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# Validation of developed analytical method for moxifloacin floating tablets by reverse phase high performance liquid chromatography

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## ABSTRACT

Aim of the present investigation was to validate a new analytical, simple, sensitive, selective and precise High Performance Layer Chromatographic (HPLC) method for the estimation of Moxifloacin in tablet dosage form. Moxifloacin is chemically1-7-[(4aS,7aS)-1,2,3,4,4a,5,7,7a-octahydropyrrolo[3,4-b]pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid. The mobile Phase comprised of Methanol: water (350:650) and set at a flow rate of 1.2ml/minute. Detection was carried out at 293nm using pre-packed Symmetry $C_{18}$; 250x4.6mm, 5µm particle size column. Detection was carried out at 222nm using pre-packed Symmetry $C_{18}$; 250x4.6mm, 5µm particle size column. The retention time of Moxifloacin was found to be 1.825. The assay was linear over concentration range of 12.5µg/ml to 75µg/ml (R=0.99995). The limit of detection and the limit of quantification were found to be 2.68µg/ml and 4.46µg/ml respectively. The amount of Moxifloacin was found to be 100.229±0.47 and the accuracy of Moxifloacin was found to be 99.460% to 100.369%. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Moxifloacinin tablet dosage form during routine analysis.

**Keywords:** Moxifloacin, RP-HPLC, Validation, Method development.

## INTRODUCTION

Moxifloxacin is a quinolone/fluoroquinolone antibiotic and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [1-2]. Moxifloxacin is used to treat infections caused by the following bacteria: Aerobic Gram-positive microorganisms: Corynebacterium species, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus warneri, Streptococcus pneumoniae, and Streptococcus viridans group. Aerobic Gram-negative microorganisms: Acinetobacter lwoffii, Haemophilus influenzae, and Haemophilus para influenzae. Other microorganisms: Chlamydia trachomatis. [3]

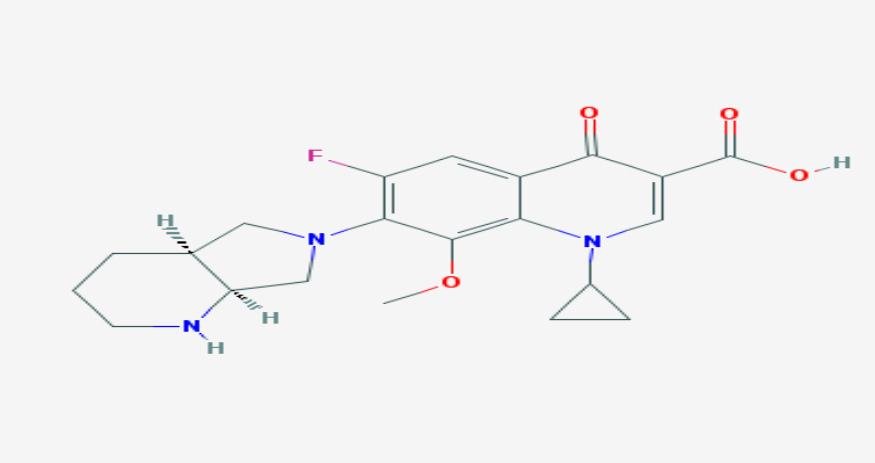


**Figure no 1: Structure of Moxifloxacin**

## MATERIALS AND METHODS

Working standard of Moxifloacin, HPLC grade Acetonitrile, Sodium hydroxide, Potassium dihydrogen Phosphate, O-Phosphoric acid, 0.45µm PVDF membrane filter and Milli-Q water were procured from the market. The separation was carried out on isocratic HPLC system Shimadzu with UV detector with pre-packed Symmetry C18 250 x 4.6mm, 5.0µ m particle size using filtered and degassed Acetonitrile: Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650 as mobile phase [4-7].

