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# NEW ANALYTICAL METHODS FOR THE QUANTITATIVE ESTIMATION OF MOXIFLOXACIN AN ANTIBACTERIAL DRUG IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

**C. Gowri, G. Dharmamoorthy, Dr. P. Jayachandra Reddy**

Department of Pharmaceutical Analysis and Quality Assurance,
Krishna Teja Pharmacy College, Tirurpati – 517506, Andhra Pradesh, India.

*Corresponding Author E-mail: gowripulicherla@gmail.com

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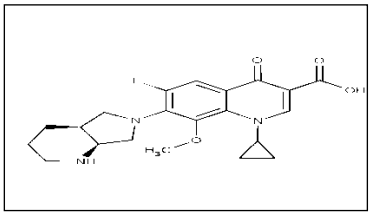
**ABSTRACT**

**Objective;** In the present investigation a rapid specific, precise and simple Reverse phase High performance liquid chromatography (RP-HPLC) method was developed and validated for Moxifloxacin in bulk and pharmaceutical dosage forms. **Materials and methods ;** The present RP-HPLC method utilized Analytical weighing balance (Sartorius), HPLC system (Agilent technologies 1200 series), Luna C18, 250X4.6mm, 5**µm column, in isocratic** mode, using mobile phase composition of Buffer (PH 2.5 with Triethylamine and Orthophosphoric acid): Methanol [55:45 (v/v)] with flow rate of 1.0 ml/min. with U.V detection of 293nm. **Results and Discussion;** The retention time of Moxifloxacin was 5.85 min. The total RP-HPLC run time was 14 min. System suitability parameters proved that the proposed method is suitable for estimation of Moxifloxacin HCl. The method was found to be satisfactory on the drug peak was found to be symmetrical as found from Asymmetry factor of 1.2 for Moxifloxacin HCl respectively. This method is validated for System suitability, specificity, precision (Repeatability,Intermediate precision), Accuracy, Linearity, Ruggedness, Robustness etc., **Conclusion;** The method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Moxifloxacin HCl in pharmaceutical formulation

## INTRODUCTION

## MOXIFLOXACIN



**Chemical formula** : $C_{21}H_{24}FN_3O_4$
**Molecular Weight** : 437.9

**IUPAC Name** : 1-cyclopropyl-7-( (s,s)-2,8-diazabicyclo(4.3.0)non-8-yl)-6-Fluoro-8-methoxy-1,4-dihydro-4-oxo-3quinoline carboxylic acid.
**Category** : Antibiotic
**Description** : Yellow to yellow crystalline substance.
**Storage** : Store in a cool and dry place protected from light and moisture.
**Solubility** : Sparingly soluble in water and practically insoluble in Acetone.

**Therapeutic Uses**[19]: Moxifloxacin can be used to treat respiratory infections, including acutesinusitis,acute exacerbations of chronic bronchitis, and community- acquired pneumonia, as well as skin and skin structure infections. Moxifloxacin is used as a second-line agent in tuberculosis (TB) and may potentially have benefits in reducing treatment duration from its current six month to four months.In ophthalmology, Moxifloxacin is available in the form of eye drops,to treat conjunctival infections caused by susceptible bacteria and to prevent infection following eye surgeries.

**Equipments and apparatus Used:** Different kinds of equipments viz Analytical weighing balance (Sartorius), HPLC system (Agilent technologies 1200 series), Column, Sonicator (Fast clean), pH meter (Poloman), Vacuum filter pump (model XI 5522050 of Millipore), Millipore filtration kit, mobile phase reseriover, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

**Collection of solvents:** HPLC grade Acetonitrile, Methanol, Orthophosphoric acid, Triethyl amine, Purified was used as solvent throughout the experiment.

**Reagents and Pharmaceutical Preparations:** Moxifloxacin was kindly supplied by MSN laboratories (Hyderabad, A.P, India). All the solvents used in HPLC method are of HPLC grade. Commercial pharmaceutical preparations of Moxifloxacin from Torrent Pharma (Mumbai, India) which were claimed to contain 400 mg of Moxifloxacin as, used in analysis

## RP-HPLC METHOD FOR THE ESTIMATION OF MOXIFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Only very few HPLC estimations have been reported in the literature for the determination of Moxifloxacin present in biological fluids. There are no reported pharmaceutical dosage forms. Hence an attempt has made to develop a HPLC method for the determination of Moxifloxacin in bulk and pharmaceutical dosage forms.

