World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Validated UV-Spectrophotometric method for quantitative estimation of Lomefloxacin HCl in bulk and pharmaceutical dosages forms

Swatantra Bahadur Singh*, Shweta Singh

Department of Pharmaceutics, Hygia Institute of Pharmaceutical Education and Research, Lucknow, Uttar Pradesh, India

Received: 14-10-2014 / Revised: 20-10-2014 / Accepted: 24-10-2014

ABSTRACT

The objective of this research was to develop and validate a simple, accurate, and precise UV spectrophotometric method for the determination of Lomefloxacin HCl in bulk and pharmaceutical dosage form .The λ_{max} of the drug was found to be 287.1 nm in water. The beer's law was obeyed in the concentration range of 2- 12µg/ml with correlation coefficient 0.9997. The developed method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of the relative standard deviation and % recovery were found to be 0.2133 and 98.54- 99.85% respectively. On the other hand estimation of the drug in marketed formulation (Lomflox 400 mg tablets) was carried out by using the above developed and validated method. The percentage assay of tables was found to be 99.61 % in good agreement with the labeled claim, indicating the absence of interference of the excipient. All the results were good, showed that the proposed method is precise, accurate, hence can be used for the routine analysis of Lomefloxacin HCl in bulk and pharmaceutical formulation in both research laboratories, and pharmaceutical and chemical industries.

Key words: Lomefloxacin HCl, Validation, UV spectrophotometry, ICH.

INTRODUCTION

Lomefloxacin is a bactericidal fluoroquinolone agent with activity against a wide range of gram-negative and gram-positive organisms. The bactericidal action of lomefloxacin results from interference with the activity of the bacterial enzymes, DNA gyrase and topoisomerase IV, which are needed for the transcription and replication of bacterial DNA. DNA gyrase appears to be the primary quinolone target for gram-negative bacteria. Topoisomerase IV appears to be the preferential target in gram-positive organisms. Interference with these two topoisomerases results in strand breakage of the bacterial chromosome, supercoiling, and resealing. As a result DNA replication and transcription are inhibited [1]. Lomefloxacin is a fluoroquinolone antibiotic used to treat chronic bronchitis, as well as complicated and uncomplicated urinary tract infections caused by *S. pneumonia*, *H. influenza*, *S. aureus*, *P. aeruginosa*, *E. cloacae*, *P. mirabilis*, *C. civersus*, *S. asprphyticus*, *E. coli*, and *K. pneumonia*. It is also used as a prophylactic or preventative treatment to prevent urinary tract infections in patients undergoing transrectal or transurethral surgical procedures [2, 3]. Lomefloxacin HCl, is a difluoroquinolone, is the monohydrochloride salt of (±) -1-ethyl-6, 8-difluoro-1, 4-dihydro-7-(3-methyl-1-piperazinyl) -4-Oxo-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{19}F_2N_3O_3$•HCl and its structural formula is-

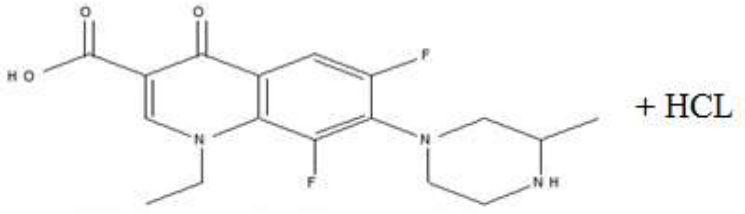

Figure 1: Structure of Lomefloxacin HCl

***Corresponding Author Address:** Mr. Swatantra Bahadur Singh, Department of Pharmaceutics, Hygia Institute of Pharmaceutical Education and Research, Lucknow, Uttar Pradesh, India; **E-mail:** swatantra48@gmail.com*

MATERIALS AND METHODS

Instrumentation: A Lab, India UV-3200 double beam spectrophotometer with 1cm matched quartz cells were used for measuring the absorbance.

