# Development and Validation of RP-HPLC Method for the Determination of Moxifloxacin in Presence of Its Degradation Products

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**Abstract:** A simple, specific and isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method with UV detection at 294 nm was developed and validated for analysis of Moxifloxacin Hydrochloride (MOXI) in presence of its degradation products. Retention time of the MOXI was found to be 7.8 min. A mobile phase consisting of 10mM sodium phosphate buffer and methanol (60:40 v/v) pH 4.4 at flow rate of 1mL/min was employed in this study. The calibration curves were linear with regression coefficient (r2) of 0.999. The method was validated in accordance with International conference on harmonization (ICH) guidelines. The proposed method was found sensitive, specific and was successfully applied for the estimation of MOXI in pharmaceutical formulations (eye drop).

**Key words:** RP-HPLC · Moxifloxacin Hydrochloride · ICH Guidelines

## INTRODUCTION

Fluoroquinolones (FQs) are among the most important antibacterial agents used in human medicine. They are active against both Gram-positive and Gram-negative bacteria through inhibition of their DNA gyrase [1] and also possess some activity against mycobacteria, mycoplasmas and rickettsias.

Moxifloxacin hydrochloride (MOXI) chemically is 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*) octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4dihydroquinoline-3-carboxylic acid (Fig. 1) [1] it is an new-generation, 8-methoxyquinolone derivative of fluoroquinolone antibacterial agent. Moxifloxacin have an azabicyclo-substitution at C-7, which is responsible for improved Gram-positive activity. Moxifloxacin is active against broad spectrum of pathogens, encompassing Gram-negative, Gram-positive bacteria [2,3] including resistant strain *Streptococcus pneumonia*. It is available for oral and parenteral administration which is specially used to treat respiratory and skin infection [3,4-9].

A wide range of analytical methods have been reported for the determination of moxifloxacin such, microbiological assay [10], electroanalytical [11,12] protonation equilibrium [13], differential pulse polarographic [14] UV spectrophotometry,
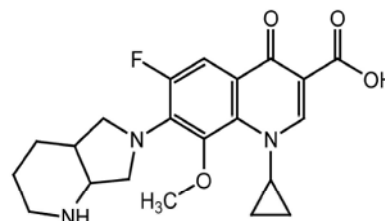


Fig. 1: Molecular structure of moxifloxacin.

HPTLC [15] HPLC involved with flouroscence detector [16-19]. Also methods are reported for determination in plasma [19, 21] and lung tissues [20]. Reported analytical methods though are sensitive but suffer from disadvantage like expensive with regards to experimental set up and sometimes involved with sample dervetzation steps as in flourimetric detection making the procedure even more complicated.

Most reported methods involve troublesome mobilephases (buffers) and difficult detection methods (fluorescence or mass detection). There are no published LC methods for analysis of MOXI in bulk samples in the presence of its degradation products and process-related impurities. This research was conducted to develop a suitable HPLC-UV methodfor analysis of moxifloxacin and its degradation products. The choice of analytical method selected was HPLC-UV due its established advantages

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such as speed of analysis and applications in routine quality control [22,23]. The method was validated for specificity, linearity, precision,accuracy and robustness to show its stability-indicating power and to ensure compliance with ICH guidelines [24].

**Experimental**

**Material:** Moxifloxacin working standard powder was provided by Hetero Drugs, Hyderabad India and was used without further purification. Mosi® eye-drop (manufactured by FDC) containing 5mg/ml MOXI as per labeled claim were obtained from local market. Water and methanol(E-Merck ltd) used were HPLC grade and were purchased from E-Merck Mumbai India, sodium hydroxide, Ortho phosphoric acid ($H_3PO_4$), Hydrochloric acid, 30% Hydrogen peroxide with analytical reagent grade or better, belonged to Sigma Aldrich Chemical Co. Mumbai, India. All chemicals were at least of analytical grade and used as received.

**Standard Preparation:** Stock solution of MOXI was prepared. An accurately weighed quantity of MOXI (50 mg) was dissolved in methanol and volume was made upto 50 mL (1000 μg/mL). Working standard solution of MOXI was prepared by withdrawing an aliquot portion of stock solution of MOXI and diluting appropriately with methanol to get concentration of 100 μg/mL.