**METHOD DEVELOPMENT:** The objective of this experiment was to optimize the assay method for estimation of Moxifloxacin based on the literature survey made. So here the trials mentioned describes how the optimization was done. An HPLC method is developed and validated for various parameters as per ICH guidelines. The system suitability parameters proved that the proposed method is suitable for estimation of Moxifloxacin HCl. The method was found to be satisfactory on Luna $C_{18}$, 250X4.6mm, 5 µm column, in isocratic mode, using mobile phase composition of Buffer ($P^H$ 2.5 with Triethylamine and Orthophosphoric acid): Methanol [55:45 (v/v)] with flow rate of 1.0 ml/min. The drug peak was found to be symmetrical as found from Asymmetry factor of 1.2 for Moxifloxacin HCl respectively.

### Trial: 1

**Buffer preparation:** Transfer 7 ml of Triethylamine into 1000 ml of Water and adjust the pH to 3.2 with Orthophosphoric acid, filter through 0.45µm nylon membrane filter and degas.

**Mobile phase: Buffer and Methanol were mixed in the ratio of 80:20 and sonicated to degas.**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Flow rate** | **:** | 1.0 ml/min |
| **Column** | **:** | Micropack |
| **Detector wavelength** | **:** | 293nm |
| **Column temperature** | **:** | Ambient |
| **Injection volume** | **:** | 10µl |
| **Run time** | **:** | **60 min** |

**Chromatogram for Trail 1**

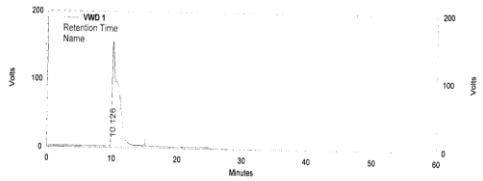



**Fig: 2**

**Observation: Theoretical plates are very less and Asymmetry is more than limit.**

### Trial: 2

**Buffer preparation:** Transfer 7 ml of Triethylamine into 1000 ml of water and

adjust the pH to 3.2 with orthophosphoric acid, filter through 0.45µm nylon membrane filter and degas.

**Mobile phase: Buffer and Methanol were mixed in the ratio of 50:50 and sonicated to degas.**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Flow rate** | **:** | 1.0 ml/min |
| **Column** | **:** | Eclipse |
| **Detector wave length** | **:** | **293nm** |
| **Column temperature** | **:** | Ambient |
| **Injection volume** | **:** | 10µl |
| **Run time** | **:** | **60 mins** |

**Chromatogram for Trail 2**

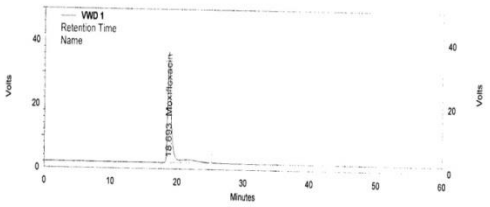



**Fig: 3**

**Observation:** The retention time is more, peak tailing and Asymmetry is high.

**Trial: 3**

**Buffer preparation:** Transfer 2 ml of orthophosphoric acid into 1000 ml of water and adjust the pH to 2.5 with triethylamine, filter through 0.45µm nylon membrane filter and degas.

**Mobile phase:** Buffer and methanol were mixed in the ratio of 70:30 and sonicated to degas.

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Flow rate** | **:** | 1.0 ml/min |
| **Column** | **:** | Luna C18, 250 x 4.6 mm, 5µ |
| **Detector wave length** | **:** | **293nm** |
| **Column temperature** | **:** | Ambient |
| **Injection volume** | **:** | 10µl |
| **Run time** | **:** | **30 mins** |
| **Diluent** | : | Buffer: Methanol (80:20) |

**Chromatogram for Trail 3**

**Observation:** Retention time is more.

**OPTIMIZED METHOD:**

**Buffer preparation:** Transfer 2 ml of orthophosphoric acid into 1000 ml of water and adjust the pH to 2.5 with triethylamine, filter through 0.45µm nylon membrane filter and degas.

