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Development and Validation of UV-Spectrophotometric Methods for Determination of Moxifloxacin HCL in Bulk and Pharmaceutical Formulations

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ABSTRACT

Two simple, precise, accurate and economical UV spectrophotometric methods have been developed and validated for the routine estimation of moxifloxacin HCL in bulk drug and pharmaceutical formulation. The drug shows maximum absorption at 293nm and obeyed Beer-Lambert's law in the concentration range of 1-20µg/ml. The same spectrum was derivatised into first order derivative. The amplitude of trough at 282nm and crest at 302nm for D_1 were measured. In D_1 method the drug showed linearity in the concentration range of 1-20µg/ml. The linear regression equations were calculated to be y=0.0966x+0.031 (R^2=0.9949) for D_0 at 293nm, y= -0.0044x+0.0004 (R^2=0.9937) for D_1 at 282nm, and y=0.0056x-0.0005 (R^2=0.9962) for D_1 at 302nm. The results of estimation of marketed tablet formulations were found to be 98.241±0.669-100.678±0.537 for tablets and 99.172±0.514-100.362±0.471 for eye drop with their SD less than 2. Recovery studies were carried out by addition of known amount of standard drug (80,100 and 120% of labeled claim of a tablet) to the preanalysed tablet solution. The % recovery was found to be 98.745-100.984 for tablets, and 98.124-100.174 which indicates accuracy and reliability of the validated method as well as noninterference from excipients to the developed method. The intraday and inter day assay was within 2%. The methods were then validated statistically as per the ICH guidelines which yielded good results concerning range, precision, accuracy, specificity and repeatability.

Keywords: Moxifloxacin HCL; UV-Spectrophotometry; first order derivative

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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. This paper describes a UV-Spectrophotometric and First Order Derivative methods for estimation of MOX. In present study, simple, economical, accurate, reproducible analytical methods with better detection range for estimation of MOX in its pure form and its pharmaceutical formulations were developed. Both these developed methods were validated as per the ICH guidelines [10]

MATERIALS AND METHODS

Chemicals

Moxifloxacin hydrochloride supplied as a gift sample by Apotex Research Pvt. Ltd, Bangalore. Tablet Moxif (Label claim: Moxifloxacin HCL 400mg/tab) and Eye drop Mosi (Label claim: Moxifloxacin HCL 5mg/mL) was purchased from local market.

Instrumentation
UV-visible double beam spectrophotometer, JASCO-V630 with spectral bandwidth of 0.5 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells were used.

Selection of solvent
Distilled water was selected as solvent for developing spectral characteristics of MOX. The selection was made after evaluating the solubility of MOX in different solvents.

Preparation of standard stock and calibration curve
The standard stock solutions of MOX was prepared by dissolving 25mg of drug in 10mL distilled water in 100mL volumetric flask, final volume was adjusted with distilled water to get 250 µg/mL. Working standard solutions of 10µg/mL were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectra as shown in **Fig. 1** The drug shows maximum absorption at 293nm. Six working standard solutions for drug having concentration 1, 2, 5, 10, 15, and 20 µg/mL were prepared in distilled water from stock solution. The absorbance of resulting solutions were measured at respective λ max and plotted a calibration curve against concentration to get the linearity and regression equation. The same spectrum was derivatised into first order derivative, the amplitude of trough at 282nm, and crest at 302nm for D_1 as shown in **Fig. 2** were measured. In Do drug shows linearity in the range of 1-20µg/ml at 293nm while in D_1 1-20µg/ml at 282nm and 302nm. The linear regression equations were calculated to y=0.0966x+0.031 (R^2=0.9949) for D_0 at 293nm as shown Fig. 3, y= -0.0044x+0.0004 (R^2=0.9937) for D_1 at 282nm, and y=0.0056x-0.0005 (R^2=0.9962) for D_1 at 302nm as shown in **Fig. 4.**

