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SPECTROPHOTOMETRIC AND RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF LEVOFLOXACIN

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ABSTRACT

In this study, a simple, sensitive and highly accurate ultraviolet spectrophotometric and RP-HPLC method has been developed and validated for determination of levofloxacin in bulk and pharmaceutical formulations. The method is based on the measurement of the absorbance of levofloxacin solution in 0.1N HCL at 294nm. Beer's law was obeyed in the concentration range of 2-12µg/ml. The slope, intercept and correlation coefficient were also calculated. For HPLC method validation the analyte was resolved by using a mobile phase [sodium acetate buffer and acetonitrile was selected in a ratio of (60:40) respectively] at a flow rate of 0.8ml/min, with a column at a wavelength of 295nm. The linear dynamic range for levofloxacin was 2µg-8µg/ml.

KEYWORDS: Levofloxacin, Spectrophotometry, HPLC, linearity, validation.

INTRODUCTION

Levofloxacin is considered as about twice as active as its isomer ofloxacin. It has broad spectrum of activity including gram +ve bacteria.[1], used mainly as Anti-Bacterial Agents, Quinolones, Nucleic Acid Synthesis Inhibitors, Anti-Infective Agents. A pale yellow or bright yellow powder, colorless needles from ethanol,[2] having solubility as slightly soluble in water &ethanol.[3] with a melting point of 225–227^{o}C and half- life of about 6-8 hours.

Literature survey revealed that chromatographic[4] and spectrophotometric methods[5] were reported for estimation of levofloxacin individually or in combination with other drugs. Here

we have made an attempt to validate both UV and HPLC methods for determination of levofloxacin.

MATERIALS AND METHODS

Chemicals and materials

Levofloxacin was obtained as a gift sample from Wockhardt Limited, Aurangabad. All chemicals and solvents were purchased from Thermofischer scientific Pvt. Ltd, Mumbai and were of analytical grade. Distilled water was used to prepare all solutions and HPLC grade water was used for HPLC method validation. Freshly prepared solutions were always employed.

Instrumentation

The UV-Visible Spectrophotometer Shimadzu UV-1800 was used. The sample solutions were recorded over the range of 200-400nm. Digital Weighing Balance of Shimadzu AX200 was used for weighing the samples. An Ultra Sonicator of Toshniwal process instruments, pvt. Ltd Ajmer was used for sonication of the drug solution.

The development and validation of the RP-HPLC method was performed on a column. The work was carried out in an air-conditioned room maintained at temperature 20 °C. The mobile phase was pumped from the respective reservoir system to the column (flow rate 0.8ml/min. Eluents were observed at 295nm and data were acquired.

Development of Analytical Methods (UV Spectrophotometric)

Preparation of standard stock solution

Standard drug solution of levofloxacin was prepared by dissolving 200mg in methanol and the volume was made upto 100ml with 0.1N HCL, from this 1ml was taken and diluted upto 100ml with 0.1N HCL to obtain stock solution of 20µg/ml concentration. Ultrasonication was done to obtain a clear solution.

Determination of analytical wavelength

From the standard stock solution, 6 ml was pipetted out into 10 ml volumetric flask. The volume was made up to 10 ml with 0.1N HCL. The resulting solution containing 12µg/ml was scanned between 200 and 400 nm.

Preparation of Calibration curve of levofloxacin

Aliquots of 01 to 06 ml portion of stock solutions were transferred to a series of 10 ml volumetric flasks, and volume made up to the mark with 0.1N HCL. The serial dilution of the range of 2, 4, 6, 8, 10, and 12 µg/ml was prepared. The absorbance was measured at 294nm.

HPLC method of analysis

Selection of mobile phase

Different solvent systems were made and levofloxacin sample was injected into HPLC system. Different mobile phases containing phosphate buffer, methanol, acetonitrile were tried and finally sodium acetate buffer and acetonitrile was selected in a ratio of (60:40) respectively.

Preparation of mobile phase

Mobile phase comprising of sodium phosphate buffer and acetonitrile was adjusted to (3.5-4) P^H and filtered through watmann filter and kept for degasification in sonicator for 10 minutes. This mobile phase was pumped from the respective reservoir system to the column (flow rate 0.8ml/min. Eluents were observed at 295nm and data were acquired.

RESULTS AND DISCUSSION

The resultant UV spectrum of levofloxacin solution 12µg/ml exhibited maximum wavelength of absorbance at 294 nm, this complies with the reported one for the drug.

The high value of correlation coefficient (R^2 = 0.9992) indicates linearity and obeys Beer's law.

Linearity and range

The linearity of the response of the drug was verified at 2 to 12 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. The equation of the calibration curve for levofloxacin was obtained. The linearity range of calibration curve and correlation coefficient (r^2) of determination was obtained.

Table 1: Absorbance at different concentration of Levofloxacin.

