Indian Journal of Pharmacy Practice., 2025; 18(1):64-70.
https://www.ijopp.org

Original Article

Development and Validation of Assay Method for the Estimation of Moxifloxacin in Bulk and Pharmaceutical Formulations by RP-HPLC

Zahid Siraj[1,2,*], Omair Adil[1,2], Bakht Amin[3], Iftikhar Ahmed Tahiri[1]

[1]Department of Chemistry, Federal Urdu University of Arts, Sciences and Technology, Gulshan-e-Iqbal, Karachi, PAKISTAN.
[2]School of Chemical and Biomolecular Sciences, Lincoln Dr, Southern Illinois University at Carbondale, USA.
[3]Department of Pharmacy, Federal Urdu University of Arts, Sciences and Technology, Gulshan-e-Iqbal, Karachi, PAKISTAN.

ABSTRACT

Background: A simple, specific and isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method with UV detection at 293 nm and column Agela Technology, C_{18}, (Venosil XBP, 4.6 mm×250 mm, 10 µm) was developed and validated for analysis of Moxifloxacin hydrochloride (MOXI) in presence of its degradation products. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines. **Materials and Methods:** The study used a mobile phase consisting of a phosphate buffer and methanol (18:7 v/v), with a flow rate of 1.3 mL/min. The temperature was maintained at 50°C using a column oven. Additionally, 0.1N HCl was used as the diluent. **Results:** The retention time of the MOXI was found at 9.99 min. The calibration curves were linear with correlation coefficient (R^2) of 0.999. The detection and quantification limit were found as 0.029 µg mL^{-1} and 0.095 µg mL^{-1} respectively. **Conclusion:** The proposed method was found to be sensitive, specific and was successfully applied for the estimation of MOXI in pharmaceutical formulations. Innumerable analytical measurements are conducted for the estimation of MOXI in pharmaceutical formulations, so the proposed analytical method leads to provision of cost effective by using low-cost diluents and reagents.

Keywords: Moxifloxacin, High pressure liquid chromatography, International conference on harmonization; validation, HPLC, ICH.

Correspondence:
Mr. Zahid Siraj[1,2]
[1]Department of Chemistry, Federal Urdu University of Arts, Sciences and Technology, Gulshan-e-Iqbal, Karachi, PAKISTAN.
[2]School of Chemical and Biomolecular Sciences, 1245 Lincoln Dr, Southern Illinois University at Carbondale, IL 62901, USA.
Email: zahid.siraj@siu.edu

Received: 05-08-2024;
Revised: 21-08-2024;
Accepted: 04-09-2024.

INTRODUCTION

Moxifloxacin is a fourth-generation antibiotic drug that belongs to the fluoroquinolone family.[1] The fluoroquinolones are quinolones with fluorine at position six of the naphthyridine ring.[2] Previous studies indicate that the presence of fluorine atom in the Moxifloxacin (Moxi) structure makes its antibacterial activity effective against both types of gram-positive and gram-negative pathogens.[3-5] Its clinical studies confirmed that it is a well-tolerated drug and has high efficacy against pneumonia, acute bacterial rhinosinusitis and chronic bronchitis.[6] Previous study was conducted for the estimation of moxifloxacin in bulk and pharmaceutical formulations by spectrophotometer.[7] Analysis of the moxifloxacin in human plasmas was also conducted by liquid chromatography-electrospray ionization tandem mass spectrometry.[8] Here, in this study we developed and validated a new, simple, precise, accurate, cost effective and reproducible method for the determination of moxifloxacin in bulk and pharmaceutical formulations by Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC) as shown in Figure 1. The developed method was validated as per the International Council for Harmonisation (ICH) guideline.[9]

MATERIALS AND METHODS

Moxifloxacin HCl was received from INDUS pharma Karachi, Pakistan and used as a working standard, batch no. EL-03/L011/M/14138, manufacturer, enaltec labs chemistry, from India. The reading of the potency of moxifloxacin HCl on anhydrous basis and their moisture content were taken from the Certificate of Analysis (CoA) provided by the vendor i.e., 99.81% and 3.59% respectively. Analytical grade reagents, potassium dihydrogen phosphate (≥99%) and sodium hydroxide (≥97%), were purchased from Merck KGaA, Germany.

Instruments

Analytical five digits balance from Sartorius CP225D, Germany, HPLC Perkin Elmer Flexar, USA Flexar Column oven, Flexar

DOI: 10.5530/ijopp.20250084

UV/VIS detector, Flexar LC pump, Flexar LC Autosampler, Flexar solvent manager. Chromera software for Perkin Elmer Flexar HPLC and pH Meter Orion, 312, USA were used in the validation study.