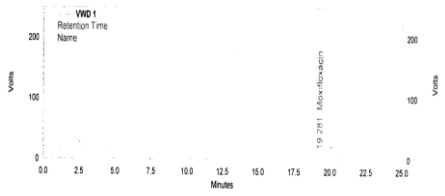



**Fig: 4**

**Mobile phase: Buffer and methanol were mixed in the ratio of 55:45 and sonicated to degas.**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Flow rate** | **:** | 1.0 ml/min |
| **Column** | **:** | Luna C18, 250 x 4.6 mm,5µ |
| **Detector wave length** | **:** | **293nm** |
| **Column temperature** | **:** | **Ambient** |
| **Injection volume** | **:** | 10µl |
| **Run time** | **:** | **15 mins** |
| **Diluent** | **:** | Buffer: Methanol (55:45) |

**Preparation of Solutions:**

**Standard Preparation:** Weigh and transfer accurately Moxifloxacin HCl equivalent to 20 mg of Moxifloxacin Working Standard into a 50 ml clean dry volumetric flask, add about 30 ml of mobile phase, sonicate for 5 minutes, and dilute to volume with mobile phase. Further dilute 5 ml to 50 ml with mobile phase.

**Sample preparation:** Weigh 20 tablets, and powder them. Transfer the powder equivalent to 400 mg of moxifloxacin into 100 ml of clean, dry, volumetric flask and, add 70 ml of mobile phase and sonicate to dissolve for about 15 minutes further make up the volume with mobile phase. Filter through 0.45 micron filter. Further dilute 1 ml of the filtrate to 100 ml with mobile phase.

**Procedure:** Separately inject 10µl of the blank, Standard and sample solution in duplicate into the HPLC system, record the chromatographs and measure the peak areas.

**Calculation**: Calculate the amount of drug by using the following formula

Assay = Spl Area x Std Wt x 5 x 100 x 100 x AvgWt x 401.4 x 100-$H_20$ x Std purity/ Std Area x 50 x 50 x Spl.Wt x 1 x 400 x 437.9x 100

**METHOD VALIDATION:**

**SYSTEM SUITABILITY:** A Standard solution was prepared by using Moxifloxacin working standards as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD of retention times and peak areas from five replicate injections

**ACCEPTANCE CRITERIA:**

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Moxifloxacin peaks is NLT 3000.
4. The Tailing factor (T) for the Moxifloxacin peaks is NMT 2.0.
5. From the system suitability studies it is observed that all the parameters are within limit, hence it is concluded that the Instrument, Reagents and Column are suitable to perform Assay. These results are expressed in Table: 8

**SPECIFICITY:**

**A) *Moxifloxacin Identification*:** Solutions of standard and Sample are prepared as per test method and injected into the chromatographic system.

**ACCEPTANCE CRITERIA:** Chromatogram of Standard and sample should be identical with near Retention time. The chromatograms of Standard and Sample are identical with nearly same Retention time, hence it is concluded that the standard and sample are same.

*B) Placebo interference:* A study to establish the interference of placebo was conducted. Samples were prepared by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and was injected into the HPLC system..

**ACCEPTANCE CRITERIA:** Chromatogram of placebo should not show any peak at the retention time of analyte peak

***C) Blank interference:***

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method.

**ACCEPTANCE CRITERIA:**

Chromatogram of blank should not show any peak at the retention time of analyte peak.

There is no interference at the due to Placebo and Sample at the retention time of Analyte, hence the method is specific.

**3. PRECISION:**

***Repeatability***: Six Sample solutions are prepared as per test method and injected as per test procedure.

**ACCEPTANCE CRITERIA:**

The all-individual assays of Moxifloxacin Hcl tablets should be within 98% to 102% Relative standard deviation of % Assay results should not more than 2.0%

***b) Intermediate precision (Analyst to Analyst variability)****:*

Two analysts as per test method conducted a study

**ACCEPTANCE CRITERIA:**

The all-individual assays of Moxifloxacin Hcl tablets should be within 98%-102%. Relative standard deviation of % assay results should not more than 2.0% by both the analysts.

