
Research Article

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LEVOFLOXACIN IN BULK AND TABLET FORMULATION

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ABSTRACT

All immediate release tablets are subjected to dissolution studies in 0.1 N HCl as recommended by SUPAC-IR guidelines or in specified dissolution medium as per their official monograph a simple, selective, rapid, and precise double beam UV-Visible spectrophotometer method has been developed and validated for the estimation of Levofloxacin in pharmaceutical dosage form. Levofloxacin is an antibiotic for treating bacterial infections. Many common infections in humans are caused by bacteria. Bacteria can grow and multiply, infecting different parts of the body. Drugs that control and eradicate these bacteria are called antibiotics. Levofloxacin is an antibiotic that stops multiplication of bacteria by preventing the reproduction and repair of their genetic material, DNA. It is in a class of antibiotics called fluoroquinolones. The standard solution of Levofloxacin in 0.1 N HCl showed maximum absorption at 293 nm. Beer-Lambert's law obeyed in the concentration range of 2-12 µg/ml, with regression, slope and intercept 0.9997, 0.058 and 0.086 respectively. The percentage recovery is between 100±2 which reflect that the method is free from interference of the impurities and other additives during the estimation of drug in formulation. The proposed method can be successfully used for analysis of Levofloxacin in marketed preparations (Livaquin). The results of analysis have been validated statistically and by recovery studies.

Keywords: Levofloxacin, UV-Visible spectrophotometer method, Validation.

INTRODUCTION

Levofloxacin (LVFX), (-)-(-S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de] [1, 4] benzoxazine-6-carboxylic acid^[1], is a new quinolone antimicrobial agent which exhibits broad-spectrum *in vitro* bactericidal activities against gram-positive and gram-negative aerobes^[2,3]. Levofloxacin is used to treat infections of the sinuses, skin, lungs, ears, airways, bones, and joints caused by susceptible bacteria. Levofloxacin also is frequently used to treat urinary infections, including those resistant to other antibiotics, as well as prostatitis (infection of the prostate). Levofloxacin is effective in treating infectious diarrhea caused by *E. coli*, *Campylobacter jejuni*, and *Shigella* bacteria. Levofloxacin also can be used to treat

various obstetric infections, including mastitis (infection of the breast).

It is an off-white crystalline powder, relatively insoluble in water, and freely soluble in acidic aqueous solutions. Levofloxacin is available in Tablet dosage form 250, 500 and 700 mg under brand name Livaquin. The structure of Levofloxacin is shown in (fig. 1).

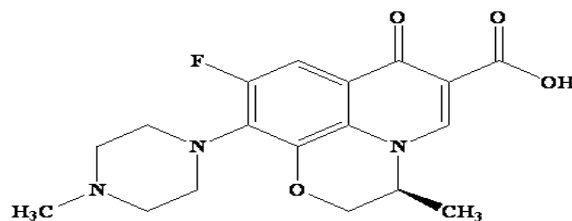


Fig. 1: Structure of Levofloxacin

According to SUPAC-IR guidelines, all immediate release tablets should be subjected to *in vitro* dissolution studies in 0.1 N HCl or other as recommended by official monograph of drug candidate^[4].

Method validation is the process of proving that an analytical method is accepted for its intended purpose. For pharmaceutical methods, guidelines from the United States of Pharmacopeia (USP), International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA) provides a framework for performing such validations. FDA defines validation as "Establish the documented evidence which provides a high degree of assurance that a specific process will consistently produce a product of pre-determined specifications and quantity attributes"^[5]. The objective of validation is to form a basis for written procedure for Production and Process control, which are designed to assure that the drug products have the Identity, Quality and Purity, Assurance of Quality and Government Regulation. Analytical testing of a pharmaceutical product is necessary to ensure its purity, stability, safety and efficacy. Analytical method validation is an integral part of the quality control system. Analytical procedures to be validated for identification tests, Quantitative tests for impurities content, Limit tests for the control of impurities, Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product, Dissolution testing for drug products and Particle size determination for drug substance^[6,7].

EXPERIMENTAL

Instrumentation

A Shimadzu UV-Visible Spectrophotometer (UV-1700) with a matched pair of 10 mm quartz cells were used for experimental purpose.

Materials and Reagents

Levofloxacin was obtained from Ranbaxy Labs, Gurgaon. Freshly prepared 0.1 N HCl, methanol and all other chemicals and reagents were of analytical grade. The commercially available two marketed tablet brands containing levofloxacin (Levaquin), in 250 and 500 mg in each tablet have been used for estimation.