Sr. No.	Concentration (µg/ml)	Absorbance ± SD (Avg)
1	2	0.177 ± 0.00626
2	4	0.439 ± 0.0177
3	6	0.675 ± 0.0251
4	8	0.952 ± 0.01852
5	10	1.154 ± 0.01418
6	12	1.452 ± 0.01692

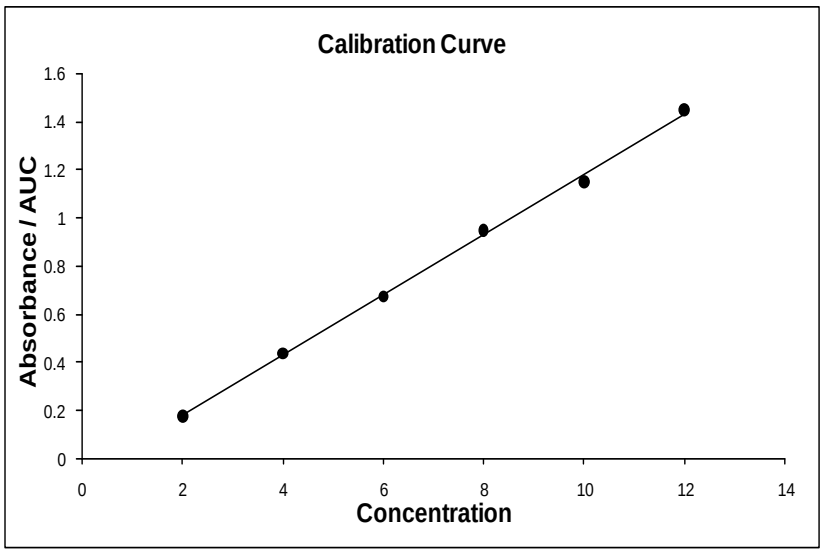

Fig. 1: Standard curve for Levofloxacin.

PRECISION

Absorbance of the prepared dilutions for calibration curve were determined for three times in a day (morning, afternoon, evening) and the percent relative standard deviation was calculated for intraday study (which should be less than 2%).

The same procedure was repeated for three consecutive days for the interday study and the percent relative standard deviation should be less than 4% for interday study.[6]

Interday precision (n=3)

Table 2: Interday precision

Concentration (µg/ml)	±Standard deviation n=3	%Relative Standard deviation
2	±0.001	0.695
6	±0.003	0.952
10	±0.002	0.478

Intraday Precision (n=3)

Table 3: Intraday precision.

Concentration (µg/ml)	±Standard deviation n=3	%Relative Standard deviation
2	±0.005	0.655
6	±0.006	0.732
10	±0.002	0.323

Accuracy

Accuracy study was done by spiking the drug (80%, 100%, 120% of the dose) into the placebo solution containing all other excipients of the formulation. The average % recovery was found to be 99.61%.

Accuracy (n=3)

Table 4: Accuracy

Tablet Amount (µg/ml)	Level of Addition (%)	Amount Added (µg)	Drug found (µg/ml)	% recovery	Mean % recovery
10	80	8	7.99	99.88	100.04
10	100	10	10.05	100.5	
10	120	12	11.97	99.75	

Limit of detection (LOD) and Limit of quantitation (LOQ)

It was calculated from standard deviation and slope value using a formulae:

$$LOD=3.3*\sigma/slope$$

$$LOQ=10*\sigma/slope$$

Where σ is standard deviation.

The limit of detection and limit of quantification was found to be 0.085µg/mL and 0.170µg/mL

Linearity study and calibration curve by HPLC method

Preparation of caliberation curve

Caliberation curve was obtained by plotting AUC Vs conc. of levofloxacin and linearity range was obtained between 2µg-8µg/ml conc. of levofloxacin Solution.

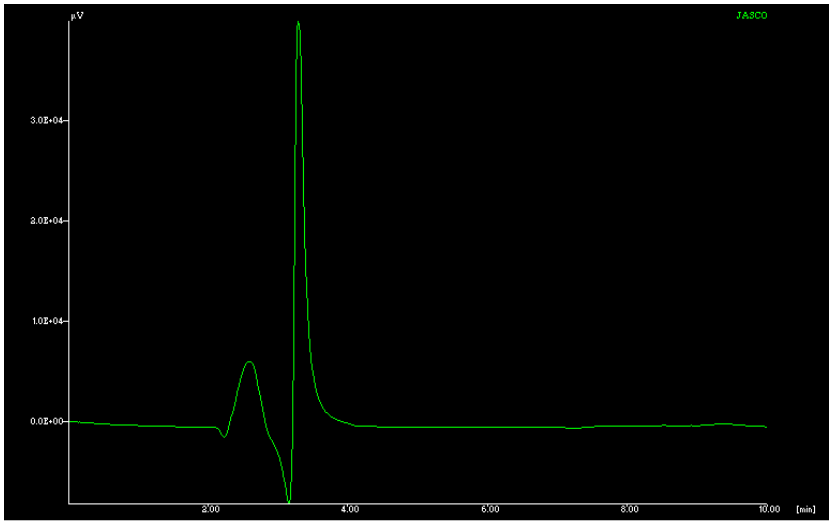

Fig.2: HPLC trial peak of 6µg/ml of levofloxacin.

