Quantitative determination of Moxifloxacin hydrochloride in bulk and ophthalmic solution by UV-spectrophotometry and first order derivative using area under curve

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ABSTRACT

Moxifloxacin Hydrochloride (MOX) is a fourth generation fluoroquinolone broad spectrum antibiotic agent used in conjunctivitis. Two simple, quick and economical methods 'Zero Order UV-Spectrophotometric (Method I)' and 'First Order Derivative spectrophotometric (Method II)' using Area Under Curve (AUC) technique have been developed for routine analysis of MOX in bulk and ophthalmic solution. In double RO water, MOX showed maximum absorbance at 288.2 nm. In method I, AUC between two wavelengths 279.0 nm and 296.4 nm were selected. In Method II, UV-spectrum of MOX was derivatized into first order and two wavelengths 289.4 nm and 305.6 nm were selected for determination of AUC. In both these methods, MOX obeyed linearity in the concentration range of 2 − 12 µg/mL (r^2 > 0.99). Proposed methods were applied for ophthalmic solution and amounts of MOX estimated by method I and method II were found to be 99.97 ± 1.21 and 99.86 ± 1.82, respectively. Both these methods were validated statistically and by recovery experiments.

Keywords: Moxifloxacin Hydrochloride; UV-Spectrophotometry; first order derivative; Area Under Curve.

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INTRODUCTION

Moxifloxacin Hydrochloride (MOX) is a fourth generation fluoroquinolone broad spectrum antibiotic agent used in conjunctivitis [1, 2]. In literature survey many analytical methods includes RP-HPLC [3-5] and UV-spectroscopic [6-8] and HPTLC [9] methods have been reported for the estimation of MOX in bulk, pharmaceutical formulation and in biological samples.

In present study, simple, economical, accurate reproducible analytical method with better detection range for estimation of MOX in its pure form and its ophthalmic solution were developed. This paper describes a UV-Spectrophotometric and First Order Derivative methods

for estimation of MOX in bulk and ophthalmic solution using Area Curve (AUC) technique. Both these developed methods were validated as per the USP guidelines [10].

MATERIALS AND METHODS

Chemicals
Moxifloxacin hydrochloride supplied as a gift sample by Cipla Pharmaceuticals Ltd, Baddi. Ophthalmic solution (Mosi) was purchased from Indian market, containing Moxifloxacin Hydrochloride 5mg/mL.

Instrumentation
A UV-Visible spectrophotometer (2450 Shimadzu, software UV Probe 2.21) with spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 10 mm was employed for all.

Selection of common solvent
Double Reversed Osmosis (R.O.) water was selected as common solvent for developing spectral characteristics of MOX. The selection was made after evaluating the solubility of MOX in different solvents.

Preparation of Stock standard solution and selection of wavelengths
A stock standard solution of MOX was prepared by dissolving 10 mg in 100 mL methanol to obtain concentrations 100 µg/mL. After proper dilutions, 10 µg/mL MOX was scanned in the UV-region i.e. 400 – 200 nm. MOX showed maximum absorbance at 288.2 nm. In Zero order UV –Spectrophotometric method (Method I), two wavelengths 279.0 nm to 296.4 nm were selected for determination of Area Under Curve [AUC] (**Figure I**). In method II, the zero order spectrum was derivatized into first order derivative ($\Delta \lambda = 2$ nm, scaling factor 2) using UV Probe software 2.21 and two wavelengths 289.4 nm to 305.6 nm were selected for determination of AUC (**Figure II**).