**Method Development:** Various solvent systems were tried for the development of suitable HPLC method for the analysis of MOXI. The suitability of the solvent system was decided on the basis of separation of drug completely from the degradation products. In the process of method development it was found that the solvent system comprising of 0.1 M sodium phosphate buffer and methanol (60:40 v/v) pH 4.4 at flow rate of 1mL/min and sample in methanol gave sharp peak at a good retention time of 7.9 min. shown in Fig.1 and hence this was selected as a solvent system for further studies.

**Instrumentation and Conditions:** The HPLC system consisted of Thermo separation products (TSP) system equipped with quaternary pump system P4000 having a membrane degasser equipped with a Spectra System UV 1000 detector, manual rheodyne injection system, The software was an Data ace software version 6.1. Chromatographic Data System was coupled to the detector via an Dataace module used to record and evaluate the data collected during chromatographic analysis. The chromatographic separation was performed

using Grace C$_{18}$column (250mm × 4.6 mm i.d., 5μm particle size) Separation was achieved using a mobile phase consisting of 10mM phosphate buffer: methanol in the ratio (60:40 v/v, pH 4.4 adjusted with o-phosphoric acid) at a flow rate of 1ml/min and UV detection at 294 nm. The column was maintained at ambient temperature with injection volume of 20 μl and the run time was 20 min. The mobile phase was filtered through 0.45 μm membrane filter and degassed in ultrasonic bath prior to use. A blank chromatogram was recorded before the studies (Fig. 1). Quantization of result was performed using peak area counts. For analysis of forced degradation samples same conditions were employed.

**Validation:** The method was validated with respect to parameters including system suitability, linearity, precision and accuracy. The specificity of the method was studied by checking the peak purity i.e presence of any hidden peaks this was done by changing the mobile phase components (Concentration of organic phase) [25].

**Linearity:** Aliquot portions of stock standard solution were diluted to 10.0 ml with mobile phase having concentrations 10 to 100μg/ ml. The chromatographic conditions were set as described earlier with a steady baseline. Standard solutions of different concentration were injected separately and the chromatograms were recorded. The calibration curve was constructed by plotting the peak area against the concentration showing excellent linearity.

**Precision:** Precision of analytical method was expressed as SD and %RSD of series of replicate measurements. Precision of estimation of MOXI by proposed method was ascertained by replicate analysis of homogeneous samples of eye-drop. Intra-day precision was assessed by injecting the 3 samples having concentration 50μg/ml standard drug on 3 different times. Inter day precision was assessed by injecting same three samples having same concentration on 3 different consecutive days. The ruggedness of the method was assessed by comparison of the intra-and inter-day assay results for MOXI undertaken by two analysts.

**Accuracy:** Accuracy of method was ascertained on the basis of recovery studies (Standard addition method) performed by standard addition method in eye drop formulation(Mosi FDC ltd). An accurately weighed quantity of formulation was taken in 10 ml volumetric flask to get a final concentration of 100 μg/ml; of each,

Table 1: Intraday and Inter day precision studies of Moxifloxacin

| Intra Day | | Amount Estimated (µg/ml) | Inter Day | | Amount Estimated (µg/ml) |
|---|---|---|---|---|---|
| 1 | T1 (50µg/ml) | 50.01 | 1 | D1 (50µg/ml) | 49.82 |
| 2 | T2 (50µg/ml) | 49.92 | 2 | D2 (50µg/ml) | 50.11 |
| 3 | T3 (50µg/ml) | 49.44 | 3 | D3 (50µg/ml) | 49.13 |
| | Mean | 49.79 | | Mean | 49.68 |
| | ±SD | 0.330 | | ±SD | 0.49 |
| | %RSD | 0.663 | | %RSD | 0.986 |

*T1, T2, T3 is Time1, 2 and 3 respectively. D1, D2, D3 is Day 1, 2, 3 respectively

