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Development and Validation of UV spectrophotometric method for determination of levofloxacin hemihydrate in marketed tablet dosage formulations

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Abstract:

A sensitive, safe, novel, precise, economic, accurate and simple UV spectrophotometric method has been developed for assessment of Levofloxacin Hemihydrate tablets by using a solvent composition of acetonitrile:water (8:2). The drug shows maximum absorbance (λ_{\max}) at 297 nm. Linearity was observed in the concentration range of 5.0–25.0 $\mu\text{g/mL}$ and exhibited good correlation coefficient of ($R^2 = 0.9983$). The proposed method has been successfully applied to analysis of Levofloxacin Hemihydrates marketed tablets. The study was proposed to confirm the validation. The method was validated as per ICH guideline and the value of accuracy, precision, stability test and other statistical analysis was found to be in good accordance with the label claim.

KEYWORDS:

Fluoroquinolone, Levofloxacin, UV Spectrophotometric method, Validation.

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1. INTRODUCTION:

Levofloxacin hemihydrate:

Levofloxacin hemihydrate (LEV) is the L-isomer of the fluoroquinolone ofloxacin which is an antimicrobial agent. Chemically it is (S)-9-Fluoro-3-methyl-10-(4-methylpiperazine-1-yl)-7-oxo-2,3-dihydro[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrates¹. The antibacterial activity is enhanced by the addition of 6-fluoro and 7-piper-aziny groups to the molecule. They are generally referred as the second generation fluoroquinolone antibacterial agents and are greatly effective against both gram-negative and gram-positive bacteria that are resistant to other antibacterial agents².

Mechanisms of action: It acts by inhibiting bacterial DNA gyrase which is needed for DNA replication and causes lyses of bacteria^{3,4}.

Spectroscopy methods:

It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm^{5,6}.

Ultraviolet-Visible spectrophotometry:

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer-Lambert law.

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer-Lambert law is expressed as

$$A = a b c$$

Where,

A= absorbance

a= absorptivity or extinction coefficient

b= path length of radiation through sample (cm)

c= concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A = A \times (1\%/1 \text{ cm}) \times bc$$

Quantification of medicinal substance using spectrophotometer may be carried out by preparing solution in transparent solvent and measuring its absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ_{\max}), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal⁷.

They are used in standard absorptivity value, calibration graph and single or double point standardization. In standard absorptivity value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. In calibration graph method, the absorbances of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The single point standardization procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

$$C_{\text{test}} = (A_{\text{test}} \times C_{\text{std}}) / A_{\text{std}}$$

Where C_{test} and C_{std} are the concentrations in the sample and standard solutions respectively and A_{test} and A_{std} are the absorbances of the sample and standard solutions respectively. For assay of substance/s in multi component samples by spectrophotometer; the following methods are being used routinely, which includes⁸,

- Simultaneous equation method.
- Derivative spectrophotometric method.
- Absorbance ratio method (Q-Absorbance method).

Difference spectrophotometry.

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation⁹:

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantification limit, linearity and range.

Furthermore revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

This work deals with the validation of the developed method for the assay of Levofloxacin Hemihydrate from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability.

The aim and scope of the proposed work are to develop suitable spectrophotometric method for assay of Levofloxacin Hemihydrate tablet and perform the validation for the method.

2. Materials and Method:

Materials

Instrumentation:

A Shimadzu UV-visible double beam spectrophotometer (UV -1700, Shimadzu) was used for all absorbance measurements and a pair of 10 mm matched quartz cells was used.

Chemicals:

All chemicals and reagents were of analytical grade. Levofloxacin reference standard drug in the form of levofloxacin hemihydrates powder was used as reference standard. Tablets brands used are Alevo 500mg (Alkem) and Alvox 500mg (Bombay Tablet). Solvent system used is acetonitrile:water (8:2).

Method

Selection of wavelength of maximum absorbance:

The dilutions were obtained and the solutions were scanned in UV range (200-400nm) in 1.0 cm cell against solvent blank. The study of spectrum reveals that levofloxacin shows a well-defined λ_{\max} at 299 nm. This wavelength was selected for development of method.

Preparation of stock solution:

10 mg of LEV pure drug was accurately weighed and taken in separate 100ml volumetric flask and dissolved with 70 ml of solvent system and shaken for 15 minutes and then it was diluted with the solvent system to obtain 100 $\mu\text{g/ml}$ of standard stock solution.

Construction of calibration curve: Various standard stock solutions were pipette out and diluted with distilled water to get the concentrations of 5-25 $\mu\text{g/ml}$. The solution was scanned in the spectrum mode from 400 nm - 200 nm wavelength range. The calibration curve was made by plotting the absorbance versus concentration (Figure No. 1) , (Figure No.2) & Table No. 1.

Various optical parameters along with regression characteristics for levofloxacin are given (Table No. 2).

Accuracy: Recovery studies were carried out to check the accuracy of proposed method as per ICH guidelines (Table No. 3).

Repeatability: Suitable statistical evaluation was carried out to check the repeatability of proposed method. The standard deviation, coefficient of variation and standard error were calculated (Table No. 3).

Analysis of formulation: Aforesaid two brands of levofloxacin tablets were analyzed using the proposed method.

