ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF LEVOFLOXACIN IN PURE AND IN PHARMACEUTICAL DOSAGE FORMS BY UV SPECTROPHOTOMETRY

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ABSTRACT

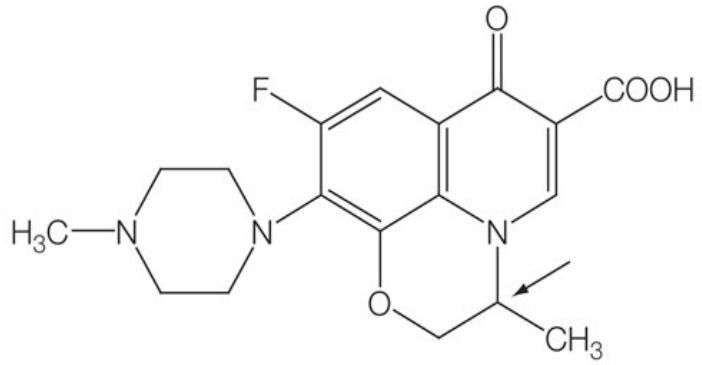
A new, simple method indicating UV spectroscopy was developed and validated for the estimation of levofloxacin in pure form and in formulation. The adequate drug solubility was found in distilled water and the maximum absorbance was measured at 290 nm in the wavelength range of (200-400nm), the linear calibration curve was obeyed in the concentration range of (2-16 µg/ml) show regression equation 0.0548 and 0.0834 respectively and correlation coefficient (R^2=0.9937). This method was validated and applied to the determination of Levofloxacin in pharmaceutical dosage form, no interference was found from excipients at the selected wavelength and analysis conditions.

Key words: UV spectroscopy, Levofloxacin, Method Development and Validation, Calibration Curve method

INTRODUCTION

Levofloxacin is a broad-spectrum antibiotic with anti-bacterial properties, The molecular formula of levofloxacin is $C_{18}H_{20}FN_3O_4$ with a molecular weight of $C_{18}H_{20}FN_3O_4$. Levofloxacin widespread through the bacterial cell wall and work by suppressing DNA gyrase, suppression of Dna Gyrase results in blockage of bacterial cell growth. It is widely used to cure mild to moderate urinary and respiratory tract infections. It belongs to drug class- anti-infective agents. The Volume of distribution of levofloxacin is found to be 74-112 L after single and multiple 500mg or 750mg doses. Levofloxacin arrive at its peak level after 3hour of dosing, the half-life of the drug is between 6-8 hours. Levofloxacin is use to cure pneumonia, sinus infection, worsening of chronic bronchitis, skin infections, chronic prostate infection, urinary tract infection, kidney infection, inhalational anthrax, plague. The dosage of drug varies from one infection to another i.e. For pneumonia, the adult dosage is 750mg taken for every 1-2 weeks, For acute bacterial sinusitis the adult dosage is 500mg taken once a day for 5 days. On the other hand, it depends on bacterial infection. For chronic bronchitis, the dosage is 500mg taken once a day for 1 week. For complicated skin infections, the adult dosage is 750mg taken once a day for 1-2 week. For

uncomplicated skin infections, the dosage is 500mg taken once a day for 7-10 days. For chronic prostate infection, the adult dosage is 500mg taken once a day for 28 days. For complicated urinary tract infections, the adult dosage is 250mg taken once a day for 10 days or 750 mg taken once a day for 5 days, for uncomplicated urinary tract infections, the adult dosage is 250mg taken once a day for 10 days or 750mg taken once a day for 5 days. For inhalational anthrax, the adult dosage is 500mg taken once a day for 60 days. For the treatment of plague, the adult dosage is 500mg taken once a day for 10-14 days.



Levofloxacin

Levofloxacin is trade under the brand name Levaquin and available in the tablet dosage form 250mg, 500mg and 750mg. The structure of levofloxacin is shown above.

Preparation of standard stock solution

To prepare stock solution of Levofloxacin, weigh accurately 50mg of drug and dissolve in 20ml of distilled water. The volume was made up to the mark with water. 10ml of this solution was transferred to 100ml vol. flask and diluted up to 100ml with water. The solution contained 100 µg of drug/ml of the solution. The drug showed maximum absorbance at 290nm.

Concentration of solvent and wavelength selection

Dilute the working solution to get solution of concentration 2, 4, 6, 8, 10, 12, 14, 16, µg /ml respectively. The resultant solution was measured at 290nm against water as a blank.

