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ORIGINAL ARTICLE

A Novel RP-HPLC Method for Simultaneous Determination of Moxifloxacin and Atorvastatin

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Keywords: *Method validation, moxifloxacin, atorvastatin, simultaneous determination.*

Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscript.

Article info.
Received: September 12, 2017
Accepted: November 26, 2017

Funding Source: *Nil*
Conflict of Interest: *Nil*

Cite this article: *Hamid F, Qamar F, Naveed S, Saleem S, Basheer S, Khan S, Sadia H, Usmanghani K. A Novel RP-HPLC Method for Simultaneous Determination of Moxifloxacin and Atorvastatin. RADS J. Pharm. Pharm. Sci. 2017;5(4):50-56.*

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ABSTRACT

Objectives: A RP-HPLC method was validated which is simple, rapid and accurate for simultaneous determination and quantification of Moxifloxacin in presence of Atorvastatin.

Methodology: Chromatographic separation was achieved using C18 having column size of 250x4.6mm, 5µm column. The mobile phase used was composed of methanol: water (95:5v/v) pumped at 1.0mL min-1 flow rate. The detection was performed at 250nm.

Result: The results presented complete separation of peaks with good resolution for both active ingredients in the analyte. The values of intra and inter-day precision were also found to be within required limits. The LOD and LOQ were estimated. Validation process also showed stability of both the drugs at altered pH, wavelength, flow rate and concentration of mobile phase also seemed to unaffected the results. The method was validated and all the parameters of method validation were found to be in good agreement.

Conclusion: Overall, the method revealed acceptable linearity and good correlation for both the drugs. The method possessed high precision value, accuracy and % recoveries of both drugs. The developed method can therefore be a valuable tool for regular simultaneous analysis of both drugs in API and bulk dosage form.

INTRODUCTION

Moxifloxacin (Figure **1**) belongs to fourth generation of Fluoroquinolones is a broad-spectrum antibiotic. Moxifloxacin (MOX) is chemical name is as 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(4As,7As)–octahydro-6H-pyrrolo-(3,4-b) pyridin-6-yl)-4-oxo-3-quinoline carboxylic acid [1]. The drug is highly comparable to other members of the same class in its activity against bacteria of Gram positive, anaerobic and atypical bacteria with low MICs [2]. The drug is highly active because of increased activity against DNA gyrase and topoisomerase IV [3]. The drug is well tolerated at doses of 400mg in the management of community acquired pneumonia (CAP), acute and chronic bronchitis (ACEB) with fewer adverse effects related to CNS and the most common being GI disturbance. A 7 day course of 400mg once daily is recommended in acute and chronic sinusitis [4]. The drug is also recommended in urogenital infections with profound long term outcomes [5].

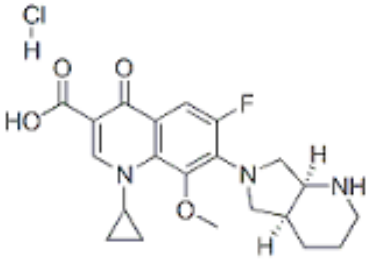

Figure 1. Moxifloxacin.

Statins

LDL lowering drugs are HMG-CoA reductase inhibitors widely known to reduce heart diseases complications in individuals with Heart complications and others at high risk like Type 2 Diabetes [6-8]. Among these Atorvastatin (Figure **2**) is one of most commonly prescribed Statin with maximum cost effectiveness [9]. Clinical data shows newer insight into concomitant use of combination of antimicrobials along with immunomodulatory agents and other adjuvant therapies like Corticosteroids and Statins in patients with severe community Acquired Pneumonia [10]. Moreover, Statins therapy has also been evaluated for COPD (Chronic Obstructive Pulmonary Disease) management [11]. and as immunomodulatory as well [12].

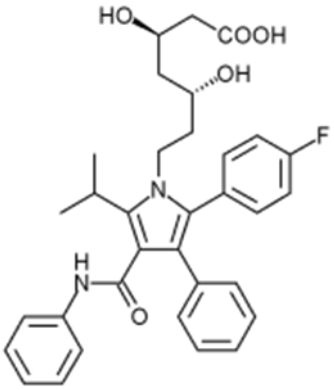

Figure 2. Atorvastatin.

