Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i01.6449

Development Of A Novel Uv Spectrophotometric Method For Estimation Of Moxifloxacin Hcl In Simulated Lacrimal Fluid (Slf) Ph 7.4 And Methanol Ar

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Submitted: 20 Jan 2022; Accepted: 17 Feb 2022; Published: 01 Mar 2022

ABSTRACT

In this study, we worked on the development and validation of a simple, accurate and precise UV spectrophotometric method for measuring Moxifloxacin HCl in simulated lacrimal fluid (SLF) pH 7.4 and methanol AR. The drug shows the λmax 285 nm in SLF and 282 nm in Methanol. This method obeys Beer-Lambert's law over the concentration range of 2µg/ml to 10µg/ml, as absorbance values for this concentration range were found to be linear. The calibration curves were plotted separately for this concentration range of Moxifloxacin HCl for both the solvents. The correlation coefficients were found to be 0.995 and 0.997 for SLF and methanol respectively. In SLF, the limit of detection (LOD) and limit of quantification (LOQ) were found 0.037µg/ml and 0.1123µg/ml and in methanol, they were found to be 0.073µg/ml and 0.221µg/ml respectively. The interday and intraday assay was conducted for both simulated lacrimal fluid and methanol and %RSD was found below 2%. The method was validated as per ICH Q2 (R1) guidelines for its accuracy, precision and sensitivity. The developed novel method was found to be less toxic, reproducible, accurate and sensitive for routine quantitative estimation of Moxifloxacin HCl in laboratory..

Keywords: Moxifloxacin HCl, UV Spectrophotometric method, Simulated Lacrimal Fluid (SLF), Validation

INTRODUCTION

Various analytical methods like HPLC, HPTLC, simultaneous chromatographic and UV spectrophotometric method of Moxifloxacin HCl in different pharmaceutical formulations have been reported 1, 2, 3, 4, 5. The Literature shows that RP-HPLC method has been developed for quantification of moxifloxacin and Flavoxate 14. A HPLC-UV method has been developed and validated for estimation of moxifloxacin in biological fluid and useful in pharmacokinetic study 15 An eco-friendly UV spectroscopic method was innovated and validated for simultaneous determination of diclofenac sodium and moxiflxacin in ophthalmic preparation 16 It was reported that various UV spectrophotometric methods have been developed for estimation of moxifloxacin in bulk and pharmaceutical formulations 18, 19, 20, 21.

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Spectroscopy is a useful technique for the studying the chemical structure of pharmaceutical compounds. The region for UV visible spectroscopy on electromagnetic spectrum ranges between 100 to 400 nm 6, 7. The UV visible regions on electromagnetic spectrum are shown in Table 1.

TABLE 1: UV Visible Regions on Electromagnetic Spectrum

Region	Wavelength
Far (or vacuum) ultra-violet	10-200 nm
Near ultraviolet	200-400 nm
Visible	400-750 nm

UV spectrophotometry is the most widely used analytical technique in pharmaceutical analysis. It involves the measurement of amount of UV radiation absorbed by drug molecules in the test solution. This method measures the ratio, or function of the ratio of the intensity of two light beams in the UV-Visible region. This technique is simple, rapid, specific and applicable for both quantitative and qualitative estimation of small quantities of pharmaceutical compounds. Beer - Lambert law govern the working principle of spectrophotometric analysis. Beer's law states that the intensity of a beam of parallel monochromatic beam decreases exponentially with the increase in number of absorbing molecules. In different words, absorbance is directly proportional to the concentration of absorbing substance in solution 8. According to Lambert's law, the intensity of a beam of monochromatic beam decreases exponentially with increase in thickness of homogeneous absorbing medium.