Table 2: Results of recovery studies of Moxifloxacin

| | | | Mosi® FDC | | |
|---|---|---|---|---|---|
| SR. No | Volume of stock sol. of eye-drop (100 ug/ml) added in 10 ml (ml) | Amount of pure drug added (ug/ml) | Peak area of standard* | Peak area of sample* | % Recovery |
| 1 | 5 | 5 | 18024.636 | 19827.136 | 98.42 |
| 2 | 5 | 10 | | 21629.639 | 99.14 |
| 3 | 5 | 15 | | 23431.636 | 99.37 |
| 4 | 5 | 20 | | 25234.436 | 99.49 |
| 5 | 5 | 25 | | 27036.836 | 99.56 |
| *Results are mean of three replicates | | | | Mean | 99.196 |
| | | | | SD | 0.462 |
| | | | | %RSD | 0.413 |

to it standard solution of MOXI was added in different proportions (5 to 25 µg/ml) as given in Table 2. The resultant solution was diluted with methanol to get final concentration and filtered through 0.45µm membrane filter and subjected to the proposed HPLC method. The amount of drug contributed by the formulation was deduced from the total amount of respective drugs estimated and the resultant quantities were assumed to be recovered from the added pure drugs. The content of drug was calculated using following formula.

The % Recovery was then calculated by using formula

$$\%Recovery = \frac{A}{B+C} \times 100$$

Where-
A = Total amount of drug estimated.
B = Amount of drug found on preanalysed basis.
C = Amount of pure drug added.

**Solution Stability:** The solution stability was studied by recording the chromatograms of standard solution kept at room temperature over a period of 30hrs.

**Forced Degradation Studies of API and Specificity Studies:** In order to determine whether the analytical method and assay were stability-indicating, MOXIactive pharmaceutical ingredient (API) powder was stressed

underacidic (0.1 N HCl), basic (0.1 N NaOH), oxidizing (3% and 6% $H_2O_2$), neutral hydrolysis, temperature stress and photo stability stress conditions to conduct forced degradation studies [22]. As MOXI is soluble in methanol and so is solvent in all studies. All solutions of MOXI prepared for use in forced degradation studies were prepared in methanol. specificity of method was performed by changing the HPLC conditions as modifying the original HPLC method can change selectivity so as to resolve previously overlapping peaks this is evidence that all compounds have been resolved and the method is specific. For this purpose the stressed samples were analyzed by changing the concentration of organic phase for presence of any hidden peaks i.e peak purity studies[23].

**Oxidation:** Oxidation studies were carried by taking 50 mg of PRF dissolved in 50ml 3 % $H_2O_2$ (1mg/ml) and 25 ml of the above solution was refluxed in round bottom flask on previously calibrated heating mental for 2 hrs at 80°C and chromatograms were taken for the same after proper dilutions.

**Acid Degradation Studies:** Two hundred and fifty mg of MOXI was dissolved in 50ml of 0.1N hydrochloric acid (5 mg/ml) and was refluxed in round bottom flask previously calibrated heating mental at 80°C for 6 hrs. chromatograms were taken for the sample after proper dilution in methanol.

Table 3: Result of estimation of Moxifloxacin in eye-drop

| Sr. No. | Wt. of eye-drop(g) | Detector response (Peak area) | | Amount Estimated (mg) | % of Labeled Claim |
|---|---|---|---|---|---|
| | | Standard* | Sample# | | |
| 1 | 1 | 18024.636 | 18019.236 | 4.99 | 99.97 |
| 2 | 1 | | 18027.754 | 5.00 | 100.01 |
| 3 | 1 | | 18032.693 | 5.00 | 100.04 |
| 4 | 1 | | 18023.794 | 4.99 | 99.99 |
| 5 | 1 | | 18024.810 | 5.00 | 100.00 |
| | | | | Mean | 100.002 |
| | | | | ±SD | 0.025 |
| | | | | %RSD | 0.025 |

*-Conc. 50 µg/ml
#-Mean of five replicate injections

**Alkali Degradation Studies:** Two hundred and fifty mg of MOXI was dissolved in 50 ml of 0.1N sodium hydroxide (5 mg/ml) and was refluxed in round bottom flask on previously calibrated heating mental at 80°C for 6 hrs. chromatograms were taken for the sample after proper dilution in methanol.

**Neutral Degradation Studies:** Two hundred and fifty mg of MOXI was dissolved in 50ml of distilled water (5 mg/ml) and above solution was refluxed in round bottom flask on previously calibrated heating mental at 80°C for 6 hr. chromatograms were taken for the sample after proper dilution in methanol.