Literature survey demonstrated different technique for direct quantification of Moxifloxacin in pharmaceutical dosage form, bulk and human plasma [13-16]. Moreover we also have methods for simultaneous determination of Moxifloxacin with drugs of same class of Fluoroquinolones [17, 18] and other drugs in pharmaceutical formulations [19, 20]. But none have been reported for the simultaneous determination of Moxifloxacin in presence of Atorvastatin. Therefore, the proposed method presents use for rapid simultaneous determination of Moxifloxacin and Atorvastatin in bulk and as Active Pharmaceutical Ingredient.

EXPERIMENTAL

Chemicals and Reagents

All the reagents used were of high purity and analytical grade. Working standards were of pharmaceutical grade. Moxifloxacin (%, w/w) and Atorvastatin (%, w/w) obtained from local pharmacy.

Instrumentation and chromatographic conditions

Shimadzu HPLC system was used that had LC 20A with UV visible detector LC-10 AT and having VP pump and C18 column having dimension of 250 x 4.6mm, 5 µm) and equipped with Pentium IV Version 5.03 for data acquirement. Mobile phase having ratio of 90:10 v/v of Methanol: Water was used for Separation. The mobile phase was then degassed and filtered through by Whatman filter paper having a membrane filter of 0.45 µm. The flow rate was set tom 1mL min^{-1} with injection volume of 20µl. At 250 nm determinations were performed. All performance was done at ambient temperature.

Preparation of reference solution

Standard stock solution containing 1.00 mg mL^{-1} of Moxifloxacin was mixed with 2.00 mg mL^{-1} of Atorvastatin using methanol as a solvent.

Working standard (50mg) was accurately weigh and transferred to a 100ml measuring flask followed by addition of 70ml of diluents. Sample was dissolved by sonicating on ultrasonic water bath for 5 minutes followed by stirring on magnetic stirrer for 5 minutes. The solution was then cooled to room temperature and with diluents volume was made up. 5 ml of the above

solution was taken to a 100 ml measuring flask and make up the volume with diluents and mixed. For HPLC, working standard solutions having concentration range of 1–10µg mL^{-1} for Moxifloxacin were prepared by diluting solution and 2-20µg mL^{-1} for Atorvastatin. The solution was filtered through 0.45-micron filter and the filtrate was used as reference solution. The prepared solutions were filtered by passing through a membrane filter of 0.45µm. An amount of 20µl of standard (five Replicates) and sample solution (two Replicates) were injected into the HPLC. The chromatogram was recorded and the concentration of both the drugs was calculated using peak area.

RESULTS AND DISCUSSION

Optimization of HPLC Method

The present study aims to validate a precise and accurate RP-HPLC method for simultaneous determination of Moxifloxacin and Atorvastatin. A number of varied parameters including solvent system, pH, and flow rate were employed to obtain desired changes in symmetry and chromatographic parameters. Initially mobile phase comprising of different solvent ratios were utilized. Later on, mobile phase having ratio of 95:5 v/v of Methanol: Water was shown to present suitable separation with minimum use of solvent. The pH of the solvent system was adjusted to with use of Acetonitrile.

The standard preparation was chromatographed and the peak areas were recorded with good resolution. The symmetry factor for the drugs were not more than 2.0, theoretical plates not less than 1000 and the relative standard deviations for 6 replicate injections of the working standard preparation was not more than 2.0. The RT i.e. retention times for Moxifloxacin and Atorvastatin were observed 3.558 ± 0.03 and 7.450 ± 0.04 min, respectively.

Wavelength selection

The wavelength for the detection was measured to be at 250 nm through a common wavelength recording of multiple UV spectra for both the drugs over 200-400 nm wavelength range leading to 250 nm most appropriate for sensitive detection of both drugs.

Method validation

The proposed method was validation as per the International Conference on the Harmonization of Pharmaceuticals for Human Use guidelines criteria.