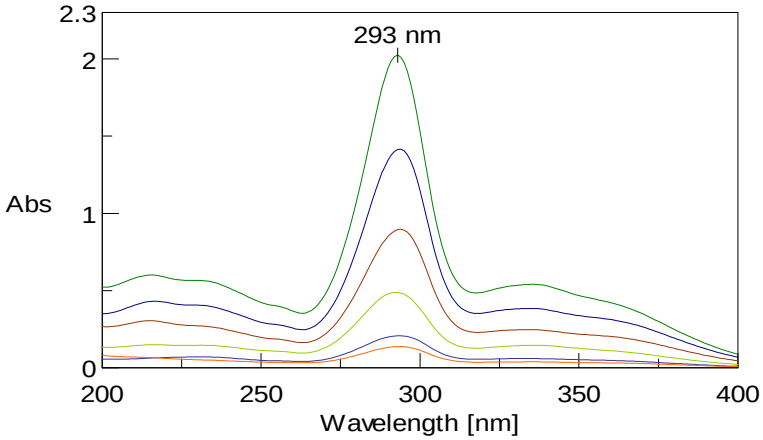

Figure 1: Overlain spectra of moxifloxacin HCL (D_0)

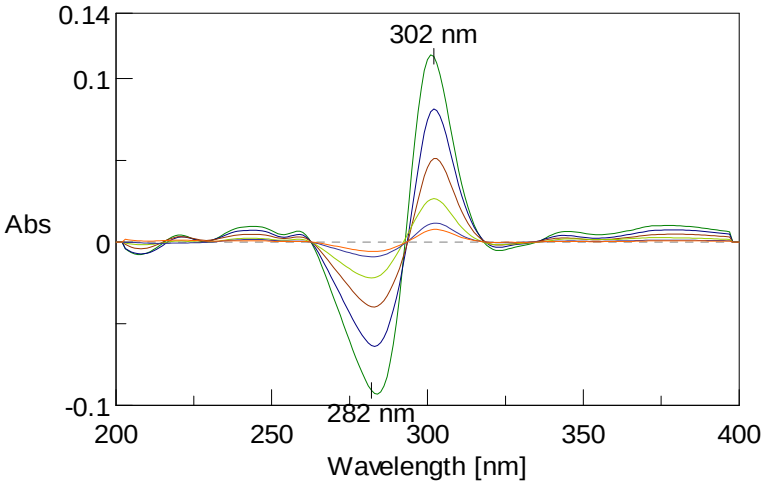

Figure 2: Overlain spectra of moxifloxacin HCL (D_1)

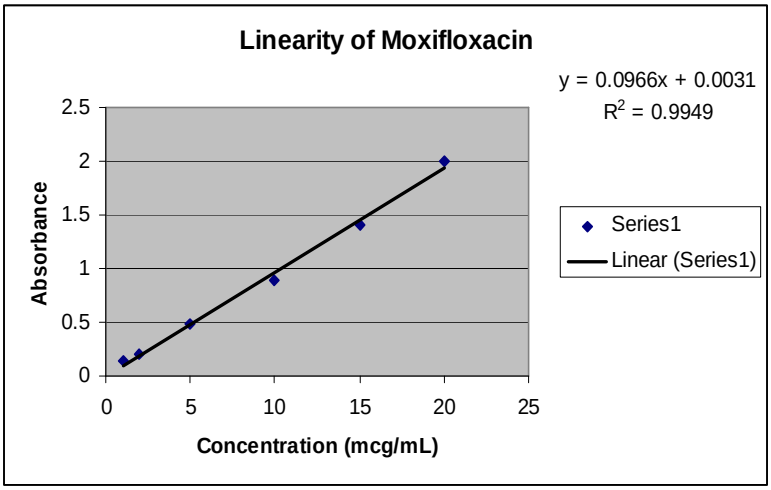

Figure 3: Linearity of moxifloxacin HCL (D_0)