Figure 1: Zero order spectrum of MOX showing wavelength selection between 279.0 and 296.4 nm.
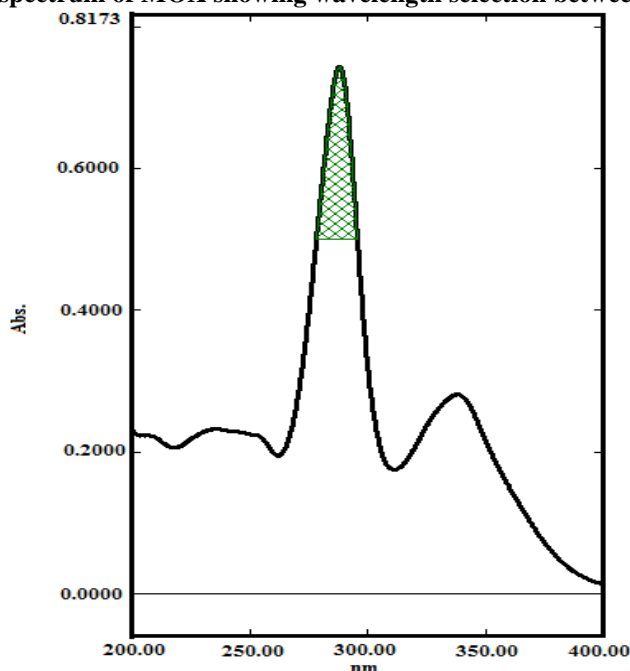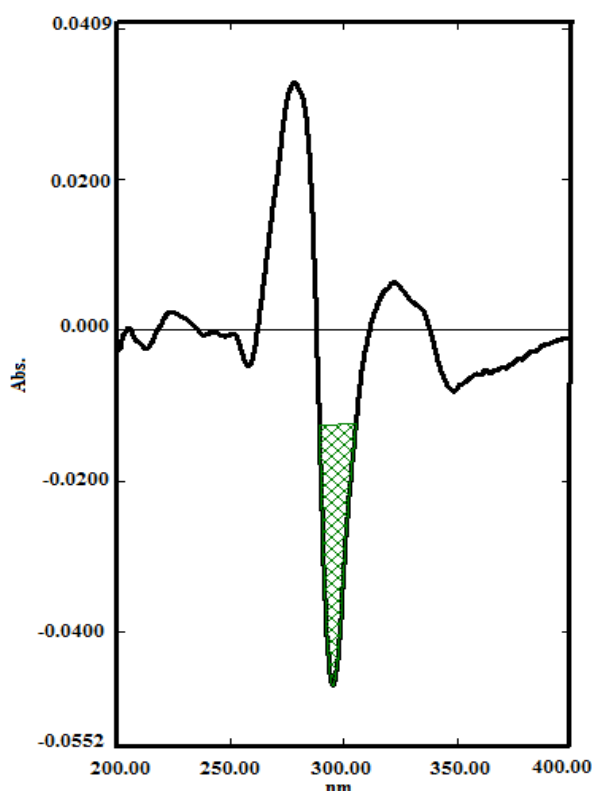
Figure 2: First derivative spectrum of MOX showing trough wavelength selection between 289.4 and 305.6 nm.

Study of linearity curves

To examine the linearity of the assay, the calibration curve for MOX at a concentration ranged from 2 – 12 µg/mL in water was prepared by putting an aliquot portions 0.2 − 1.2 mL into series of six separate 10 mL volumetric flasks and volume was adjusted to mark with water. The AUC between the selected wavelengths were determined and calibration curves were constructed by plotting concentration *versus* AUC between the selected wavelengths. The optical characteristic and statistical data is shown in **table 1.**

Analysis of marketed formulation

An accurately measured volume of ophthalmic solution equivalent to 10 mg of MOX was transfer into 100 mL volumetric flask and volume was made up to the mark with water, filtered through 0.45 µm Whatmann filter paper. A suitable volume of solution was further diluted with water to obtain concentration 8 µg/mL of MOX. AUC were recorded in between the selected wavelengths and the concentrations were determined using respective linear regression equations. The analysis procedure was repeated for six times with ophthalmic solution.

RESULTS AND DISCUSSION

In double R.O. water, MOX showed maximum absorbance at 288.2 nm. In method I, and Method II, MOX followed linearity in the concentration range of 02 − 12 µg/mL. The amounts of MOX estimated by Method I and Method II were found to be 99.97 ± 1.21 and 99.86 ± 1.82, respectively. Results acquired indicate that there was no interference from the excipients commonly present in ophthalmic solution. Both these methods were validated for accuracy, precision and ruggedness. The accuracy of the methods were studied at multiple levels i.e. 80%, 100% and 120 % level using standard addition method. The precision of the methods were

studied as repeatability and intra-day and inter-day variation; the results were expressed in terms of % RSD. Both these methods were found to accurate and precise as shown by small values of % RSD. Ruggedness of the both these methods were studied by two different analysts and on two different make of instruments. Methods proved to be rugged as demonstrated by small values of % RSD. The results from the validation of methods are given in **table I.**

Table 1: Results of optical characteristics and validation of MOX by proposed methods

Parameter	Method I	Method II
Linearity range [µg]	2 - 12	2 - 12
Linearity equation	Y = 0.331 X +0.008	Y= 0.040 X +0.002
Coefficient Correlation [r^2]	0.999	0.999
% Recovery [n = 3]	100.04	101.18
% RSD	0.54	0.35
Precision [% RSD]		
Intra-day [n = 3]	0.63 – 1.77	1.12 – 1.90
Inter-day [n = 3]	0.64 – 1.62	0.60 – 1.67
Repeatability [n = 6]	1.83	1.68
Ruggedness [% RSD]		
Analyst – I [n = 6]	1.87	1.68
Analyst – II [n = 6]	1.11	1.17
UV-Vis- Spectrophotometer -2450 [n = 6]	1.04	0.98
UV-Vis Spectrophotometer –1700 [n = 6]	1.59	1.48

CONCLUSION

Both these developed methods are simple, economical, accurate and precise and can be used for routine estimation of MOX from its pharmaceutical formulation.

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