For **Analyst-1** Refer Precision (Repeatability)

**4. ACCURACY (RECOVERY):**

Assay was performed in triplicate as per test method for various concentrations of Moxifloxacin equivalent to 50%, 75%, 100%, 125% and 150% of the labeled amount as per the test method. The average % recovery of Moxifloxacin was calculated.

**ACCEPTANCE CRITERIA:** The mean % recovery of the Moxifloxacin at each spike level should be not less than 98.0% and not more than 102.0%.

Amount Added = Std wt x Std diln x 401.4x 99.5 x 1000/50 x 50 x 437.9 x 100

Amount found = Amount found Area x Amount found factor/ Avg Standard Area

% Recovery = Amount found/ Amount Added x 100.

**Standard Chromatogram for Optimized method**

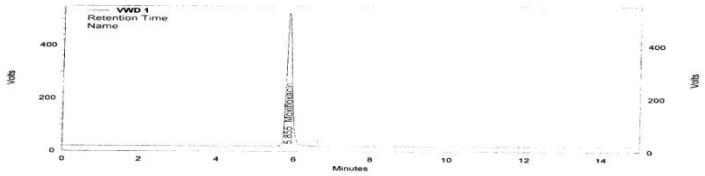



**Fig: 5**
**Sample Chromatogram for Optimized method**

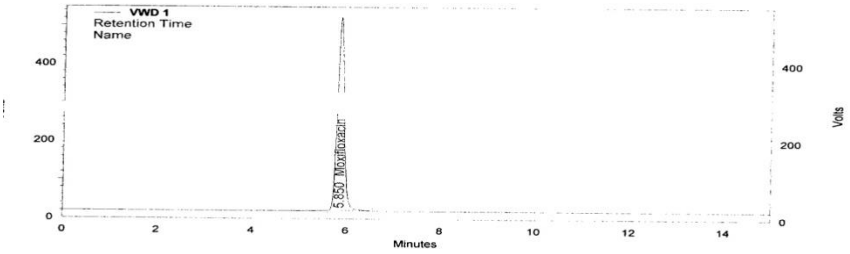



