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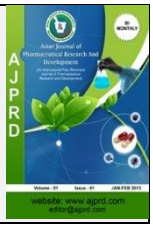

Open Access Research Article

Method Development, Validation and Forced Degradation Studies For the Determination of Moxifloxacin in Bulk and Pharmaceutical Dosage Forms Using UV Spectroscopy

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A B S T R A C T

The objective of this work was to develop and validate spectrophotometric method for moxifloxacin for analysis. A very simple, unique, novel, reliable and easy method of spectrophotometric estimation in UV-region has been developed for the assay of moxifloxacin tablet formulation and also to perform forced degradation studies.

Method: water was used as diluents to perform all the validation parameters and stress studies.

Results: ICH guidelines were adopted during the method development and the method was validated statistically by calculating RSD and %RSD. The drug obeys Beer's lamberts law at 1-10 mcg/ml concentration. Accuracy, Linearity, precision and robustness LOD at 3.3 ppb levels, LOQ at 11.2 ppb levels was performed. % of drug Degradations at different parameters were determined.

Conclusion: - This method can be used for the routine laboratory analysis and can extend the studies to chromatographic techniques.

Keyword: moxifloxacin, validation parameters, Forced degradation.

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INTRODUCTION

Moxifloxacin HCl is a fourth generation fluroquinolone, the antimicrobial activity of which depends upon inhibition of DNA gyrase (bacterial topoisomerase II), an enzyme necessary for DNA replication, transcription, repair and recombination[1-6]. Moxifloxacin has in-vitro and in-vivo activities against wide range of gram+ve and gram-ve bacteria. Moxifloxacin HCl (MOXI) is 1- Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS) octahydro-6H- pyrrole[3,4-b]pyridin-6-yl]-4-oxo-1,4 dihydroquinoline-3- carboxylic acid hydrochloride. a simple and cost effective analytical method is preferred[6-12]. The objective of the present study was to develop a simple precise, accurate and economic analytical method with better detection range, for the estimation of moxifloxacin HCl in bulk and pharmaceutical formulation. In the analytical method developed, water was used as analytical media, it was found to be stable in water , & also water is economic as compare to other media so this method is simple precise, accurate and economic. The developed method was validated as per ICH guidelines and suitable statistical tests were performed on validation data.

MATERIALS AND EQUIPMENTS

Chemicals:

Moxifloxacin (pure API), Avelox (formulation), methanol, and water.

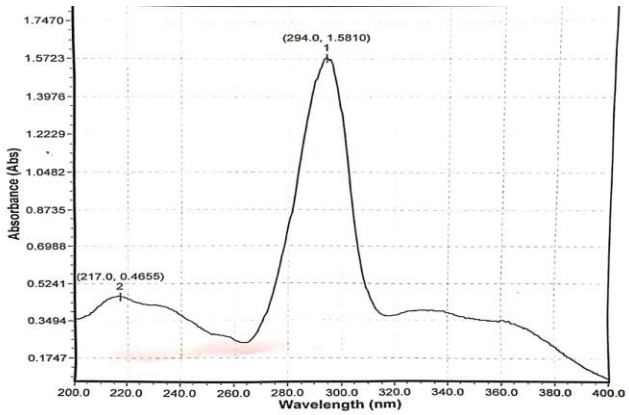
Instruments used:

Weighing balance, Double beam UV-Spectrophotometer (make ELICO SL 210), Volumetric flasks, pipette.

METHODOLOGY

TRAIL-1:

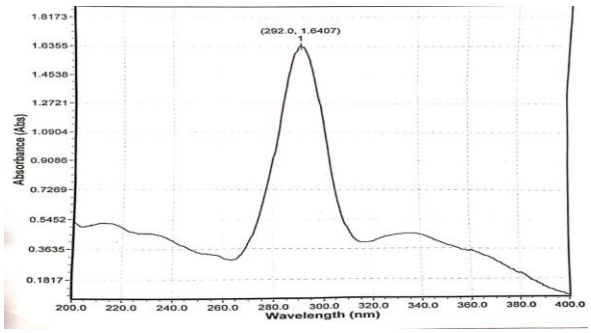
10 mg of Moxifloxacin was weighed and taken in 10 ml volumetric flask and it was diluted with water and volume was made to mark with water (1000ppm). From this 0.1ml was pipetted out into 10ml volumetric flask and the volume was made up to the mark (10ppm). The solutions were scanned at the range of 200-400nm.



Method Parameters
Diluent : Acetonitrile
Wave length : 294 nm
Absorbance : 1.5810
Concentration : 10 ppm.

TRAIL-2:

10 mg of Moxifloxacin was weighed and taken in 10 ml volumetric flask and it was diluted with water and volume was made to mark with water (1000ppm). From this 0.1ml was pipetted out into 10ml volumetric flask and the volume was made up to the mark(10ppm).the solutions were scanned at the range of 200-400nm.