Moxifloxacin hydrochloride ophthalmic solution 0.5%, under the trade name Vegamox®, was introduced into Japan in 2006, with approval for the treatment of bacterial conjunctivitis, keratitis and surgical prophylaxis9, 10. It is a BCS class I drug with a wide therapeutic index 16. It has also been shown to be effective in the treatment of chronic bronchitis, acute bacterial sinusitis, commonly acquired pneumonia and skin infections. The moxifloxacin is act through inhibiting topoisomerase IV and DNA gyrase and effective against wide varieties of bacteria including both gram positive and negative organisms. Moxifloxacin HCL ophthalmic solution 0.5 % shows elevated tissue concentration and moderate to high rate of clinical success against common ocular pathogens 17. The moxifloxacin ($C_{21}H_{24}FN_3O_4$) is almost white to yellowish crystalline powder 11, 12. Chemically, it is 1-cyclopropyl-7-[(S, S)-2, 8-diazabicyclo [4.3.0] non-8-yl]-6-fluoro-8-methoxy-1, 4- dihydro-4-oxo-3 quinoline carboxylic acid 13. A specific, accurate and precise analytical method is always needed for routine estimation of moxifloxacin HCl in given samples.

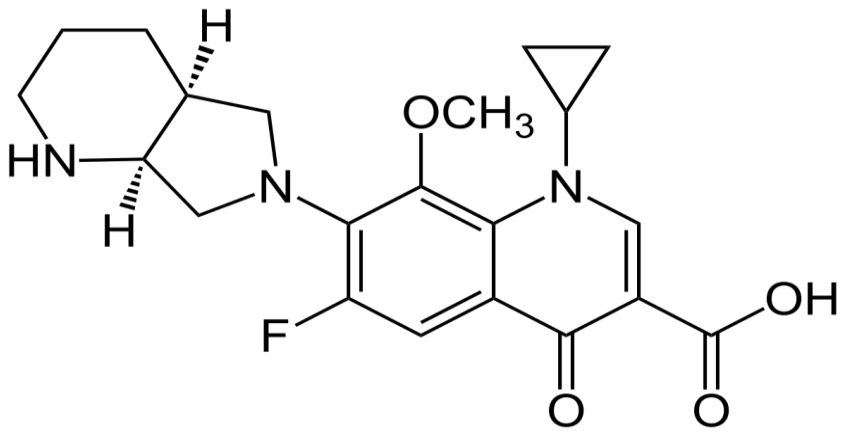

FIGURE 1: Chemical Structure of Moxifloxacin

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Using this method, the drug can be quantified by preparing a solution in a in a clear solvent and measuring its absorbance at an appropriate wavelength. The wavelength is usually selected as the wavelength of λ max, where a small error in setting the wavelength scale will have little effect on the measured absorbance. Preferably, the concentration should be adjusted to give an absorbance of about 0.9 nm, around which the measurement accuracy and precision will be optimal 12.

In this study, simple, accurate and precise UV spectroscopy methods were developed, which could be useful for estimating Moxifloxacin HCl in SLF and methanol AR. These developed methods have been validated according to ICH Q2 R1 guidelines 13.

MATERIAL AND METHODS

Chemicals

The Moxifloxacin HCl pure sample was gifted by Cureworth Drugs & Intermediates Pvt. Ltd. and used as reference standard. The Methanol AR grade supplied by Loba Chemie Pvt Ltd, India was used as the solvent for the preparation of stock and working standard solutions for this study. All chemicals and reagents were of analytical grade.

Instruments

A UV-Visible double-beam spectrophotometer (3000, Lab India) having a spectral bandwidth of 0.1 nm, wavelength accuracy of 0.5 nm, and a pair of matched quartz cell of 10 mm was used. The list of chemicals & instruments with their suppliers is provided below Table 2.

TABLE 2: List of Materials and Equipments used in Study

Materials	Manufacturer & supplier
Moxifloxacin HCl	Cureworth Drugs & Intermediates Pvt. Ltd.
Sodium chloride	Loba Chemie Pvt Ltd
Sodium bicarbonate	Loba Chemie Pvt Ltd
Sodium hydroxide	Loba Chemie Pvt Ltd
Calcium chloride dihydrate	Loba Chemie Pvt Ltd
Methanol AR	Loba Chemie Pvt Ltd

TABLE 3: List of equipments used in Study

Equipments	Model and Maker
Analytical weighing balance	ATY224, Shimadzu, Japan
Ultracentrifuge	OptimaTM MAX-XP, Beckmann Coulter, USA
pH meter	Universal Enterprises, Mumbai, India
UV-Visible Spectrophotometer	V -1900, Shimadzu, Japan
Cyclomixer	CM-101 Plus, Remi, Mumbai, India
Magnetic Stirrer	1MLH, Remi, Mumbai, India