**Fig: 6- Blank Chromatogram for Optimized method**

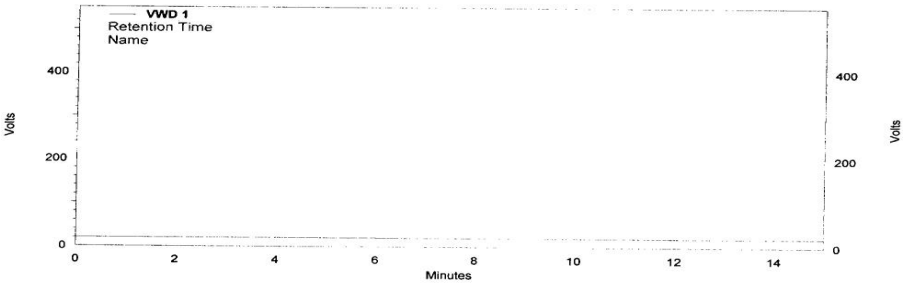



**Fig: 7 - Standard Chromatogram for System suitability**

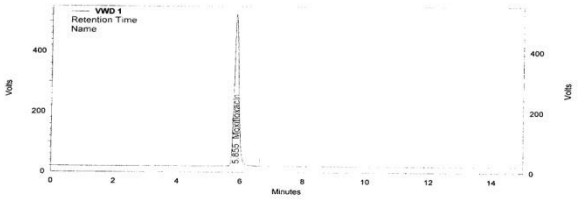



**Fig: 8- Standard Chromatogram for Moxifloxacin Identification**

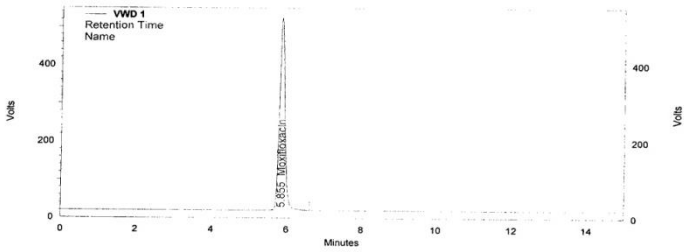



**Fig: 9- Sample Chromatogram for Moxifloxacin Identification**

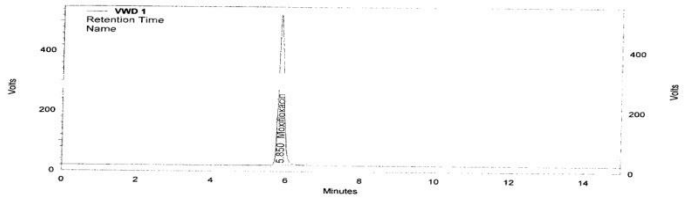



**Fig: 10- Chromatogram of Placebo interference**

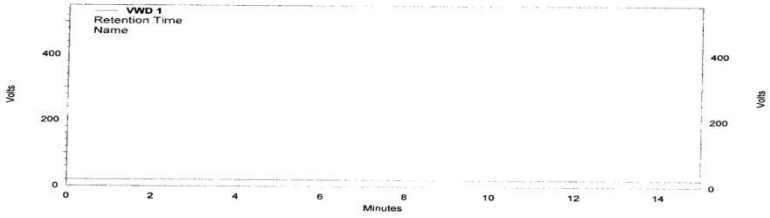



**Fig: 11- Chromatogram of Blank interference**

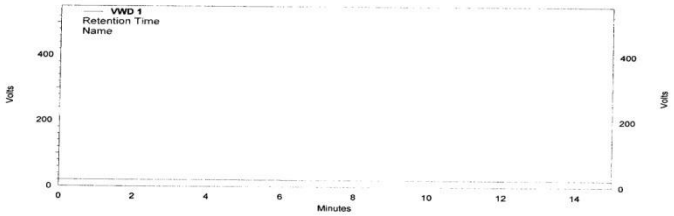



**Fig: 12- Standard Chromatogram of Repeatability**

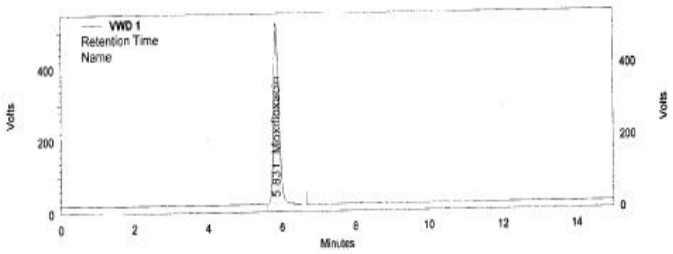



**Fig: 13 - Sample Chromatograms of Repeatability**

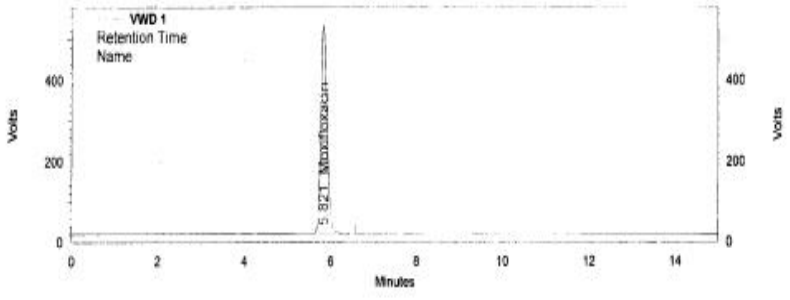