**Temperature Stress Studies:** MOXI API powder was exposed to dry heat in an oven for 0,7,15,30 days at a fixed temperature (60°C). The powder was removed from the oven on intervals mentioned. Removed samples were dissolved for proper dilutions and chromatograms were taken for the sample.

**Photostability Stress Studies:** MOXI API was exposed to day light in photo stability chamber to determine the effects of daylight irradiation on the stability of MOXI in the solid state. 1 g powder of MOXI was evenly spread in thin layer in a petri-dish with thickness less than 2mm and was kept in photo stability chamber for different time intervals (0, 7,15 and 30days). The powder was removed from dish on intervals mentioned. Removed samples were dissolved in methanol for proper dilutions and chromatograms were taken for the samples.

**Method Application on Marketed Formulation Estimation of Moxifloxacin in Formulation (Eye-drop) by Proposed HPLC Method:** The proposed method was applied for estimation of drug content in formulation i.e eye drop (Mosi® FDC) having strength 5mg/ml. the amount of eye drop was taken via formula of density.An accurately weighed quantity of eye-drop equivalent to 5 mg (1ml) was sonicated with 50 ml methanol for 15 minutes and the volume was made to 100.0 ml (conc.50µg/ml) with methanol the resultant was filtered through membrane filter. Five replicate sample solutions were prepared in similar manner.After equilibration of stationary phase, three replicate injection of standard solution and each of five sample solutions were made separately and chromatograms were recorded and amount of drug estimated in sample was calculated using formula.

$$Ew = \frac{Au}{As} \times Cs$$

Where $E_w$ = Drug estimated in sample weight (µg)
$Cs$ = concentration of standard (µg/ml)
$Au$ = Peak area of unknown
$As$ = Peak area of standard

Amount of drug present in dosage form as percent of labeled claim was calculated using following formula and is given in Table No. 3

$$\% \text{ of labeled claim} = \frac{Ew \times Wav}{Lc \times Ws} \times 100$$

Where,
$E_w$= Amount estimated in sample weight (mg)
$W_{AV}$= weight of eye drop (mg)
$W_s$= Sample weight (mg)
$L_c$= Labeled claim (mg / ml)

**RESULTS**

**Calibration Curve:** The calibration curve was plotted between concentration and peak area and it was found to be linear over the concentration range of 10 to 100 µg/mL with regression coefficient (r2) 0.999. The retention time and asymmetry factor were found to be 7.84 min and 1.12 respectively.
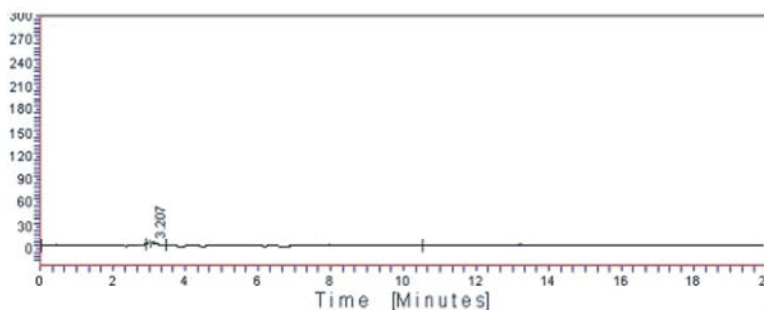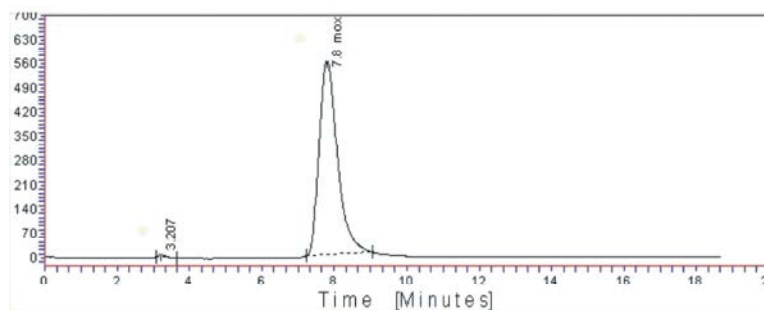
Fig. 2: Blank chromatogram
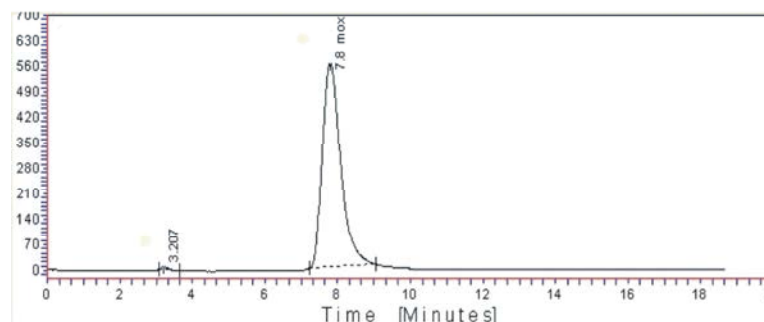