Preparation of Standard Stock Solutions

The Standard stock solution of moxifloxacin is prepared by dissolving 100 mg of the drug in 100 ml of methanol and simulated lacrimal fluid pH 7.4 separately in a 10 ml volumetric flask to obtain a 1 mg/ml solution. The drug was dissolved in two separate volumetric flasks containing methanol and simulated lacrimal fluid of pH 7.4, with vigorous shaking, final volume adjusted with each solvent and sonicated for approximately 10 minutes. A 10 ml solution was taken from this stock solution and diluted to 100 ml with suitable solvents to get another stock solution containing 100 µg/ml of drug. The stock solution was filtered using Whatman filter paper No.41. This stock solution was further diluted to create a working solutions with a concentration from 2 µg/ml to10 µg/ml.

Determination of Maximum Absorbance (λmax)

The λ max was determined by scanning working standard solution of 10 µg/ml in simulated lacrimal fluid pH 7.4 and methanol AR in entire UV range of 200-400 nm using UV-visible

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spectrophotometer. In addition, typical UV spectra of moxifloxacin were obtained for both the solvents.

Preparation of Calibration curve

The working standard solutions of moxifloxacin with a concentration of 2, 4, 6, 8, and 10 µg/ml were prepared in triplicate using methanol and simulated lacrimal fluid pH 7.4 from their respective stock solutions. The absorbance of various drug concentrations were measured at λ max and the mean absorbance for each concentration was calculated. A calibration curve was plotted between mean absorbance and concentration of each solvent to obtain the standard plot equations and regressions coefficients.

RESULT AND DISCUSSION

The λ max of Moxifloxacin was found to be 285 nm and 282 nm in simulated lacrimal fluid pH 7.4 and methanol AR respectively as shown in Figure 2 and 3.

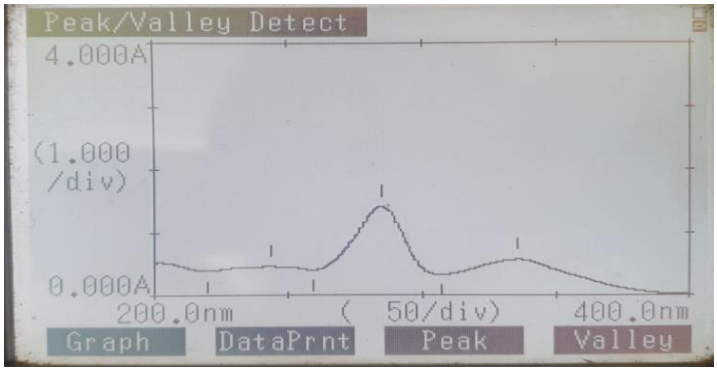

FIGURE 2: UV Spectrum of Moxifloxacin in SLF pH 7.4

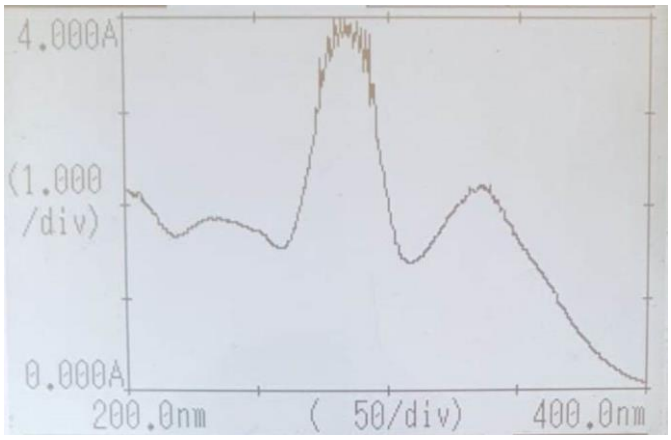

FIGURE 3: UV Spectrum of Moxifloxacin in Methanol AR

A calibration curves was created between the mean absorbance and concentrations for both the solvents as shown in Figures 4 and 5. The absorbance value of the drug was found to be linear over a particular concentration range. The regression equation was y = 0.069x + 0.052 with a correlation coefficient (R2) of 0.997 for methanol and y = 0.089x + 0.065 with R2 value of 0.995 in case of SLF pH 7.4.