Table No. 1: Concentration Vs Absorbance table for linearity study of Levofloxacin

Table
Various

S. No.	Concentration	Absorbance (at wl 299 nm)	Weight Factor
1	0	0.0006	1.0000
2	5	0.4614	1.0000
3	10	0.8329	1.0000
4	15	1.3110	1.0000
5	20	1.6755	1.0000
6	25	2.1351	1.0000

No.2:
optical

parameters

Parameter	Data
Beer-Lambert's law range (Linearity)	5-25
λ_{\max}	299 nm
Regression equation ($y=mx+c$)	$y=0.0838x+0.0262$
Slope	0.0838
Intercept	0.0262
R^2	0.9983

Table No. 3: Accuracy study

% Level of standard drug added	Recovery (%)
80	99.45
90	99.00
100	100.02
110	99.23
120	99.36

Table No 4: Repeatability Study

Concentration (µg/ml)	Absorbance (at 299 nm)	Amount (%)	S.D.= 0.273453 COV= 0.002739 S.E.= 0.111637
20	1.6752	99.95	
20	1.6711	99.56	
20	1.6698	100.13	
20	1.6717	99.49	
20	1.6745	99.74	
20	1.6723	100.10	
	Average =	99.83	

3. Results & Discussion:

The proposed method for determination of LEV in marketed dosage formulation was found to be rapid, simple, accurate and economic. The UV spectra of LEV were obtained; the absorption maximum was found to be 299 nm. The Beer- Lambert's law was obeyed which was confirmed by the linearity of the calibration curve of LEV in the concentration range of 5-25 µg/ml producing the regression equation as $y=0.0838x+0.0262$ and $R^2=0.9983$. The accuracy was found within the range of 99.00-100.02%. The estimation of LEV in tablet formulation was carried out by taking concentrations of 5-25 µg/ml. The

percentage purity values were found to be 98.6 % and 100.2 % in Alevo and Alvox respectively.

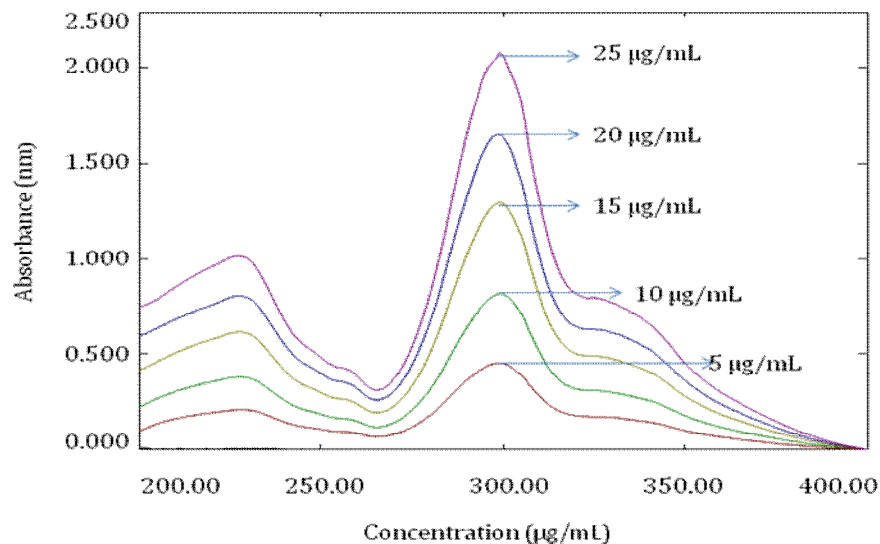


Figure No. 1: Spectra for various dilutions in linearity range

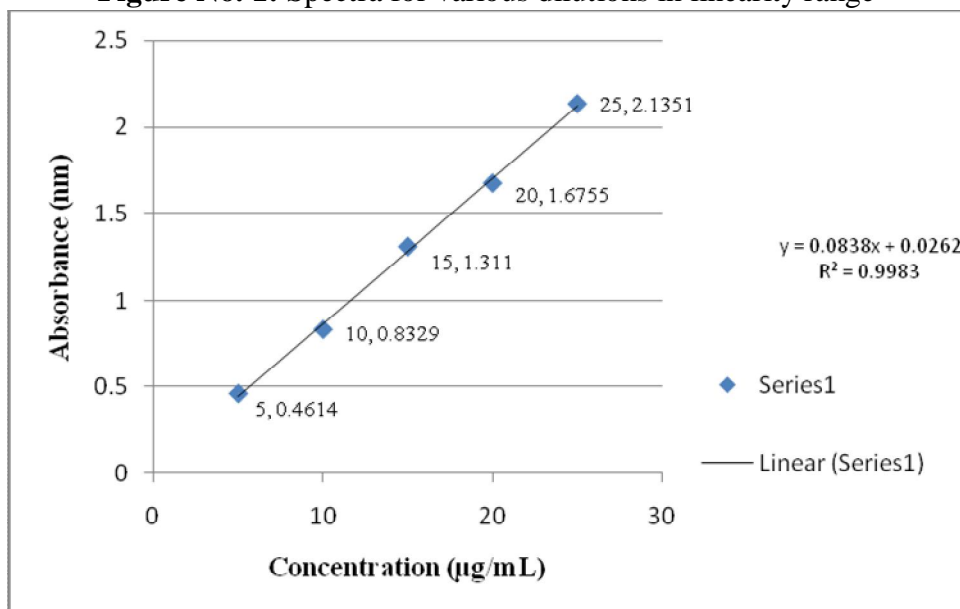


Figure No. 2: Calibration curve for Levofloxacin

4. Conclusion:

The proposed UV spectrophotometric method is rapid, simple, accurate and economic. Hence, it can be routinely used for determination of levofloxacin hemihydrate in tablet formulation using acetonitrile:water (8:2) as a solvent system.

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