713	139.80
	2	0.712		
	3	0.715		

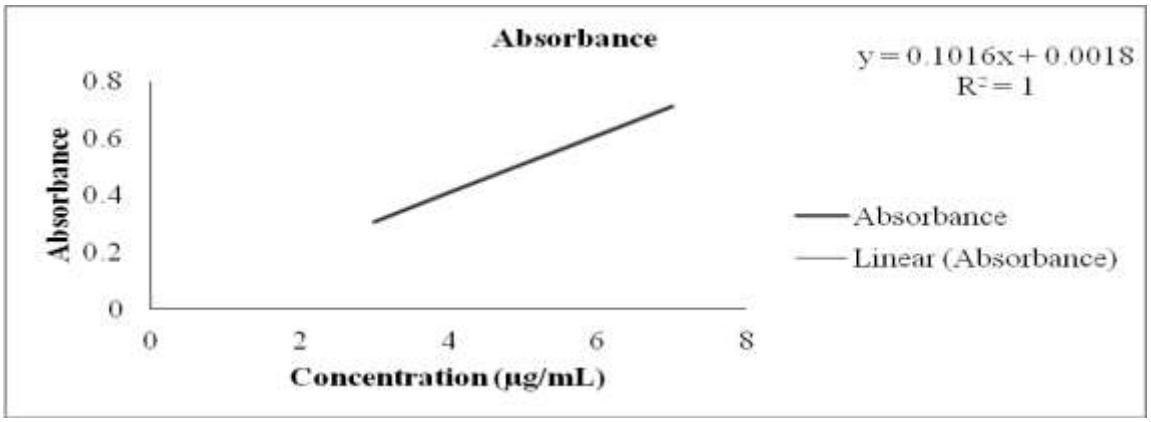

Fig. 3: Calibration curve of levofloxacin at 293 nm

Calibration curve for levofloxacin was observed for linearity in the range of 3-7µgmL^{-1}. The linearity of the calibration curve was validated by the value of correlation coefficients (r^2). The test results were found to be within limit 98 to 110% shown in table 3 and Fig. 3.

Accuracy (% Recovery)

The % and mean % recoveries are given in the table 4.

Table IV: Accuracy (% Recovery) study

Amount of levofloxacin in sample (mg/100mL)	Amount of levofloxacin added in sample (mg)	Total amount of levofloxacin (mg)	Amount (mg) recovered at 293nm	% Recovery (n = 3)	Mean % Recovery
500	10.00	510	510.95	100.18	100.05
500	10.00	510	509.23	99.84	
500	10.00	510	510.75	100.14	
500	20.00	520	519.91	99.98	100.05
500	20.00	520	520.98	100.18	
500	20.00	520	520.00	100.00	
500	30.00	530	529.90	99.98	99.92
500	30.00	530	528.96	99.80	
500	30.00	530	529.97	99.99	

The standard addition method was used for the measurement of accuracy. The % recoveries for levofloxacin were found in the range of 99.84 - 100.18%. The means of % recovery assay was found to be 100 % that is close to standard value of levofloxacin (table 4) demonstrating that the developed method is a precise method for the validation of levofloxacin and also indicating that there is non-interference with the excipients of injection.

System precision

The % RSD of these samples should be within 2% according to ICH as given in table 5.

Table V: System precision study

No. of samples	Absorbance	%Assay
1	0.510	100.00
2	0.511	100.19
3	0.512	100.39
4	0.511	100.19
5	0.513	100.58
Mean	0.5114	100.27
% RSD	**0.00223**	

Intraday precision (repeatability) and intraday precision (intermediate precision)

Mean, %SD and % RSD were calculated and shown in table 6 for repeatability and intermediate precision.

Table VI: Intraday and intraday precision study

Intraday and intermediate precision				Intraday assay (n = 5)		Analyst-to-Analyst (n = 10)		
	Preparations	Absorbance	Mean	%SD	%RSD	Mean	%SD	%RSD
Analyst 1	1	0.509						
	2	0.511						
	3	0.510	0.5098	0.000837	0.001641			
	4	0.510						
Levofloxacin	5	0.509						
Analyst 2	1	0.509				0.510	0.000816	0.00160
	2	0.510						
	3	0.511	0.5102	0.000837	0.001639			
	4	0.510						
Levofloxacin	5	0.511						

The precision of the proposed method for five samples of levofloxacin for same concentration (5µg/mL) and also in the same day (same times) was analyzed (table 5 and 6) and found that the developed method to be in the high degree of precision (98 - 110%).

Robustness

With small variation in wavelength, % assay was calculated with in limit 98 to 110% (table 7).

Table VII: Robustness

Samples	Concentration (µg mL^{-1})	λ_{max} (λ_{293})		λ_{max+1} (λ_{294})		λ_{max-1} (λ_{292})	
		Absorbance	% Assay	Absorbance	% Assay	Absorbance	% Assay
Sample	5	0.511	100.19	0.511	100.00	0.513	100.19
Standard	5	0.510		0.511		0.512	

Ruggedness

Ruggedness was determined with the same concentrations by different analysts on same instrument and same day (different time). The % RSD was calculated and found to be 0.001639-0.001641% that is less than 3%, showing the ruggedness of the developed method (table 6).

CONCLUSIONS

An efficient, fast and simple UV-Spectrophotometric method was developed and validated for levofloxacin which showed absorbance, maxima at 293nm. The concentration range of 3 to 7µg/mL obeyed Beer's Lambert's law. The proposed method was validated in terms of linearity, precision, accuracy, ruggedness and robustness. The findings of these validation parameters revealed that the developed method was simple, precise and economically feasible. The proposed method was fruitfully applied in Pharmaceutical laboratories for the validation of levofloxacin injection for the routine analysis without interference of the excipients and other additives.

Robustness was carried out to

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