In addition, this method has been validated according to ICH Q2B guidelines to determine the linearity, sensitivity, accuracy and precision for both qualitative and quantitative estimation of drug analyte for routine laboratory practice.

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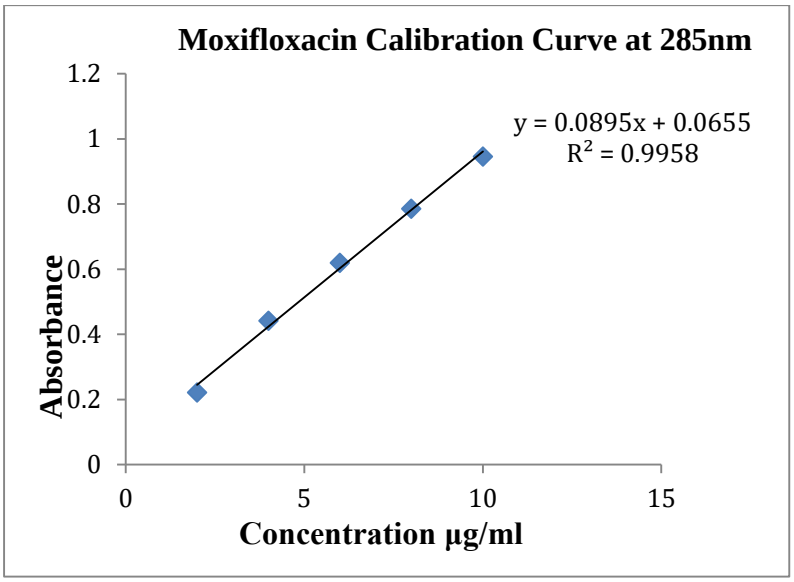

FIGURE 4. Standard Plot of Moxifloxacin in SLF pH 7.4

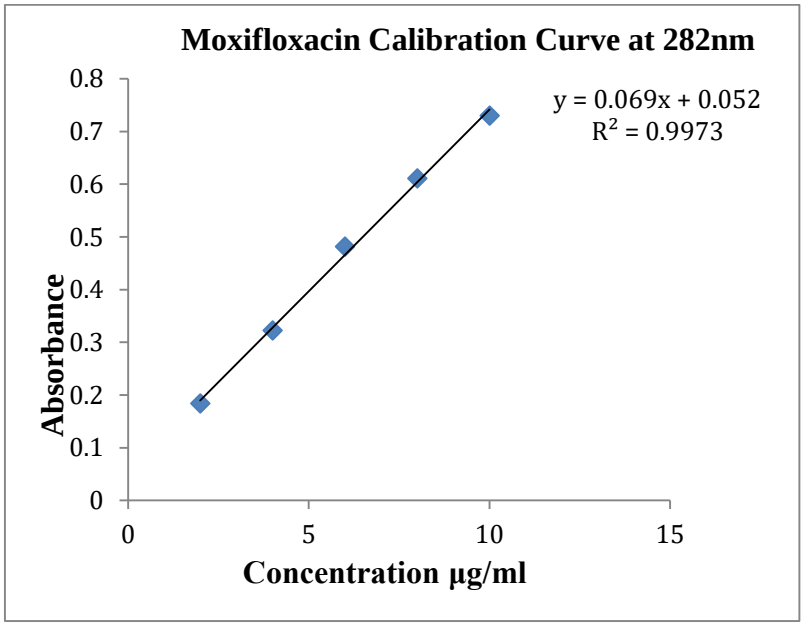

FIGURE 5: Standard Plot of Moxifloxacin in Methanol AR

TABLE 4: Inter-Day Precision Data of Moxifloxacin in SLF pH 7.4

Concentration (µg/ml)	Absorbance (285 nm)			Mean	SD	%RSD
	Day1	Day2	Day3			
2	0.222	0.225	0.218	0.221	0.003511	1.58
4	0.442	0.436	0.439	0.439	0.003	0.68
6	0.613	0.618	0.611	0.614	0.003605	0.59
8	0.785	0.778	0.781	0.781	0.003511	0.45
10	0.941	0.946	0.942	0.943	0.002645	0.28