Validation Procedure

Method was validated according to ICH guidelines, In terms of linearity, range accuracy, precision, Limit of detection (LOD), Limit of quantification (LOQ).

Linearity and Range

Linearity is the ability of the method to obtain test results, which is directly proportional to analyte concentration with in a given range.Range is the interval between upper and lower level of analyte. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte at the extreme of the specified range of the analytical procedure.

Accuracy

Accuracy is closeness of the test results obtained by the method to the true value. It should be assessed using a minimum of 3 concentration levels, each in triplicate (total of 9 determinations) and should be established across specified range of analytical procedure.

Precision

Precision is the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample. I t should be determined from a minimum of 9 concentrations.

Limit of detection (LOD)

LOD can be estimated by Signal to Noise ratio of 3:1,

LOD is lowest amount of analyte in a sample that can be detected but not quantitated.

Limit of quantitation (LOQ)

LOQ can be estimated by Signal to Noise ratio of 10:1,

LOD is lowest amount of analyte in a sample that can be quantified with suitable accuracy and precision

Table-1

Parameter	Observation
Linearity and Range	2-16µg/ml
Regression equation	0.0548x+0.0834
Correlation coefficient	0.9937
Slope	0.0548
Intercept	0.0834

Table-2

Result of recovery studies

Amount taken (µg/ml)	% (Recovery ± S.D.)
2	99.3568 ± 0.03875
8	98.1422 ± 0.06812
16	100.315 ± 0.08941

Table -3

Intra Day and Inter Day precision

Intra Day (n=3)

Normal concentration (μg/ml)	Mean ± S.D.	Precision (% RSD)
2	1.98±0.079	0.937
8	7.63±0.045	0.503
16	15.167±0.057	0.546

Inter Day (n=3)

Normal concentration (μg/ml)	Mean ± S.D.	Precision (% RSD)
2	1.98±0.0181	0.856
8	7.63±0.0240	0.348
16	15.167±0.0643	0.545

Table -4

DRUG	Label claim (mg)	Amount found	% of drug co ntent	Standard deviation
Levofloxacin(Levaquin)	250	249.39	99.75	0.427
Levofloxacin(Levaquin)	500	493.95	99.8	0.677

Result and Discussion

Linearity and Range

The calibration curve was evaluated by its correlation coefficient. Linearity was observed over the concentration range **(**2-16μg/ml)) with regression coefficient ($r^{2)}$ =0.9937) are shown in Table1 and fig. 2.

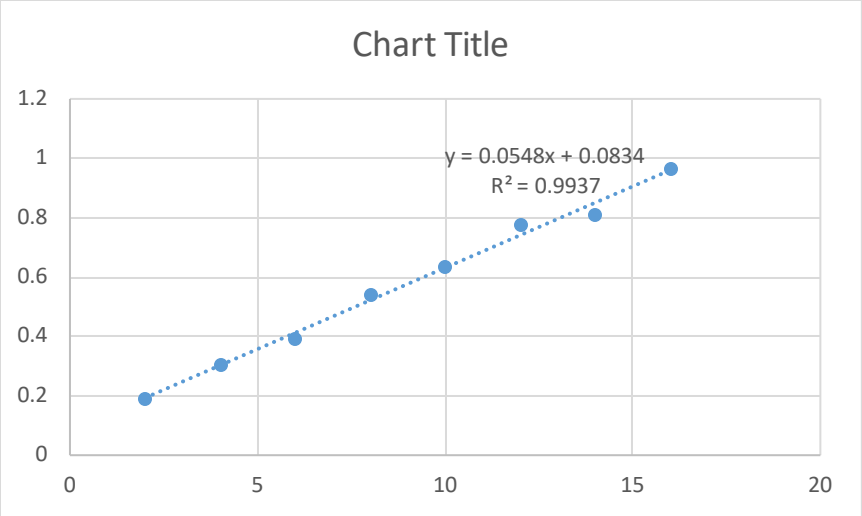


Fig. 2: Calibration Curve of Levofloxacin

Accuracy

Recovery studies

To perform accuracy study of developed method, analytical recovery experiment were carried out by standard addition method. All values come under 100±2which indicates that the method is accurate.

Precision

The precision was determined by intra-day and inter-day. Results of Table-3 indicates the repeatability is good and having low inter-day variability.

Analysis of marketed dosage form of levofloxacin

The amount of levofloxacin were calculated by extrapolating the absorbance from the calibration plot shown in table-4.

CONCLUSION

The proposed UV method is simple, accurate, precise, specific and highly sensitive developed and validated for the determination of levofloxacin in pure and pharmaceutical 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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