Fig. 3: HPLC chromatogram of Moxifloxacin



Fig. 4: HPLC chromatogram of Moxifloxacin eye drop Mosi®

**Validation of Method**

**Linearity:** The linearity was calculated by least squares linear regression analysis of calibration curve. The constructed calibration curve was linear over the concentration range of 10 to 100 μg/mL. The linear regression equation was Y= 324.8 x +1201 with regression co-efficient of 0.999.

**Accuracy as Recovery:** The proposed method afforded recovery of 98.42 to 99.56 % after spiking the additional standard drug concentration to the previously analyzed test solution. The values of % recovery, % RSD and are shown in Table 2, in each case all the values for % RSD were found to be less than 1% which indicated that the proposed method was accurate and precise.

**Precision:** Results representing Intra and Inter day precision studies aredepicted in table.no.1. Results of intraday show SD of 0.330 and % RSD of 0.663 while inter day results were SD of 0.49 and %RSD of 0.986. The values were < 2% thus demonstrating good repeatability and precision of method.

**Solution Stability:** The drug was stable over a period of 30hrs at room temperature in optimized mobile phase. More than 99% of the MOXI remained unchanged, on the basis of comparison of peak areas with those obtained from a freshly prepared solution of MOXI.

**Forced Degradation Studies of API and Specificity Studies:** The drug was submitted to stress degradation studies as per the ICH recommended guidelines.
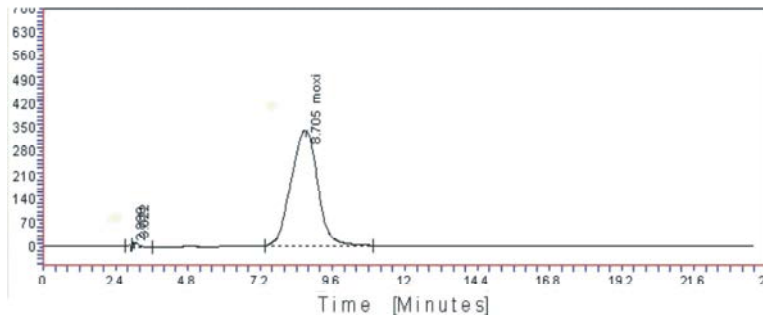
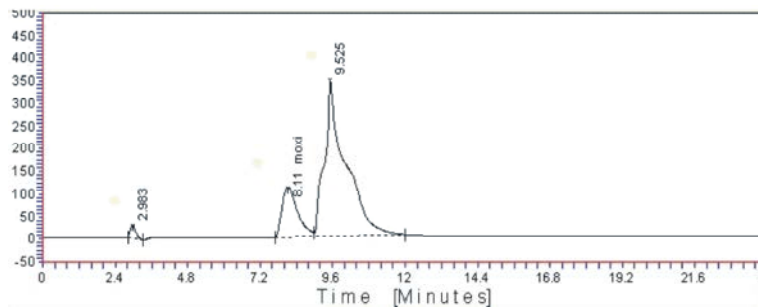Fig. 5: Moxifloxacin of Moxifloxacin in 0.1N HCL after 6 hrs