Chemicals and reagents: Lomefloxacin HCl pure drug was obtained as a gift sample. Tablets of 400 mg strength were procured from the local market under the commercially available brand name **Lomflox-400.** All the chemicals used were of analytical grade.

Determination of maximum wavelength (λ_{max})

Preparation of stock solution: Standard stock solution of Lomefloxacin HCl was prepared by dissolving accurately weighed 100 mg of Lomefloxacin HCl in water in a 100ml volumetric flask to give a concentration of 1000µg/ml. From this, 10ml of the solution was transferred to a 100ml volumetric flask and made up the volume with water to give a concentration of 100µg/ml which is the mother stock solution. From the above stock solution, pipette out 2ml and 3ml in to 10ml volumetric flask and finally made up the volume with water to produce a concentration of 20µg/ml and 30µg/ml respectively. The samples were then scanned in UV spectrophotometer from a range of 200-400nm against water as reference and the wavelength corresponding to maximum absorbance in water was found at 287.1nm **(Figure 2)**

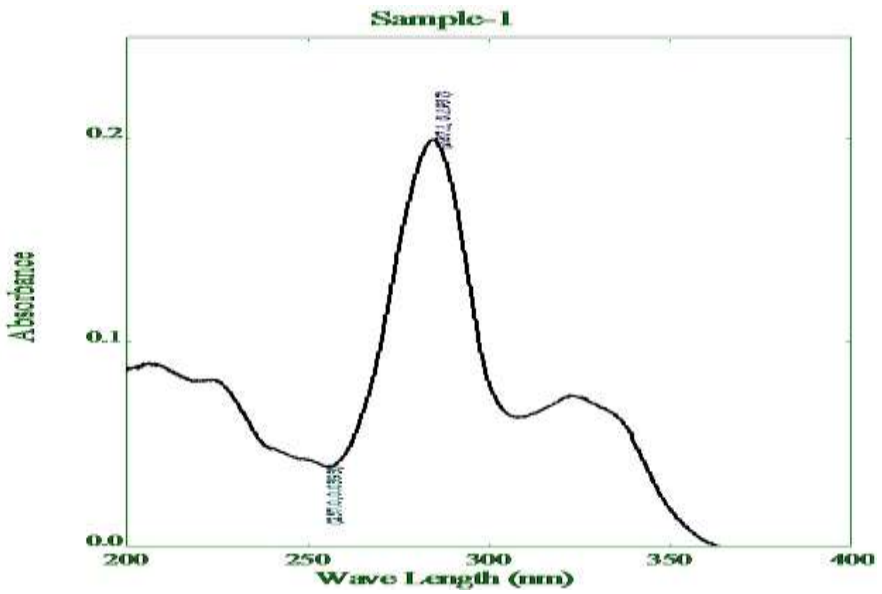

Figure 2: UV Spectrum of Lomefloxacin HCl in water

Preparation of standard calibration curve: For the preparation of standard calibration curve, concentrations of 2-12µg were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml of the 100µg/ml solution into a 10ml volumetric flask and made up the volume with water. The absorbance of each solution was measured at 287.1 nm taking water as reference. The calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis **(Figure 3).** The curve showed linearity in the range of 2-12µg/ml with correlation coefficient 0.9997.

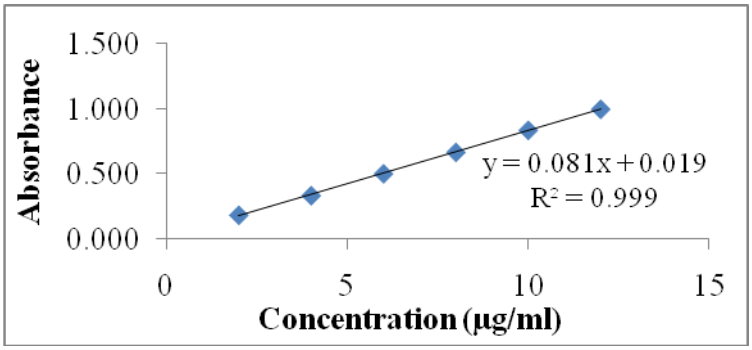

Figure 3: Calibration curve of Lomefloxacin HCl

VALIDATION

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection (LOD), and Limit of quantification (LOQ) according to ICH guidelines [2, 3].