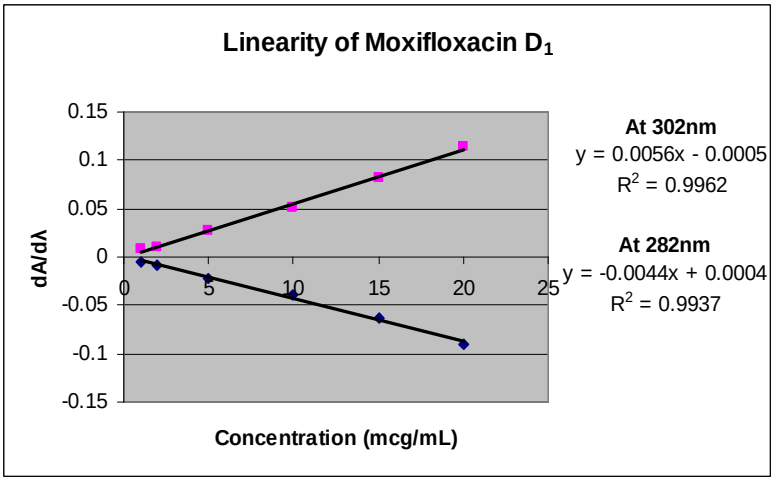

Figure 4: Linearity of moxifloxacin HCL (D_1)

Analysis of formulations

Tablets

Twenty tablets were weighed accurately and reduced to fine powder, drug equivalent to 25mg of MOX was weighed and dissolved in 10ml of distilled water in a 100ml volumetric flask, final volume was made with distilled water. The above solution was filtered by using Whattmann filter paper No.:41. From the above filtrate 0.4 mL of solution was diluted to 10 mL with distilled water to get 10µg/mL of MOX. Analysis procedure was repeated six times with tablet formulation. Aliquot was scanned in the UV range (200-400nm). The same spectrum was derivatised into first order, amplitude of the trough at 282nm, and crest at 302nm for D_1. The amount of drug present in the tablets was calculated from the standard graphs as given in **Table 1.**

Eye drop

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 Whatmann filter paper No.:41 A suitable volume of solution was further diluted with water to obtain concentration 10µg/mL of MOX. Analysis procedure was repeated six times with eye drop formulation. Aliquot was scanned in the UV range (200-400nm). The same spectrum was derivatised into first order, amplitude of the trough at 282nm,and crest at 302nm for D_1. The amount of drug present in the eye drop was calculated from the standard graphs. Results are given in **Table 1.**

Table 1: Analysis of formulations, Statistical Validation and Recovery studies

*S.D.: Standard deviation., COV: Coefficient of variation., S.E.: Standard error *Average of six estimation of tablet formulation., # Average of three estimation at each level*

Formulation	Method		Label Claim	Amount found mg/tab	Label Claim (%)	S.D.*	% COV	S.E.*	Amount Added		% Recovery #
									%	mg/ml	
Tablet	D_0	At 293 nm	400 mg/tab	397.036 mg/tab	99.259	0.669	0.276	0.274	80	320	99.045
									100	400	98.954
									120	480	100.984
	D_1	At 282 nm		399.984 mg/tab	99.996	0.538	0.221	0.220	80	320	98.745
									100	400	99.458
									120	480	100.458
		At 302 nm		398.352 mg/tab	99.588	0.369	0.152	0.151	80	320	98.884
									100	400	99.485
									120	480	100.024
Eye Drop	D_0	At 293 nm	5 mg/mL	4.987 mg/mL	99.759	0.514	0.211	0.210	80	4	99.741
									100	5	98.124
									120	6	100.174
	D_1	At 282 nm		5.027 mg/mL	100.534	1.06	0.432	0.435	80	4	99.783
									100	5	100.122
									120	6	100.117
		At 302 nm		4.980 mg/mL	99.618	0.471	0.194	0.193	80	4	99.824
									100	5	99.174
									120	6	98.9217

Method Validation:

Linearity

Appropriate concentration of stock solution was assayed as per developed methods. Beer-Lambert's concentration range was found to be 1- 20µg/ml. The linearity data for both methods are presented in **Table 2.**