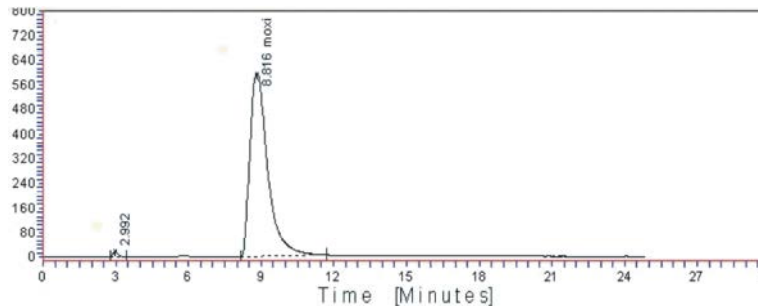Fig. 6: Chromatogram of Moxifloxacin in 1N NaOH after 6 hrs.



Fig. 7: Chromatogram of Moxifloxacin in hydrogen peroxide (3%) at room temp.after 24 hrs
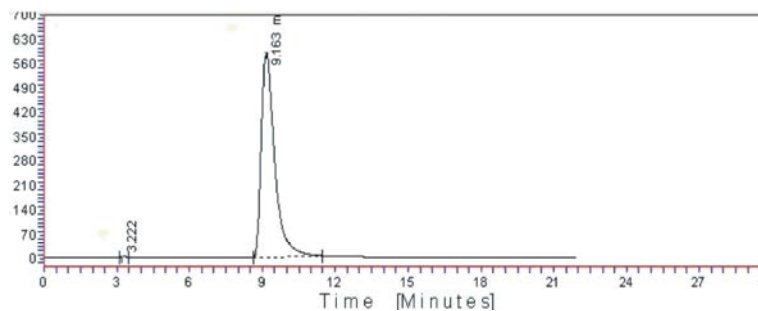


Fig. 8: Chromatogram of Moxifloxacin after Neutral hydrolysis of (after 6hrs)

The drug was found to stable under acidic conditions, neutral and oxidation stress (3% $H_2O_2$) also the drug was stable under photo stability studies and to thermal stress. The drug degraded in 0.1N NaOHshowing one major degradation product at RT 9.525 min. the chromatographs of various stress degradation are shown in Figs 4-11. Also the method was checked for specificity by further changing the ratio of organic phase for any hidden peaks the results do not showed presence of any other peaks establishing the method to be specific.
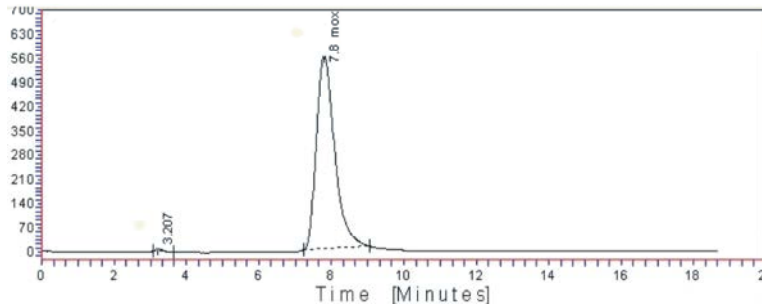
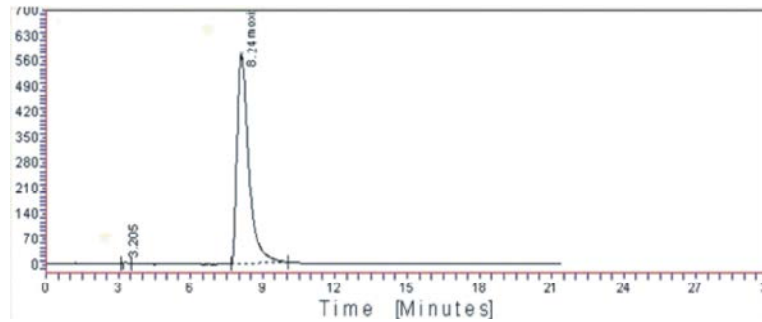Fig. 9: Chromatogram of Moxifloxacin in daylight on 1st day