Linearity: The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analyzed. The drug showed linearity in the range of 2-12µg/ml with correlation coefficient 0.9997. Linearity data are shown in **Table1.**

Precision: Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing six replicates of the same concentration of the sample and the absorbance was measured. The Intraday precision study was carried out by preparing a drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision. The result was reported as %RSD [4, 5]. The precision result showed a good reproducibility **(Table 2)** with percent relative standard deviation less than 2. The results of Intraday and interday precision studies are shown in (**Table 3 and Table 4)**.

Accuracy: The accuracy of measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "True value" is known) is analyzed and the measured value should ideally be identical to the true values. Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of the pure drug to the pre analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in (**Table 5) [6].**

Ruggedness: Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents, and so on. Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the result was indicated as % RSD **(Table 6).**

Robustness: Analysis was carried out at two different temperatures, room temperature and at 18 ^{0}C to determine the robustness of the method and the respective absorbance was measured. The result was indicated as %RSD [5, 6].

LOQ and LOD: Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined using the following equation LOQ-10s/m, LOD-3.3s/m where s is the standard deviation of the response and m is the slope of the related calibration curve. The values of LOQ and LOD were found to be 4.26 and 1.40µg/ml respectively [7, 8]. The results of various parameters of the developed method are shown in (**Table 8).**

Quantification in dosage form: To analyze the concentration of drug in the pharmaceutical formulation, twenty tablets were accurately weighed and powdered. Tablet powder equivalent

to 100mg was accurately weighed and transferred to a 100ml volumetric flask, dissolved in water, sonicated, and finally made up the volume with water up to mark. The solution was centrifuged for the excipients to settled down and the resulting solution was filtered using Whatsmann filter paper no.1.The solution was suitably diluted so as to obtain a concentration in the linearity range and the absorbance was measured at 287.1nm against water as blank. The results of analysis are shown in (**Table 9)**.

RESULTS AND DISCUSSION

The proposed method provides a simple, accurate, economical and convenient method for the analysis of Lomefloxacin HCl using UV spectrophotometer. The wavelength corresponding to maximum absorbance of drug in water was found at 287.1 nm. Beer's law was obeyed in the concentration range of 10-60µg/ml with correlation coefficient 0.9997. Accuracy of the proposed method was determined by the recovery studies, and was found to be 98.54- 99.85%, indicated that the method is accurate. The method was found to be precise as %RSD values for interday and intraday was found to be less than 2. The method was also found to be rugged and robust as the % RSD values were found to be less than 2. The limit of detection and limit of quantification of the proposed method was found to be 0.217and 0.245µg/ml indicating that the method developed is sensitive. The result of assay obtained was found to be in good agreement with the labeled claim, indicating the absence of interference of the excipient.

CONCLUSION

The developed method can be concluded to be simple, economic, easy, accurate, precise, reproducible and highly sensitive. The proposed method is specific without and interference of excipient and hence can be used for the routine estimation of Lomefloxacin HCl in bulk and in pharmaceutical formulation.

TABLE 1: Linearity table of Lomefloxacin HCl

Concentration	Absorbance
2	0.190±0.0015*
4	0.340±0.0020*
6	0.507±0.0011*
8	0.671±0.0036*
10	0.840±0.0035*
12	1.001±0.0040*

**SD (Standard deviation)*

TABLE 2: Precision results showing repeatability

Conc. (µg/ml)	Absorbance	Statistical Analysis
6	0.508	
6	0.509	**Mean-** 0.5083
6	0.508	**SD-** 0.0011
6	0.510	**%RSD-** 0.2133
6	0.507	
6	0.508	