Table 2: Optical Characteristics for Moxifloxacin HCL

Parameters	Values		
	(D_0)	(D_1)	
Working λmax	293nm	282nm	302nm
Beer's law limit (µg/ml)	1-20	1-20	1-20
Correlation coefficient*	0.9949	0.9937	0.9962
Intercept*	0.031	0.0004	-0.0005
Slope*	0.0966	-0.0044	0.0056

** Average of six estimation*

Accuracy

The accuracy of the methods was determined by performing recovery studies on tablet formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels 80%.100% and 120% as per ICH guidelines. The recovery study performed three times at each level. The results are shown in **Table 1.**

Precision

To check the degree of repeatability of methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variance and standard error was calculated. The results of statistical evaluation are shown in **Table 1.**

Selectivity

The selectivity of the methods was checked by monitoring a standard solution of MOX in presence of excipients at the same concentration level as used in tablet using the method described in the procedure for calibration curve in pharmaceutical tablets.

Intermediate Precision (Interday and Intraday precision)

The experiments were repeated three times in a day to determine intraday precision and on three different days to determine interday precision. The results of the same are presented in **Table 3**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of MOX by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in **Table 3**.

Table 3: Validation Parameters

Formulation	Method		LOD* µg/ml	LOQ* µg/ml	Precision(%COV)			
					Intraday n=3	Interday*		
						First day	Second day	Third day
Tablet	D0	At 293 nm	0.588	1.764	0.1171	0.7781	0.1258	0.9431
	D1	At 282 nm	0.542	1.626	0.0941	0.9879	0.9065	0.8278
		At 302 nm	0.642	1.926	0.1249	0.1547	0.1893	0.1299
Eye Drop	D0	At 293 nm	0.512	1.536	0.9016	0.1027	0.9924	0.8945
	D1	At 282 nm	0.715	2.145	0.9124	0.9471	1.1455	1.6847
		At 302 nm	0.845	2.535	1.1547	0.9879	1.4789	0.5471

*COV: Coefficient of variation., *Average of six determination*

RESULTS AND DISCUSSION

The proposed methods are simple, rapid and precise and do not suffer from any interference due to excipients of tablet. Various optical characteristics are shown in the Table 1. The proposed spectrophotometric methods were found to be linear in the range of 1- 20µg/ml at 293nm in Do with correlation coefficients (R^2) 0.9949 while in D_1 1-20 µg/ml at 282nm, and 302nm. with correlation coefficients (R^2) for D_1were found to be 0.9937 and 0.9962 respectively. The methods were validated in terms of accuracy, precision, repeatability and the results are recorded in **Table 2 and 3**. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels. Values of recovery greater than 98.0% indicate that proposed method is accurate for the analysis of the drug. The precision of the proposed method was estimated in terms of interday precision and intraday precision wherein the method was repeated on three different days and repeated for three different time periods in the same day respectively. The results shown in Table 3. SD less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of the drug.

The selectivity of the method was checked by monitoring a standard solution of MOX in presence of excipients at the same concentration level as used in tablets and eye drop using the method described in the procedure for calibration curve in pharmaceutical tablets. The excipients did not show any effect on the estimation of MOX. Hence, the determination of MOX in the tablets and eye drop were considered to be free from interference due to the excipients. Rigorous analysis of the results indicates that the presence of excipients in tablets and eye drop formulations did not interference with the final determination of the active component. This reveals that the potential utility of this method for the routine analysis of MOX in pharmaceutical preparations.

CONCLUSION

Two new, simple precise, accurate and selective spectrophotometric methods were developed for the analysis of MOX in bulk and in pharmaceutical formulation. The D_0 method is useful for formulations where there is no interference of excipients in the absorbance of MOX and method D_1 can be utilized for formulations containing any interfering excipients. The developed methods were also validated and from the statistical data, it was found that methods were accurate, precise, reproducible and can be successfully applied to the pharmaceutical formulations without interference of excipients.

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