### Mobile phase preparation

### Diluent

Mix Acetonitrile: Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650

### Mobile phase

Filtered and degassed mixture of Acetonitrile: Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650

### Standard preparation

Weighed accurately about 0.100g of Moxifloacin working standard into a 100ml volumetric flask, added 70ml of diluent, shaked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

### Assay preparation

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask. Added 70ml of diluent sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

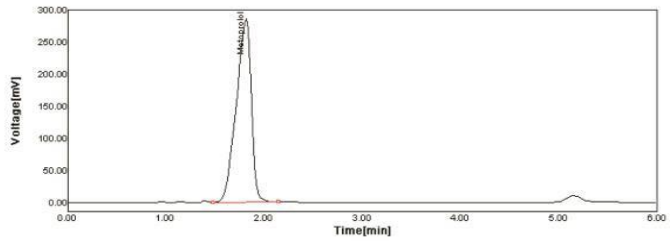
### Chromatographic conditions

Flow rate 1.0ml/min; detection wavelength 222nm; injection volume 20µl; column used symmetry column (5µm, 250x4.6mm); column temperature: 25$^{o}$C; Acetonitrile: Phosphate buffer (350:650).

### Method development [09-13]

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

**Chromatogram**




**Result**

| No. | Name | RT[min] | Area[mV*s] | Area% | TP | Height% | TF |
|---|---|---|---|---|---|---|---|
| 1 | Metoprolol | 1.81 | 372414.96 | 100.00 | 4125 | 100.00 | 1.13 |
| Sum | | | 372414.96 | | | | |

## CHROMATOGRAM OF MOXIFLOACIN

### Validation of developed method [09-16]: System Suitability Preparation

### Weighed

20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask. Added 70ml of diluent sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

### Procedure

Separately injected equal volumes (about 20 µl) of the standard preparation and the assay preparation into the chromatograph, recorded the chromatograms, and measured the responses for the Moxifloacin peak.

### Precision

To establish the precision of the analytical method by using the following two methods.

### System Precision

Establish the repeatability of the analytical method by estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six- sample preparations and report the %RSD for the same.

**Preparation of Blank:** Use diluent as blank**.**

### Preparation of standard solution

Weighed accurately about 0.100g of Moxifloacin working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

### Preparation of sample solutions

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloxacin) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

### Procedure

Injected separately 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculated the content of Moxifloxacin in the individual solutions.

### Intermediate precision (Ruggedness)

A different analyst using a different HPLC system with a different similar column on a different day should carry out this experiment. Estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six-sample preparations and report the %RSD for the same.

**Blank preparation:** Use diluent as blank.

## Preparation of standard solution

Weighed accurately about 0.100g of Moxifloacin working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

**Preparation of sample solutions:** Weighed

20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

## Procedure

Separately injected 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculated the content of Moxifloacin in the individual solutions

## Linearity & Range

## Objective

To establish the linearity of the analytical method for assay using the following two methods.

## Linearity & range for Moxifloacin working standard

Demonstrate the linearity of the analytical method for assay by injecting the various concentrations of standard preparations prepared in the range of 25% to 150% into the chromatograph, covering 6 different concentrations. Draw a plot between the Concentrations vs. peak response of Moxifloacin. Report the slope, intercept and regression coefficient from the plot obtained for concentration vs. Peak response of Moxifloacin in standard preparation.

## Preparation of analytical solutions for linearity & range for Moxifloacin standard preparations

**a.** **Blank preparation:** Use diluent as blank.

**b.** **Standard stock solution preparation:** Transfer an accurately weighed quantity of about 100 mg of Moxifloacin working standard into 100 ml volumetric flask , add 20ml of diluent, sonicated for 10 minutes to dissolve and made to volume with mobile phase. From the stock solution 10 ml was pippeted out into the 100 ml volumetric flask and made to volume with mobile phase.

**a)** **25%Linearity Standard solution preparation (12.5 ppm):** Pipette out 6.25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

**b)** **50%Linearity Standard solution preparation (25.0 ppm):** Pipette out 12.50 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

**c)** **75% Linearity Standard solution preparation (37.5 ppm):** Pipette out 18.75 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

**d)** **100% Linearity Standard solution preparation (50 ppm):** Pipette out 25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

**e)** **125% Linearity Standard solution preparation (62.5 ppm):** Pipette out 31.25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

**f)** **150% Linearity Standard solution preparation (75.0 ppm):** Pipette out 37.5 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

## Calculations

Draw a plot between the concentrations vs. the average peak responses of Moxifloacin peak for all the above studies. Calculate slope, intercept and regression coefficient from the plot obtained.

# ACCURACY / RECOVERY

## Objective

To establish the accuracy of the analytical method is the closeness of sample results obtained by method to the true value by using recovery study.

## Procedure

Perform the recovery studies by adding known quantity of Moxifloacin working standard to known quantity of placebo (MoxifloacinTablet 100 mg excipient mixture) in the range of 50% to 150% of the sample concentration. Report the percentage recovery in relative standard deviation for all the values of % recovery.