✓ This trial was Optimized method

Method Parameters
Diluent : Water
Wave length : 292 nm
Absorbance : 1.6407
Concentration : 10 ppm

RESULTS AND DISCUSSIONS

FORCED DEGRADATION

1. ACID DEGRADATION:

From 10ppm of drug solution, 1 ml of 10ppm solution was taken into 10 ml volumetric flask, and then 1ml of 0.1N HCl was added and the solution was kept aside for 24 hours. After 24 hours the solution was neutralized with 1 ml of 0.1N NaOH the absorbance value was measured at 292nm.

Absorbance after 24hrs was =1.3220

%degradation=standard absorbance-observed absorbance/standard absorbance ×100

=1.6407-1.3220/1.6407×100

=19.42%

2. ALKALI DEGRADATION:

From 10ppm of drug solution, 1 ml of 10ppm solution was taken into 10 ml volumetric flask and then 1 ml of 0.1N NaOH was added and the solution was kept aside for 24 hours. After 24 hours neutralized with 1 ml of 0.1 N HCl and measure its absorbance value was measured at 292nm.

Absorbance after 24hrs was =1.2672

=1.6407-1.2672/1.6407×100

=22.4%.

3. PHOTOLYTIC DEGRADATION:

10mg of drug was exposed to UV light in UV chamber for 3hrs by placing the drug in watch glass. after 3hrs Sample was diluted to get concentration of 10 µg/ml and absorbance was measured at292nm

Absorbance after 3hrs=1.3992

= 1.6407-1.3992/1.6407×100

=15.1%

4. THERMAL DEGRADATION:

Drug was exposed to dry heat at 40ºC in oven for 3hrs by placing the drugs in watch glass. For every one-hour frequency for 3 hours 10mg of drug weighed and diluted to get a final concentration of 10 µg/ml and absorbance was measured at292nm.

Absorbance after 3hrs was =1.2100

=1.6407-1.2100/1.6407×100

=26.2%

5. PEROXIDE DEGRADATION

From the 10ppm of drug solution, 1 ml of the drug solution was taken into 10 ml volumetric flask and 1 ml of 3% hydrogen peroxide solution was added. then kept aside for 24 hours, after 24 hours the solution was diluted with water to get concentration of 10 µg/ml and absorbance was measured at292nm.

Absorbance after 24hrs was =1.2475

=1.6407-1.2475/1.6407×100

=23.9%.

Table 1: Moxifloxacin Forced degradation studies results in different parameters

1	Acid degradation (0.1N HCl)	19.42%
2	Alkali degradation(0.1N NaOH)	22.4%
3	Peroxide degradation (3%H2O2)	23.9%
4	Thermal degradation(hot air oven)	26.2%
5	Photolytic degradation(UV light exposure)	15.1%

VALIDATION PARAMETERS

1. Linearity (Calibration Curve)

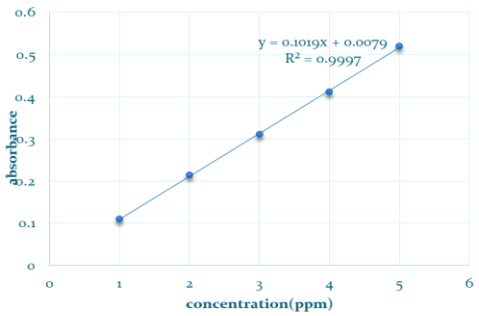
An accurately weighed quantity of moxifloxacin was taken in 10 ml volumetric flask and it was dissolved by using water and volume was made to mark with distilled water to get 1000 µg/ml solution. from standard stock solution of moxifloxacin sub stock (100ppm) was prepared by taking 1ml in 10 ml of water to obtain concentration 100 µg/ml. Then from this 1,2,3,4, and 5ppm were prepared. The solutions were scanned in range of 200 - 400 nm against blank.

The calibration curves were constructed by plotting absorbance versus concentrations. and the regression equations were calculated.

Calibration curve were shown in regression coefficient (R2) is 0.999 for moxifloxacin.

Table 2: Linearity Results

Concentration(ppm)	Absorbance
1	0.1097
2	0.2144
3	0.3116
4	0.4122
5	0.5204



Limits: Correlation coefficient (r^2) ≥ 0.999.

Result: Correlation coefficient (r^2) was found to be 0.9997and it was found to be within the limits.

2. Precision:

5ppm standard solution of moxifloxacin pure drug is selected for precision study. From the standard stock solution 0.05ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using distilled water to give 5ppm solution.