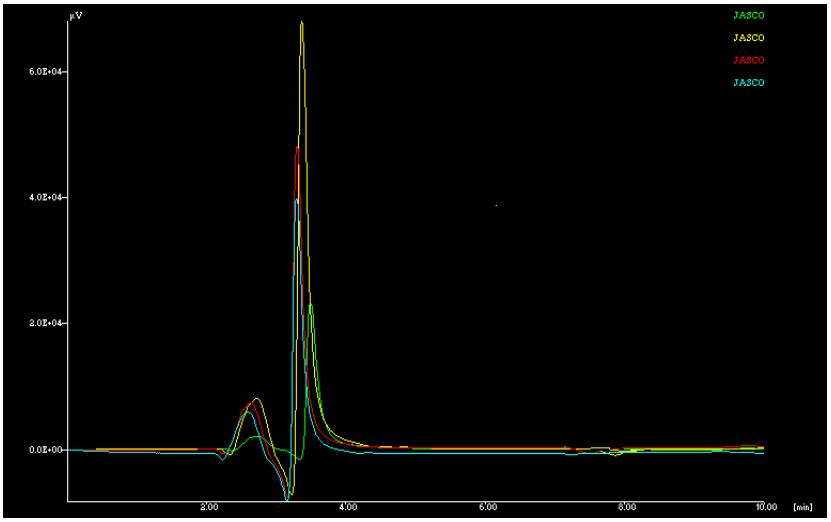

Fig.3: Overlay of peaks for Caliberation curve of levofloxacin.

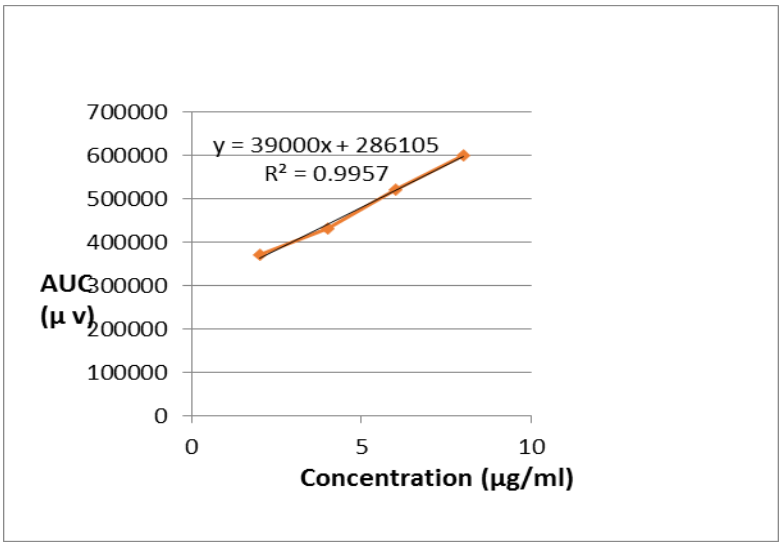

Fig.4: Calibration curve of levofloxacin by HPLC.

Precision

Precision was determined by interday and intraday studies. Intraday studies were performed in the same day, whereas interday studies were done for three consecutive days. Response factor i.e area of the peak was determined in each study and % RSD of them were determined, for doing so the dilutions were prepared of particular concentration and injected to the column of HPLC. The data reveals that the RP-HPLC method for levofloxacin was precise.

Table 5: Intraday and interday % RSD.

Sr.No.	%RSD	Observed value
1	intraday	0.754
2	interday	0.896

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value.

The accuracy of the method can be determined by adding known amount of analyte to cover both above and below (80,100, 120%) the normal levels expected in the sample.

Accuracy should be assayed using a minimum of nine determinations over a minimum of 3 concentration levels. It should be reported as %recovery by assay of known added amount of analyte in the sample or as the difference between the mean and accepted true value together with the confidence intervals. RSD of each level must not be more than 2-3%.[7]

Table 6: Accuracy study.

Tablet Amount (µg/ml)	Level of Addition (%)	Amount Added (µg)	Drug found (µg/ml)	%recovery	Mean % recovery
10	80	8	7.91	98.87	96.99
10	100	10	9.31	93.1	
10	120	12	11.88	99	

CONCLUSION

UV spectrophotometric and RP-HPLC methods were found to be accurate precise and reproducible. Validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. So the above stated methods can be best used for validation of levofloxacin in bulk and any dosage form.

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