TABLE 5: Intra-Day Precision Data of Moxifloxacin in SLF pH 7.4

Conc. (µg/ml)	Absorbance (285nm)			Mean	SD	%RSD
	Morning	Afternoon	Evening			
2	0.223	0.226	0.219	0.222	0.003511	1.58
4	0.441	0.438	0.446	0.441	0.004041	0.92
6	0.611	0.614	0.612	0.612	0.001527	0.25
8	0.783	0.788	0.778	0.783	0.005	0.64
10	0.939	0.933	0.941	0.937	0.004163	0.44

TABLE 6: Accuracy Data Analysis of Moxifloxacin in SLF pH 7.4

Stock solution 1 (µg/ml)	Stock Solution 2 (µg/ml)	Theoretical conc. Of final solution (µg/ml)	Absorbance	Observed Conc. (µg/ml)	% Recovery
2 (3ml)	12 (2ml)	6	0.404	5.9251	98.751
2 (1ml)	12 (1ml)	7	0.465	6.8300	97.571
2 (2ml)	12 (3ml)	8	0.533	7.8388	97.985

Accuracy:

The percent recovery was calculated at the level of 120%, 100%, and 80% of standard drug and accuracy was found between 90%-110%. Hence, the developed analytical method was found to be accurate.

Limit of detection (LOD)

The limit of detection of this developed analytical method for moxifloxacin was calculated from calibration curve using formula and it is found to be 0.037 µg/ml.

Limit of quantification (LOQ): The minimum amount of drug that can be quantified by the developed method is calculated according to the formula and is 0.1123 µg/ml.

TABLE 7: Inter-Day Precision Data of Moxifloxacin in Methanol AR

Stock solution 1 (µg/ml)	Stock Solution 2 (µg/ml)	Theoretical conc. Of final solution (µg/ml)	Absorbance	Observed Conc. (µg/ml)	% Recovery
2 (3ml)	12 (2ml)	6	0.410	5.89	98.16
2 (1ml)	12 (1ml)	7	0.482	6.91	98.71
2 (2ml)	12 (3ml)	8	0.542	7.89	98.62

TABLE 8: Intra-Day Precision Data of Moxifloxacin in Methanol AR

Conc. (µg/ml)	Absorbance (282nm)			Mean	SD	%RSD
	Set-1	Set-2	Set-3			
2	0.216	0.210	0.214	0.213	0.003055	1.43
4	0.431	0.417	0.422	0.417	0.005	1.20
6	0.503	0.496	0.505	0.501	0.004725	0.94
8	0.642	0.622	0.633	0.632	0.010016	1.58
10	0.704	0.698	0.702	0.701	0.003055	0.44

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TABLE 9: Accuracy Data Analysis of Moxifloxacin in Methanol AR

Conc. (µg/ml)	Absorbance (282nm)			Mean	SD	%RSD
	Set 1	Set 2	Set 3			
2	0.191	0.186	0.185	0.187	0.003214	1.72%
4	0.326	0.330	0.331	0.329	0.002645	0.80%
6	0.468	0.475	0.482	0.475	0.007	1.47%
8	0.629	0.615	0.612	0.6186	0.009073	1.47%
10	0.753	0.730	0.729	0.7373	0.013576	1.84%

Accuracy

The percent recovery was calculated at the level of 120%, 100%, and 80% of standard drug and accuracy was found between 90%-110%. Hence, the developed analytical method was found to be accurate.

Limit of detection (LOD)

The LOD of developed analytical method for moxifloxacin in methanol AR was calculated using calibration curve by applying formula and it was found to be 0.037 µg/ml.

Limit of quantification (LOQ)

The limit of detection was calculated as per the formula and was found to be 0.1123 µg/ml.

CONCLUSION

This UV-spectrophotometric method is simple, precise, and accurate and gives reproducible results. This developed method is suitable for analysis of moxifloxacin in formulation using simulated lacrimal fluid pH 7.4 and methanol AR. The statistical data of validation confirms that this method was found to be precise, accurate and appropriate for the quantification of moxifloxacin in their formulation. Therefore, this analytical technique can also be safely and satisfactorily used for routine analysis of moxifloxacin in laboratory.

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