## Blank preparation

Use diluent as blank.

## Standard preparation

Weighed accurately about 0.100g of Moxifloacin working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

## 50% recovery solution preparation

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask containing 50 mg of Moxifloacin add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45 membrane filter. Diluted the above solution as 10ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.

## 100% recovery solution preparation

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask containing 100 mg of Moxifloacin add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45µ membrane filter. Dilute the above solution as 10ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.

## 150% recovery solution preparation

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask containing 150 mg of Moxifloacin add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45 µ membrane filter. Dilute the above solution as 10ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.

## Procedure

Separately inject 20µl of standard and sample preparations of recovery solutions into the chromatograph and measure the peak responses for the major peak. Calculate the % recovery in recovery solutions using the following expression.

## Recovery Calculation

% of recovery = mg of Moxifloacin / working standard addedX100

## Stability of Analytical solutions

## Objective

To establish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 32hrs.

## Preparation of analytical solutions

**Blank preparation:** Use diluent as blank.

## Standard solution preparation

Weighed accurately about 0.100g of Moxifloacin working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipette out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

## Sample solution preparation

Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Moxifloacin) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

## Procedure

Inject 20µl of blank, resolution solution, standard preparation and sample preparations into the chromatograph and record the chromatograms. Measure the peak responses for major peak for all

solutions. Continue the chromatography with periodic injections in duplicate for standard and sample preparations in the interval of 4hrs or suitable interval depending on the instrument utilization and sequence of injections.

## Calculation

Calculate the average peak response and %RSD for initial 5 replicate injections of standard preparations. Calculate the %RSD for average peak responses of standard and sample preparations for periodical intervals.

# RESULTS AND DISCUSSION

The assay values for Moxifloacin tablets 100mg obtained from six samples were found to be within the acceptance criteria. The RSD of assay values from 6 samples is not more than 2.0%. Therefore, the method is considered precise.

**Table no 1: Data for system suitability**

| | Injections | RT | Peak area | USP Plate | USP Tailing |
|---|---|---|---|---|---|
| 1 | | 1.81 | 375841.38 | 4005 | 1.12 |
| 2 | | 1.83 | 365756.27 | 4025 | 1.14 |
| 3 | | 1.81 | 366748.63 | 4054 | 1.15 |
| 4 | | 1.83 | 364785.35 | 4056 | 1.16 |
| 5 | | 1.85 | 372726.96 | 4012 | 1.13 |
| 6 | | 1.82 | 369765.32 | 4089 | 1.12 |
| | **Mean** | **1.825** | **369270.652** | **4040.167** | **1.137** |
| | **Std deviation** | **0.015** | **4340.383** | **31.896** | **0.016** |
| | **% RSD** | **0.831** | **1.175** | **0.789** | **1.437** |

The % RSD of all the parameters like retention time, area, theoretical plates and tailing factor was within the limit. So the method passes these system suitability parameters.

**Table no 2: Data for System Precision**

| | Injections | Retention | Peak Area |
|---|---|---|---|
| 1 | | 1.79 | 365725.36 |
| 2 | | 1.82 | 368558.69 |
| 3 | | 1.83 | 375485.65 |
| 4 | | 1.81 | 372754.21 |
| 5 | | 1.82 | 375675.24 |
| | **Mean** | **1.814** | **371639.830** |
| | **Std deviation** | **0.015** | **4381.526** |
| | **% RSD** | **0.836** | **1.179** |

The % RSD of five replicate injections of standard solution is within the specified acceptance criteria.