Linearity

Drug concentrations against peak areas is plotted and linear relationships were observed, our data illustrate linear response in the concentration range of 1 – 10 µg mL^{-1} for Moxifloxacin and 2 – 20 µg mL^{-1} for Atorvastatin. The linear regression equation was y=11710x + 5281 and y=3832.x + 2499 with square of correlation coefficient (R^2) of 0.997 and 0.994 for Moxifloxacin and Atorvastatin respectively (Table **1**, Figure **3** and **4**).

Table 1. Regression Equation.

Drugs	Regression equations	r^2
Moxifloxacin	y = 11710x + 5281	0.997
Atorvastatin	y = 3832.x + 2499	0.994

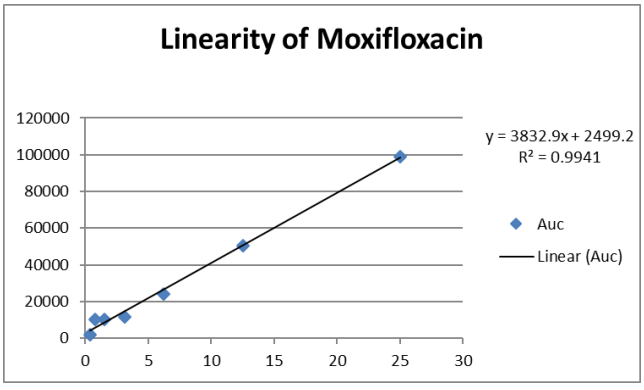

Figure 3. Linearity of Moxifloxacin.

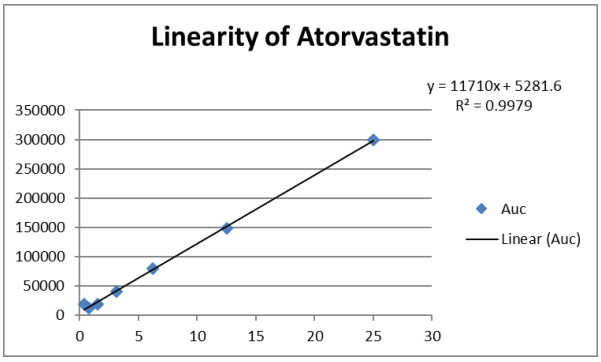

Figure 4. Linearity of Atorvastatin.

The Limit of Detection or LOD and the Limit of Quantitation or LOQ were found to be 0.162ng mL^{-1} and 0.489ng mL^{-1} for Moxifloxacin and 0.493 ng mL^{-1} and 2.569 ng mL^{-1} for Atorvastatin respectively. Results are shown in Table **2**.

Precision

For evaluate the precision of the method repeatability studies were carried out by investigation of 3 different concentrations at a range of 25 µg mL^{-1} to 0.39 µg mL^{-1} for Moxifloxacin and Atorvastatin, respectively by HPLC. By RSD% method repeatability was attained, values found by repeating the assay 7 times on the same day for intra-day precision. For inter-day precision, all performance was carried out on different days maintain the same experimental conditions.

Interday and Intraday precision results are shown in Table **3**. The developed methods were found to be precise and accurate as the RSD values for repeatability and intermediate precision studies were <2%, respectively.

Table 2. LOD and LOQ.

ng	Moxifloxacin	Atorvastatin
LOD	0.16	0.48
LOQ	0.49	2.56

Table 3. Interday and Intraday Precision.

Drugs	Conc Injected	Inter-day		Intra-day	
	mg mL^{-1}	%RSD	%Recovery	%RSD	%Recovery
Moxifloxacin	25	0.05	99.93	0.04	100
	12.5	0.05	100	0.05	100
	6.25	0.03	100	0.03	99.99
	3.125	0.01	99.99	0.01	100
	1.56	0.01	100	0.01	99.99
	0.78	0.06	100	0.06	100
	0.39	0.01	100	0.01	99.99
Atorvastatin	25	0.01	99.99	0.02	100
	12.5	0.01	99.99	0.04	100
	6.25	0.03	99.99	0.02	100
	3.125	0.01	100	0.04	100.03
	1.56	0.03	100	0.09	99.88
	0.78	0.06	100	0.10	100
	0.39	0.07	99.99	0.04	100

Table 4. Robustness.