Preparation of standard and sample solutions

LEVOFLOXACIN (100 µg) were weighed accurately and transferred to separate 1 ml Eppendorf dissolved in 1 ml 0.1 N HCl to prepare standard stock solution of 100 µg/ml. To study the linearity range of each component, serial dilutions of levofloxacin were made from 2 - 10 µg ml⁻¹ in 0.1 N HCl. Calibration curves were plotted as concentration of drug versus absorbance. For analysis of dosage form of levofloxacin (Levaquin) (Label claim: 250 and 500 mg).

Validation Procedure

Method was validated according to ICH Guidelines^[8], in terms of linearity and range, accuracy and precision, limit of detection (LOD), limit of quantitation (LOQ).

Linearity and range

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Range is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

Accuracy

Accuracy is the closeness of the test results obtained by the analytical method to the true value. For assay methods, samples are prepared in triplicate at three concentration levels covering the specified three levels over a range of 50–150% of the target concentration.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent standard deviation (RSD) for a statistically significant number of samples.

It should be determined from a minimum of nine determinations covering the specified range of the procedure.

Limit of detection

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample can be detected, not quantitated.

The limit of quantitation

The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

Table 1: Data for calibration curve of levofloxacin

Parameter	Levofloxacin
Linearity and range	2-12 µg/ml
Regression equation	0.058x-0.086
Correlation coefficient	0.9997
Slope	0.058
Intercept	0.086

Table 2: Result of recovery studies

Amount taken (µg/ml)	% (Recovery ± S.D.)
2	99.4067±0.0223
8	98.127±0.0409
12	100.405±0.0655

Table 3: Intra-day and inter-day precision

Normal concentration (µg/ml)	Intra-day (n=3)		Inter-day (n=3)	
	Mean±S.D.	Precision (% RSD)	Mean±S.D.	Precision (% RSD)
2	1.98±0.086	0.937	1.98±0.0116	0.804
8	7.85±0.039	0.503	7.85±0.0266	0.339
12	12.060±0.0629	0.522	12.060±0.0629	0.521

Table 4: Result of bench top stability

Concentration (µg/ml)	Initial amount	Amount after 20hr	% Deviation
2	2.012	2.016	-0.198
8	7.981	7.971	1.25
12	12.002	11.911	0.758

Table 5: Study of levofloxacin in marketed formulation

DRUG	Label claim (mg)	Amount found	% of drug content	Standard deviation
Levofloxacin(Levaquin)	250	249.43	99.775	0.427
Levofloxacin(Levaquin)	500	249.002	99.800	0.677

Stability studies

Stability experiments were performed with low, medium and high quality control samples to evaluate the levofloxacin solution stability under different conditions. Experiments were performed to determine stability of bench top (20 hr). The sample is found stable if % deviations are within $\pm 15\%$.

RESULT AND DISCUSSION**Linearity and range**

The calibration curve constructed was evaluated by its correlation coefficient. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over wide range. Good linearity was observed over the concentration range evaluated (2-12 $\mu\text{g}/\text{ml}$) with regression coefficient (r^2) = 0.9997) are shown in Table 1 and fig. 2.

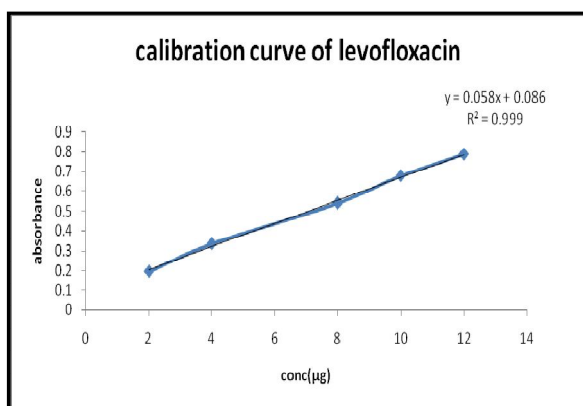


Fig. 2: Calibration Curve of Levofloxacin in 0.1N HCl

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.656 and 1.98 $\mu\text{g}/\text{ml}$.

ACCURACY**Recovery studies**

To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. All value come under 100 ± 2 (Table 2) that indicate method is accurate.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as relative standard deviations (RSD) of a set of results. Results presented in Table 3 indicate good repeatability and low inter-day variability (RSD maximum 1.0%).

Bench top stability

The sample was found bench top stable as % deviation was not more than $\pm 15\%$ (Table 4).

Analysis of marketed dosage form of Levofloxacin

Three replicates of the required dilutions were prepared from tablet stock solution and sonicated. The amounts of Levofloxacin were calculated by extrapolating the absorbance from the calibration plot. Results of analysis are reported in Table 5.

CONCLUSION

The proposed UV method is simple, accurate, precise, specific and highly sensitive developed and validated for the determination of levofloxacin in bulk and tablet dosage form. The method is economical rapid and do not require any sophisticated apparatus in contrast to chromatographic methods. Hence, the proposed method can be successfully used for routine quality control analysis of drug in marketed preparations.

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