TABLE 3: Intraday precision

Conc. (µg/ml)	Abs 1	Abs 2	Abs 3	Average %RSD
6	0.510	0.513	0.509	
6	0.512	0.509	0.508	
6	0.513	0.507	0.509	
6	0.509	0.512	0.510	
6	0.510	0.510	0.507	
6	0.508	0.512	0.507	**0.4291**

TABLE 4: Interday precision

Concentration (µg/ml)	%RSD			
	DAY1	DAY2	DAY3	Average %RSD
	0.4402	0.4351	0.426	**0.4338**

TABLE 5: Accuracy readings of Lomefloxacin HCl

Conc. of drug in formulation (µg/ml)	Conc. of pure drug added (µg/ml)	Total concentration of drug (µg/ml)	Amount found (µg/ml)	% of Drug recovery
400	80	480	472.97	98.54
400	100	500	496.81	99.36
400	120	520	519.24	99.85

TABLE 6: Results showing Ruggedness

Analyst 1		Statistical analysis		
Conc. (µg/ml)	**Absorbance**			
6	0.506			
6	0.512			
6	0.507	**Mean**	**SD**	**% RSD**
6	0.509	0.509	0.002	**0.455**
6	0.511			
6	0.508			
Analyst 2				
Conc. (µg/ml)	**Absorbance**			
6	0.507			
6	0.509	**Mean**	**SD**	**% RSD**
6	0.508	0.509	0.003	**0.508**
6	0.505			
6	0.512			
6	0.511			

TABLE 7: Results showing robustness

Analyst 1		Statistical analysis		
Room Temp	**Absorbance**			
6	0.506			
6	0.512			
6	0.507	**Mean**	**SD**	**% RSD**
6	0.509	0.509	0.002	**0.455**
6	0.511			
6	0.508			

Analyst 2				
	Absorbance			
Tempe 18^0 C				
6	0.507			
6	0.509	**Mean**	**SD**	**% RSD**
6	0.508	0.508	0.004	**0.478**
6	0.504			
6	0.514			
6	0.511			

TABLE 8: Summary of the method developed

Parameter	Result
Absorption maxima	287.1nm
Beer's law range	2-10µg/ml
Correlation coefficient	0.999
Regression equation	y = 0.081x + 0.019
Slope	0.0817
Intercept	0.0198
Accuracy	98.54-100.05%
Precision (%RSD)	Intraday (0.4291) Interday (0.4338)
LOD, µg/ml	0.217
LOQ, µg/ml	0.245

TABLE 9: Quantification in dosage form

Formulation	Label claim (mg)	Estimated amount of drug (mg)	% Labeled claim
Lomflox-400	400	398.45	99.61

REFERENCES

1. Jain Ruchi, Jain Vaibhav, Jain Nilesh, Jain Deepak Kumar, Jain Surendra. Eco friendly spectrophotometric method for quantitative estimation of lomefloxacin using hydrotropic approach .Journal of Applied Pharmaceutical Science. 2012; 02 (04): 111-114.
2. Greici Cristiani Gomes, Hérida Regina Nunes SALGADO. Validation of UV Spectrophotometric Method for Determination of Lomefloxacin in Pharmaceutical Dosage Form. Acta Farm.Bonaerense.2005; 24 (3): 406-8.
3. Drug Bank http://www.drugbank.ca/drugs/DB00978 (accessed on 18/07/14).
4. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: text & methodology, Q2 (R), Nov 2005.
5. Gurudeep R. Chatwal, Sham K. Anand. Instrumental methods of chemical analysis. Himalaya Publishing House.2013; pp.2.149 - 2.182.
6. Lloyd, Snyder, Joseph, J, Kirkland, Joseph L. Glajch. Practical HPLC method development. Wiley Publishing Inc. 2nd ed.; 1997; pp. 686-695.
7. Wilson & Griswold's Text book of Organic Medical & Pharmaceutical Chemistry.11th ed.; Lippincott Williams & Wilkins publisher.2005; pp.724-725.
8. Green, J.M. A practical guide to analytical method validation, *Analytical Chemistry*, 1996; 68: 305A-309A.