Variations	Level	tR	K'	T	R
	A: pH change				
3.1	-0.2	2.5	2940.901	5.907	1.921
3.3	0	2.9	2914.176	5.962	1.928
3.2	0.2	3.1	2919.1	5.95	1.93
S.D (n=7)					
	B: Flow rate ml/min				
0.8	-0.2	3.1	2935	5.9	1.89
1	0	2.9	2940.901	5.907	1.921
1.2	0.2	2.6	2949.514	5.98	1.78
S.D (n=7)					
	C: % of water in mobile phase V/V				
80/20	-10	2.9	2950	5.93	1.88
90/10	0	2.8	2949.514	5.98	1.78
70/30	-20	2.4	2945.321	5.98	1.75
S.D (n=7)					
	D: Wavelength (nm)				
245	-5	2.8	2911	5.97	3.7
250	0	2.9	2914.258	5.92	3.2
255	5	2.9	2942.357	5.94	3.4
S.D (n=7)					

Robustness

The robustness was carried out to estimate the effect of minute but deliberate deviation in the chromatographic conditions. This was followed by evaluation of results for the effect of change.

Robustness was performed by intentionally varying parameters like slightly pH changes, flow rate variate ±0.1 mL min^{-1}, mobile phase composition differs ±1 mL) and altered wavelength ±5. RSD was found to be less than 2%. And the standard deviation of the peak areas was calculated for each parameter. Results are shown in Table **4**.

Specificity

For the detection of impurities, degradants and excipients specificity can be evaluated. The peaks obtained were sharp with no interference with any other ingredient for both the drugs (Figure **5**).

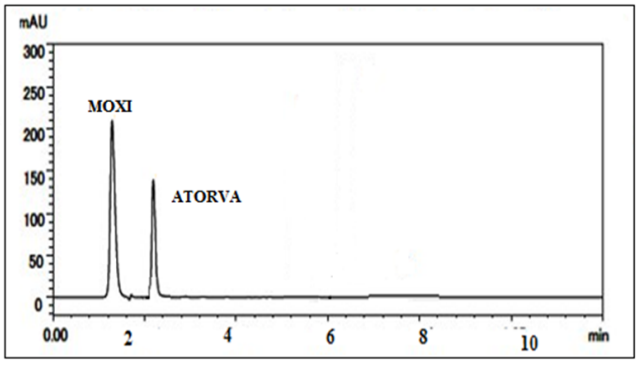

Figure 5. Chromatogram.

Accuracy

The proposed method was carried out for accuracy by utilizing three different concentrations of drug samples of Moxifloxacin and Atorvastatin. For this purpose, known amount of Moxifloxacin and Atorvastatin corresponding to 80, 100 and 120% had been used. The absolute recovery was estimated by comparing the peak areas acquire from standard

solution of Moxifloxacin and Atorvastatin with the peak areas of samples of different concentration.

Table 5. Accuracy of Moxifloxacin and Atorvastatin.

Drugs	Conc μgmL^{-1}	% RSD	% Recovery
Moxifloxacin	80%	0.07	99.99
	100%	0.06	97.377
	120%	0.03	98
Atorvastatin	80%	0.02	100
	100%	0.05	100
	120%	0.45	102

As shown in Table **5**, % recoveries with slight difference of relative standard deviations (RSD %) were obtained from different concentrations and under required limits of 98% - 102% for both the drugs. This indicates that the accuracy of the method for simultaneous quantification of the two drugs.

CONCLUSION

The validated method is simple, rapid and accurate for the quantification of Moxifloxacin and Atorvastatin in bulk and API. The proposed method can therefore be functional effectively for simultaneous quantification of Moxifloxacin along with Atorvastatin.

CONFLICT OF INTEREST

There is no affiliation with or involvement in any organization or entity with any financial interest.

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