**Fig: 14 -** Standard Chromatogram for intermediate Precision

**(Analyst-to-Analyst variability)**

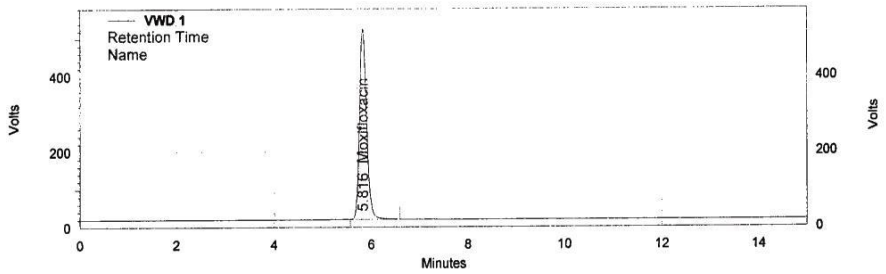



**Fig: 15**
Sample Chromatogram for intermediate Precision
**(Analyst-to-Analyst variability)**

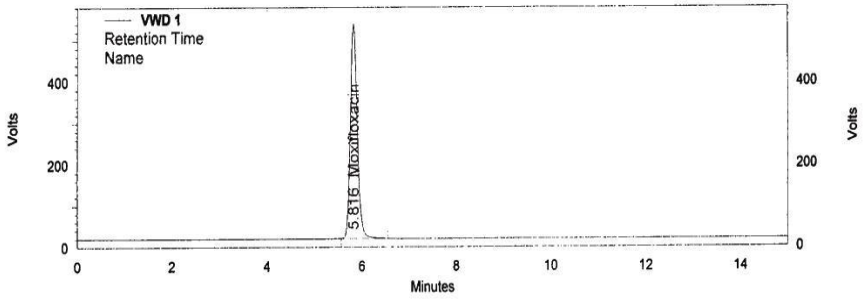



**Fig: 16**

## Standard Chromatogram of Accuracy

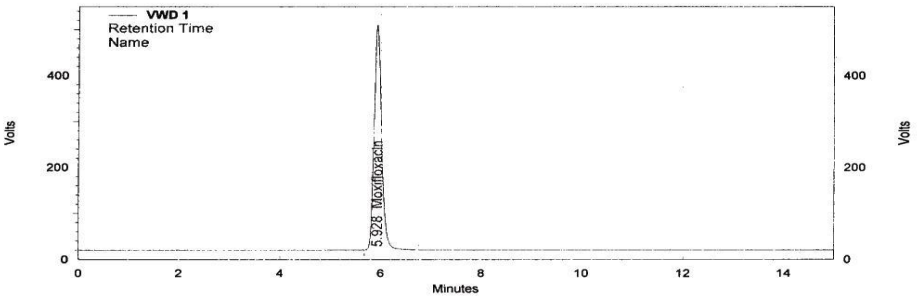



**Fig: 17**
Chromatogram of Accuracy 50% Conc.

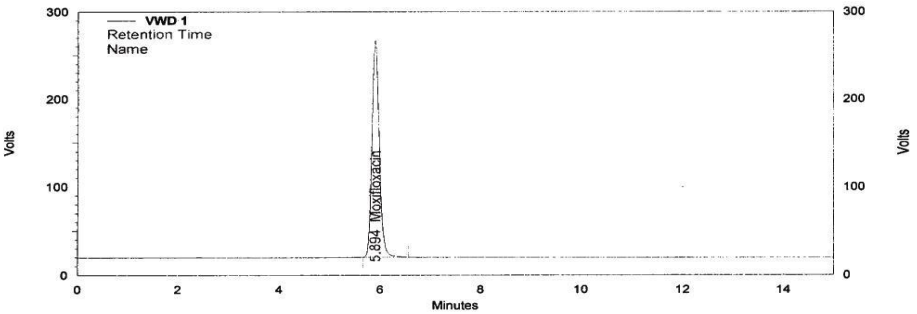



**Fig: 18**
Chromatogram of Accuracy 75% Conc.

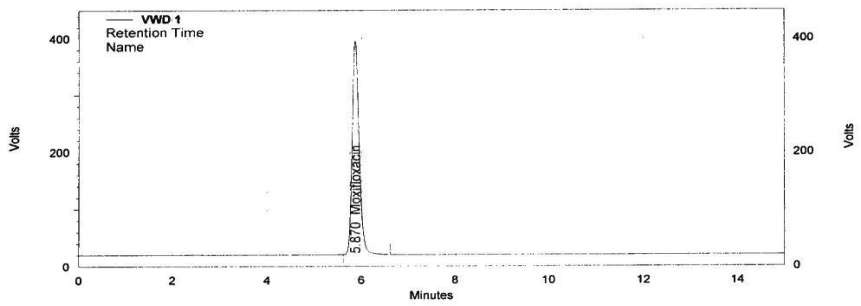



Fig: 19

**Chromatogram of Accuracy 100% Conc.**

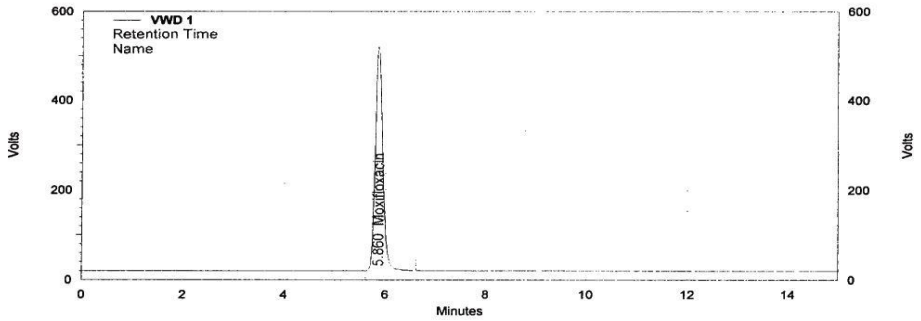



**Fig: 20- Chromatogram of Accuracy 125% Conc.**

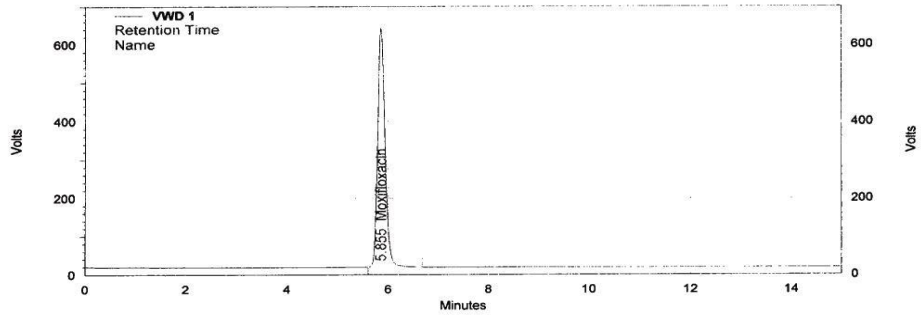



**Fig: 21- Chromatogram of Accuracy 150% Conc.**

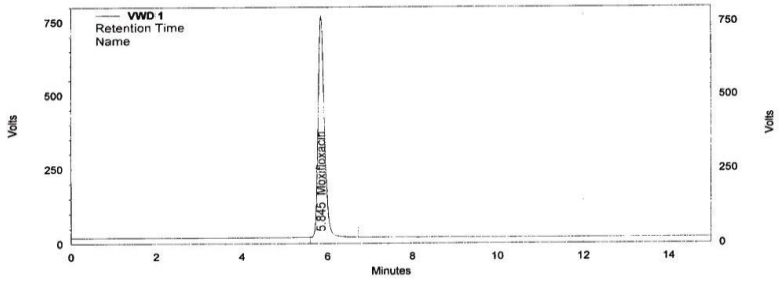



**Fig: 22**

**5. LINEARITY OF TEST METHOD:**

A Series of solutions are prepared using Moxifloxacin working standard at concentration levels from 20µg/ml to 60µg/ml (20, 24, 32, 40, 48, 56, 60) and injected as per test procedure (20µg/ml & 60µg/ml) are injected six times and the remaining are two times. Plot a graph to concentration versus peak area.

**ACCEPTANCE CRITERIA:**

Correlation Coefficient should be not less than 0.9990.

% Of RSD for Solution 1 and Solution 7 should be not more than 2.0%. Correlation coefficient = 1000

**7. ROBUSTNESS:**

***a) Effect of variation of flow rate:***

A study was conducted to determine the effect of variation in flow rate. Standard solution is prepared as per the test method was injected into the HPLC system by keeping flow rates, 0.8ml/min, 1ml/min and 1.2ml/min. Evaluate the effect of variation of flow rate for 0.8ml/min, 1 ml/min and 1.2ml/min flow.