**Table no 3: Data for Method Precision**

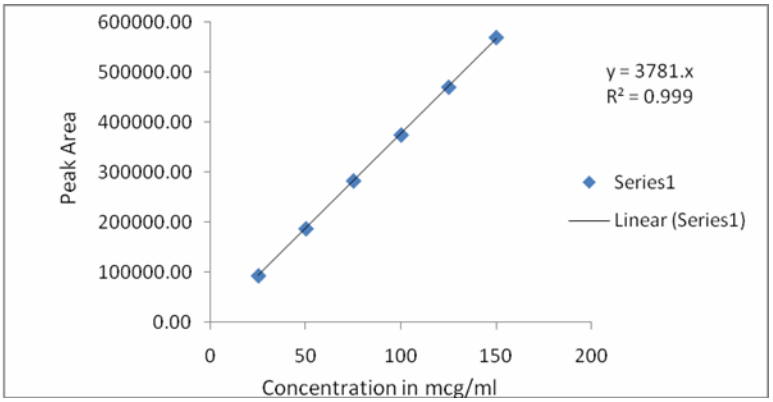
| Samples | Peak Area | Weight of sample | Assay | |
|---|---|---|---|---|
| | | | **in mg** | **in %** |
| 1 | 375656.25 | 300.25 | 99.99 | 99.99 |
| 2 | 382745.68 | 300.25 | 101.88 | 101.88 |
| 3 | 372414.96 | 300.56 | 99.13 | 99.13 |
| 4 | 382985.69 | 300.48 | 101.94 | 101.94 |
| 5 | 372774.46 | 300.95 | 99.22 | 99.22 |
| 6 | 372762.24 | 300.56 | 99.22 | 99.22 |
| | **Mean** | | **100.229** | **100.229** |
| | **Std deviation** | | **1.338** | **1.338** |
| | **% RSD** | | **1.335** | **1.335** |

**Table no 4: Data for Intermediate Precision**

| Day | Sample Inj | Peak Area | Weight Of Sample | Assay | |
|---|---|---|---|---|---|
| | | | | **in mg** | in % |
| 1 | 1 | 375754.26 | 300.25 | 100.02 | 100.02 |
| | 2 | 372785.65 | 300.65 | 99.23 | 99.23 |
| 2 | 3 | 382749.38 | 300.48 | 101.88 | 101.88 |
| | 4 | 376715.34 | 300.95 | 100.27 | 100.27 |
| 3 | 5 | 373784.59 | 300.48 | 99.49 | 99.49 |
| | 6 | 372765.18 | 300.48 | 99.22 | 99.22 |
| Mean | | | | 100.017 | 100.017 |
| Std deviation | | | | 1.006 | 1.006 |
| % RSD | | | | 1.006 | 1.006 |

**Table no 5: Linearity study for Moxifloxacin**

| Sample no | %level | concentration (µg/ml) | area |
|---|---|---|---|
| 1 | 25 | 12.5 | 94182.59 |
| 2 | 50 | 25.0 | 188105.18 |
| 3 | 75 | 37.5 | 283874.76 |
| 4 | 100 | 50.0 | 375694.35 |
| 5 | 125 | 62.5 | 471130.94 |
| 6 | 150 | 75.0 | 570373.53 |




**Linearity Curve of Moxifloxacin**

**Table No 6: Method accuracy study of Moxifloxacin**

| Sample No | Theoretical (%) | Mean Peak area | Recovery | | Mean (%) Recovery | %RSD |
|---|---|---|---|---|---|---|
| | | | In (mg) | In (%) | | |
| 1 | 50 | 188638.175 | 50.21 | 100.42 | | |
| 2 | 50 | 191452.675 | 50.96 | 101.92 | 100.369 | 1.571 |
| 3 | 50 | 185530.62 | 49.38 | 98.77 | | |
| 1 | 100 | 377164.35 | 100.39 | 100.39 | | |
| 2 | 100 | 372769.35 | 99.22 | 99.22 | 99.460 | 0.843 |
| 3 | 100 | 371061.24 | 98.77 | 98.77 | | |
| 1 | 150 | 566461.525 | 150.78 | 100.52 | | |
| 2 | 150 | 568809.025 | 151.40 | 100.93 | 99.856 | 1.525 |
| 3 | 150 | 552911.86 | 147.17 | 98.11 | | |

**Table No 7: Data for LOD and LOQ**

| Sample no | %Level | Concentration (µg/ml) | Area |
|---|---|---|---|
| 1 | 25 | 12.5 | 94182.59 |
| 2 | 50 | 25.0 | 188105.18 |
| 3 | 75 | 37.5 | 283874.76 |
| 4 | 100 | 50.0 | 375694.35 |
| 5 | 125 | 62.5 | 471130.94 |
| 6 | 150 | 75.0 | 570373.53 |
| | Slope | | 3796.40 |
| | Standard Deviation | | 46.77 |
| | Correlation co-efficient | | 0.99995 |
| | LOD | | 2.68 µg/ml |
| | LOQ | | 4.46 µg/ml |

**Table No 8: Data for standard solution stability**

| S. No. | Time In Hours | RT | Peak Area |
|---|---|---|---|
| 1 | 0 | 1.83 | 383364.25 |
| 2 | 4 | 1.85 | 374568.36 |
| 3 | 8 | 1.81 | 372715.56 |
| 4 | 12 | 1.81 | 366256.35 |

| | | | |
|---|---|---|---|
| 5 | 16 | 1.83 | 375568.25 |
| | **Mean** | **2.94** | **2.398** |
| | **Std deviation** | **0.05** | **0.034** |
| | **% RSD** | **1.76** | **1.426** |

The RSD of obtained standard area is not more than 2.0%. Therefore, the solution is considered stable.