This procedure is repeated 6 time and absorbance of all were measured at 292nm using distilled water as blank and its %RSD was calculated by using the formula:

%RSD = (Standard Deviation of The Measurement / Mean Value of Measurement) × 100

Table 3: Precision study

S. No.	x	x̄-x	$(x-x)^2$
1.	0.5210	0.5205-0.5210=-0.0005	0.00000025
2.	0.5209	0.5205-0.5209=-0.0004	0.00000016
3.	0.5206	0.5205-0.5206=-0.0001	0.00000001
4.	0.5206	0.5205-0.5206=-0.0001	0.00000001
5.	0.5202	0.5205-0.5202=-0.0003	0.00000008
6.	0.5201	0.5205-0.5201=0.0003	0.00000016
x̄	0.5205		$\Sigma(x-x)^2$ =0.00000067

$$SD = \frac{\sqrt{\Sigma(\bar{x} - x)^2}}{n}$$

$$SD = \frac{\sqrt{0.00000067}}{6}$$

$$SD = 0.0003641$$

$$\%RSD = \frac{SD}{mean} * 100$$

$$\%RSD = \frac{0.0003641}{0.5205} * 100$$

$$\%RSD = 0.069\%$$

Limit: %RSD was found to be within the limits i.e. less than 2%

Result: %RSD was found to be 0.069%

3. Accuracy :(Recovery Study)

Recovery study was done by standard addition method.

Standard quantity equal into 50%, 100% and 150 % is to be prepared by adding 2ml of 5ppm of standard solution was spiked with 2ml of 5ppm of sample solution to give 100%, and 2ml of 2.5ppm of standard solution was spiked with 2ml of 5ppm sample solution to give50%,and 2ml of 7.5ppm standard solution was spiked with 2ml of 5ppm sample solution to give150%.

Absorbance was measured for three times at 292nm.Repeated three times and absorbance were measured at 292nm.The %recovery is calculated by using the formula:

% Recovery = spiked-unspiked/unspiked × 100

Preparation of standard solutions:

10 mg drug was weighed accurately in 10 ml volumetric flask and it was dissolved by using water and volume was made to mark with distilled water to get 1000 µg/ml solution. From standard stock solution of moxifloxacin sub stock (100ppm) was prepared by diluting1ml in 10 ml of water to obtain concentration 100 µg/ml.

Form this 0.5ml in10ml gives 5 ppm and 1ml in 10ml gives 10ppm.and 1.5ml in 10 ml gives 15ppm.

Preparation of sample for accuracy:

5tablets were weighed and powdered. Powdered tablet equivalent to 10 mg was weighed and taken into 10ml volumetric flask then volume was made up to the mark with distilled water to get 1000ppm.from that 1 ml of solution was withdrawn, taken in 10ml volumetric flask and volume was adjusted with distilled water up to 10ml to get 100ppm solution. From that take 0.5ml of solution was taken in 10ml volumetric flask and volume adjusted with up to the mark to get 5ppm solution. The absorbance was measured at 292nm

Table 4: Accuracy study

% Recovery Level	% Recovery	% Mean Recovery
50%(5ppm+2.5ppm)	99.3% 99.1% 99.1%	99.1%
100%(5ppm+5ppm)	99.5% 99.4% 99.4%	99.4%
150%(5ppm+7.5ppm)	99.6% 99.5% 99.5%	99.5%

Limits: The % Mean recovery value should be between 98-102%.

Result: %Mean recovery was found to be 99.1%-99.5%

4. Limit of Detection (LOD)

The LOD is the smallest concentration of the analyte that gives a measurable response.

Following equation designated by International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 ×σ/S

LOD=3.3 × 0.00036/0.5205

LOD =0.00228ppm

5. Limit of Quantitation (LOQ)

The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified.

LOQ = 10 ×σ/S

LOQ=10 × 0.00036/0.5205

LOQ =0.00691ppm.

6. Robustness:

6 aliquots of 10ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax (i e. 291nm and 293nm). The absorbance was noted down.

Table 5: Robustness study

S.NO	Concentration (ppm)	Absorbance (nm) 291nm	293nm
1	10ppm	1.6481	1.6440
2	10ppm	1.6466	1.6426
3	10ppm	1.6445	1.6411
4	mean	1.6464	1.6425
5	SD	0.00808	0.001450
6	%RSD=SD/mean×100	0.109%	0.088%

7. Assay

5 tablets were weighed and their average weight was calculated and powdered.10mg of equivalent weight (18.3mg) of moxifloxacin was taken into 10 ml volumetric flack volume made up to the mark by using distilled water.

from that 0.1 ml of solution was withdrawn and taken in to 10ml volumetric flask. The volume was adjusted with Distilled water up to 10ml to get 10ppm solution and its absorbance was measured at 292 nm.

Weight of 5tablets = 3.673gm

Average weight = 3.673/5

=0.7346gm

400mg of drug is present in each tablet (0.7346gm)

10mg of drug is present in=? (x)

x=10× 0.7346/400

=18.3mg.

Absorbance value was=1.61

% Assay = (obtained concentration/original concentration) ×100

% Assay = 1.61/1.64 ×100

% Assay = 98.1%

Limits: The assay value should be between 98-102%.

Result: The %assay was found to be 98.1% and it was found to be within the limits.

CONCLUSION

- A Novel simple UV spectrophotometric method was developed for the moxifloxacin, has been validated according to Q2 (R1) ICH guide lines.
- This method can be used for the routine quality control analysis.
- And also used to determine the %assay of the marketed formulation.
- And also used to determine the % degradation of the drug in different parameter

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