**ACCEPTANCE CRITERIA:**

The Tailing Factor of Moxifloxacin standards should be NMT 2.0 for Variation in flow.

The % RSD of Area and Asymmetry of Moxifloxacin standards should be NMT 2.0 for variation in flow.

***b) Effect of variation of temperature:***

A study was conducted to determine the effect of variation in temperature. Standard solution is prepared as per the test method and was injected into the HPLC system at 20ºC, 25ºC at 30ºC temperature. The system suitability parameters were evaluated at 20ºC, 25ºC and 30ºc.

**ACCEPTANCE CRITERIA:**

The Tailing Factor of Moxifloxacin standard should be NMT 2.0 for Variation in temperature.The % RSD of Moxifloxacin standards should be NMT 2.0 for Variation in temperature.

**Discussion:**

1. **System suitability:** From the system suitability studies it is observed that % RSD of retention time was found to be 0.06, % RSD of peak area was found to be 0.28.Theoritical plates were found to be more than 7500.USP tailing factor was found to be 1.2.All the parameters are with in the limit. The results of system suitability studies are expressed in Table:
2. **Specificity:** The chromatograms of Standard and Sample are identical with nearly same Retention time, blank & placebo should not show any peak at the retention time of analyte peak. No interference between Placebo and Sample at the retention time of analyte shows that the method is specific. The results of specificity studies are expressed in Fig: 9, 10, and 11
3. **Precision:** The %RSD of assay results was found to be 0.58.The assay of Moxifloxacin Hydrochloride tablets was found to be 100%.All individual five assays and % RSD of assay are within limit; hence the method passes Repeatability test. The results of Precision are expressed in Table: 9,10.%RSD of assay results was found to be 0.29%, the assay of Moxifloxacin hydrochloride tablets was found to be 100.1.All individual % Assays and %RSD of assay are within limit; it passes Intermediate precision. The results of Precision are expressed in Table: 11, 12
4. **Accuracy:** The recoveries of pure drug from the analyzed solution of formulation were 99.3% to 100.2%, which shows that the method is accurate. The summary of Accuracy results are expressed in Table: 19
5. **Linearity:** From the Linearity data it is observed that the method is showing linearity in the concentration range of 20-60 µg/ml.coefficient of correlation was found to be 1.000. The data of linearity was expressed in Table: 22
6. **Ruggedness:** Comparison of both the results obtained on two different systems, shows that the assay test method are rugged for system-to-system variability. The results of Ruggedness are expressed in Table: 24. Comparison of both the results obtained on two different Columns, shows that the assay test method are rugged for Column-to-Column variability. The results of Ruggedness are expressed in Table: 26
7. **Robustness:** As the %RSD 0f Tailing factor and Peak area are with in limits for variation in flow rate i.e.0.8 ml to 1.2 ml, hence the allowable variation in flow rate is 0.8 ml to 1.2 ml. The results of robustness for effect of variation of floe rate are expressed in Table: 27. As the %RSD 0f Tailing

factor and Peak area are within limits for variation in Temperature i.e. $20^{O}C$ to $30^{O}C$, hence the allowable variation in flow rate is $20^{O}C$ to $30^{O}C$. The results of robustness for effect of variation in temperature are expressed in Table: 28. Validation summary results are expressed in Table: 29

## CONCLUSION

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The run time of the HPLC procedure is only 15 minutes. The method was validated for system suitability, specificity, precision, accuracy, linearity, ruggedness and robustness. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. As there was no interference due to excepeints and mobile phase, the method was found to be specific. The percentage recovery of Moxifloxacin was found to be in the range of 99.3%-102%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Temperature separately and analysis being performed by different analysts, on different systems and by using different columns respectively.

Good agreement was seen in the assay results of pharmaceutical formulation by developed method. We concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Moxifloxacin HCl in pharmaceutical formulation.

**Acknowledgement:** The author expresses sincere thanks to Spectrum research solution, Hyderabad to provide gift samples of Moxifloxacin HCl drug sample.

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