**Table no 9: Data for sample solution stability**

| S. no. | Time In Hours | RT | Peak Area |
|---|---|---|---|
| 1 | 0 | 1.83 | 362769.26 |
| 2 | 4 | 1.83 | 374154.35 |
| 3 | 8 | 1.82 | 371745.25 |
| 4 | 12 | 1.83 | 377755.65 |
| 5 | 16 | 1.85 | 372749.78 |
| | Mean | 2.84 | 2.256 |
| | Std deviation | 0.27 | 0.032 |
| | % RSD | 9.66 | 1.423 |

The RSD of obtained sample area is not more than 2.0%. Therefore, the solution is considered stable.

**Table no 10: Data of validation parameters for Moxifloxacin**

| PARAMETERS | MOXIFLOXACIN |
|---|---|
| Specificity | No interference between blank, standard and sample peak |
| **System Suitability** | |
| Retention time | 1.825 |
| Peak Area | 369270.652 |
| Theoretical Plates | 4040.167 |
| Tailing Factor | 1.137 |
| **Precision** | |
| System precision (% RSD) | 1.179% |
| Method precision (% RSD) | 1.335% |
| Intermediate Precision (% RSD) | 1.006% |
| **Linearity and Range** Slope | 12.5 µg/ml to 75.0 µg/ml |
| Standard deviation Correlation co-efficient | 3796.40<br>46.7707<br>0.99995 |
| **% Recovery** | |
| 50% | 100.369% |
| 100% | 99.460% |
| 150% | 99.856% |
| LOD | 2.68 µg/ml |
| LOQ | 4.46 µg/ml |
| **Solution Stability** | |
| Standard (% RSD) | 0.916% |
| Sample (% RSD) | 0.598% |

The assay values for Moxifloacin tablets 100mg obtained from six samples were found to be within the acceptance criteria. The RSD of assay values from 6 samples is not more than 2.0%. Therefore, the

method is considered precise. The assay values for the range of recovery levels from 50%-150% of the Moxifloacin working concentration (25micron/ml) conform to the acceptance criteria. The percentage Moxifloacin recovered at each of the levels falls between 99.460%- 100.369% and the %RSD of all determinations at each level was not more than 2.0% .therefore the method is considered accurate.

The LOD and LOQ of the Moxifloacin was calculated from the following formula,

**LOD = 3.3σ / S**

Where the σ = the standard deviation of the response,

S = the slope of the calibration curve

**LOQ = 10 σ / S**

Where the σ = the standard deviation of the response,

S = the slope of the calibration curve

## SUMMARY AND CONCLUSION

The validated method was to quantitatively estimate the amount of Moxifloacin in Pharmaceutical tablet dosage form using HPLC method. The calibration curve for Moxifloacin was found to be linear in the range of 12.5µg/ml to 75µg/ml (r2=0.99995) indicating a good linearity. The percentage recovery of sample was found to 99.460 to 100.369%w/w for Moxifloacin indicating the good accuracy of the method. To evaluate the validity and reproducibility of the method, known amount of pure drug was added to previously analysed samples and these samples were reanalysed by proposed method, the percentage recovery was found to be close to 100% for all the methods. The limit of detection and limit of quantification was done by using linearity data, slope and standard deviation of the linearity samples were found to 2.68µg/ml and 4.46µg/ml respectively. The % relative standard deviation (%RSD) values for system precision was 1.179%, method precision was 1.335% and Intermediate precision was 1.006. The system precision and method precision were found to be less than 2% and so the method is said to be precise. The developed RP-HPLC method is simple and selective for estimation of Moxifloacinin tablet dosage form was found to be accurate, rapid and sensitive. The values of coefficient of variance were satisfactory low and recovery was close to 100% indicating reproducibility of the method. The linearity was observed within limit hence method is linear.

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