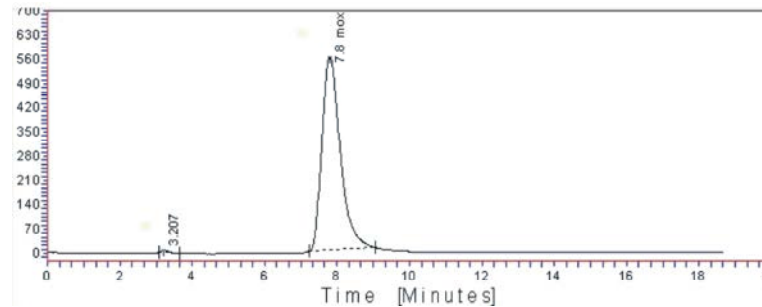Fig. 10: Chromatogram of Moxifloxacin in daylight on 30th day



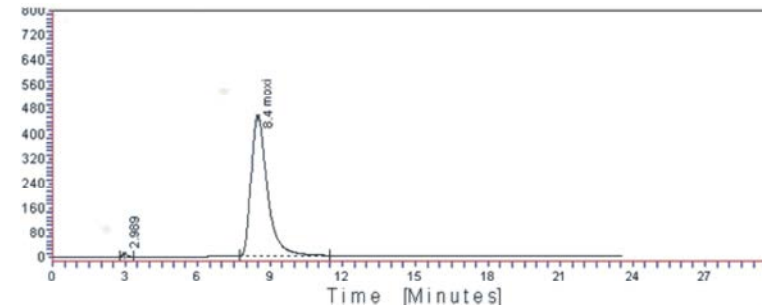Fig. 11: Chromatogram of Moxifloxacin in dry heat on 1st   day



Fig. 12: Chromatogram of Moxifloxacin in dry heat on 30th day

**Application**

**Assay of Marketed Formulation of MOXI Eye Drop:**
The validated method was applied for determination of MOXI in a commercially available marketed formulation of Eye drop of MOXI under brand name Mosi® marketed by FDC ltd. Prior to application of method placebo studies were done by injecting placebos of the eye drop diluents (sodium chloride as tonicity modifier, methyl and propyl paraben as preservatives) after filtering it through 0.45µm membrane filter in developed chromatographic conditions as shown in fig.no.1. The results are shown in Table 3; Fig. 4. illustrates a typical type of HPLC chromatogram of

Mosi® at a retention time 7.8min with no interference of excipients. The results of the assay (n=5) yielded 100.002% and %RSD = 0.025 of labeled claim. The results of the assay indicate that the method is selective for the analysis MOXI without interference of the excipients as seen in placebo studies.

## DISCUSSION

In the present study, an attempt was made to develop a simple, accurate, selective and specific RP-HPLC method of MOXI in. Grace RP C-18 column (5 μm, 250 mm x 4.6 mm i.d.), maintained at ambient temperature (25 ± 2 °C) was used for the method development. The developed method was involved with isocratic mode of separation for MOXI and its degradation products as compared to reported methods which employ complex gradient programming [26]. The developed method was involved with UV detection of analyte instead of flourimetry [27] making the application of method to routine quality control of pharmaceuticals without any prior derivetization steps.

The method was validated for specificity, accuracy, linearity and precision (interday and intraday) inaccordance with ICH guidelines. The peak areas of the drug were reproducible as indicated by the low values of % RSD. The sample recoveries in formulation were in good agreement with their respective label claim which suggested non-interference of formulation excipients in the estimation. Also the % RSD for analysis of formulationin recovery studieswas less than 1 % indicating good precision of the proposed method.

The results from stress testing, including separation of the degradation product from MOXI after exposure to stress conditions show the method is stability-indicating and capable of determining MOXI in presence of its degradation products. Also the method was specific as peak purity studies by changing the organic phase concentration do not showed presence of any hidden peaks. MOXI solution decomposed upto70% under basic stress (0.1N NaOH) conditions showing a major degradation product at RT 9.5min.

## CONCLUSIONS

The proposed method is simple isocratic method. The method was validated for accuracy and precision showing good results. The method has the ability to separate the drug from degradation products. The method is suitable for use for the routine analysis of MOXI in API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in any quality control

laboratories. The developed HPLC method can be applied for the analysis of samples obtained during accelerated stability studies to predict expiry dates of pharmaceuticals.

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