Chromatography

The analytical method of moxifloxacin was validated in accordance with the International Conference on Harmonization (ICH) guidelines. A simple, specific and isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method with UV detection at 293 nm and column Agela Technology, Venosil XBP C18, 10 µm, 4.6 mm×250 mm was developed and validated for analysis of moxifloxacin hydrochloride (MOXI) in presence of its degradation products. The composition of the mobile phase was phosphate buffer and methanol (18:7 v/v) at flow rate of 1.3 mL/min, temperature 50°C maintained by using column oven was employed throughout the study. While 0.1N HCl was used as diluent.

Analytical Method Validation

System Suitability

For the System Suitability Test (SST), a primary standard stock solution with a concentration of 1000 µg/mL was prepared. This was subsequently diluted to obtain a working standard solution with a concentration of 100 µg/mL. The working standard solution was then used as a system suitability standard to assess the precision of the HPLC system.

Accuracy

Three different concentrations of moxifloxacin-90 µg/mL, 100 µg/mL and 110 µg/mL-were prepared from the standard stock solution. Three replicates of each of these three dilutions were injected and the % recovery was calculated. The following formula was used to determine the % recovery.

$$\%Recovery = \frac{Area\ of\ Sample\ \times Concentration\ of\ Standard}{Area\ of\ Standard\ \times Concentration\ of\ Sample} \times 100$$

Precision

Six replicates of 100 µg/mL of moxifloxacin (100% of theoretical concentration) were injected and calculated the % RSD for precision.

Specificity

The specificity test involved the use of a secondary placebo, a standard solution of moxifloxacin at a concentration of 100 µg/mL and a blank solution of 0.1N HCl. Two replicates of each concentration were injected. The list of excipients used in the placebo is mention in the Table 1.

Linearity and Range

Five different concentrations of moxifloxacin were prepared as the standards for linearity i.e. 80%, 90%, 100%, 100% and 120%. Two replicates of each of five dilutions of equal volume were injected. The average area was used as an instrument response. Linearity obtained by graphical representation of the concentration versus instrument response as area.

Limit of Detection and Limit of Quantification

For the determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ), serial dilutions of the sample were prepared from higher to lower concentrations: 100 µg/mL, 5 µg/mL, 4 µg/mL, 3 µg/mL, 2 µg/mL and 1 µg/mL. The coefficient of variance among the serial dilutions was calculated. These standards were injected in duplicate in sequence and the correlation coefficient was determined.

RESULTS

System Suitability Test (SST)

The System Suitability Testing (SST) test is used to determine the suitability of chromatographic system on a day-to-day basis.[10-12] A standard stock solution of moxifloxacin was prepared at a concentration of 1000 µg/mL using 0.1N HCl as the diluent. A working solution of moxifloxacin at a concentration of 100 µg/mL was then prepared from this stock solution. Five replicates of the working solution were used to calculate the Relative Standard Deviation (RSD) of the standard area, which was found to be 0.14. All HPLC chromatogram areas for SST analysis are included in Table S2 of the supplementary information (SI).

Accuracy

Stock standard placebo solution was prepared. Further, secondary stock solution was prepared from the stock. Moreover, three different concentrations (90 µg/mL, 100 µg/mL, 110 µg/mL) were prepared from the secondary stock solution. While the dilution is carried out with 0.1 N HCl. The chosen concentration levels (90%, 100% and 110% of theoretical concentration) provide a range to assess the accuracy of the analytical method. Three replicates for each concentration were used to enhance the statistical reliability of the results and provide a measure of precision. The average result of each concentration 90 µg/mL, 100 µg/mL, 110 µg/mL were obtained as 100.69%, 100.04%, 99.52% and shown in Figure 2. All HPLC chromatogram areas for accuracy analysis are included in Table S3 of the SI. The chromatogram for the 100 µg/mL concentration is shown in Figure S2 of the SI.

Precision

Six replicates of 100 µg/mL of moxifloxacin (100% of theoretical concentration) were injected and the calculated % RSD for precision was 0.46 % as shown in Table 2.

Specificity

For specificity three solutions (Working placebo, standard solution of moxifloxacin with 100 µg/mL and 0.1 N diluent) were

Table 1: List of Excipient Used in Placebo for 5000 Tablet Batch Size.

Excipient	Quantity/ batch (kg)	Wt./ tab (400 mg)	Wt./ tab (100 mg)	Excipient wt. used in placebo (mg)
Microcrystalline cellulose 101	7.82	156.4	39.1	782
Lactose crystalline (tablettose 80 molkerei meggle GmbH) for LM.	2.85	57	14.25	285
Croscarmellose Sodium (JRS/Germany).	1	20	5	100
Polyvinyl povidone (PVP K-30).	1	20	5	100
Aerosil 200 pharma (Evonik/Germany).	0.25	5	1.25	25
Talcum (Great chem minerals/China).	0.3	6	1.5	30
Magnesium stearate (Huzhou city linghu/ peter greven/Abbott).	0.2	4	1	20
Insta moistshield	1.5	30	7.5	150
Brown lake color	0.15	3	0.75	15
Erythricine red color	0.1	2	0.5	10

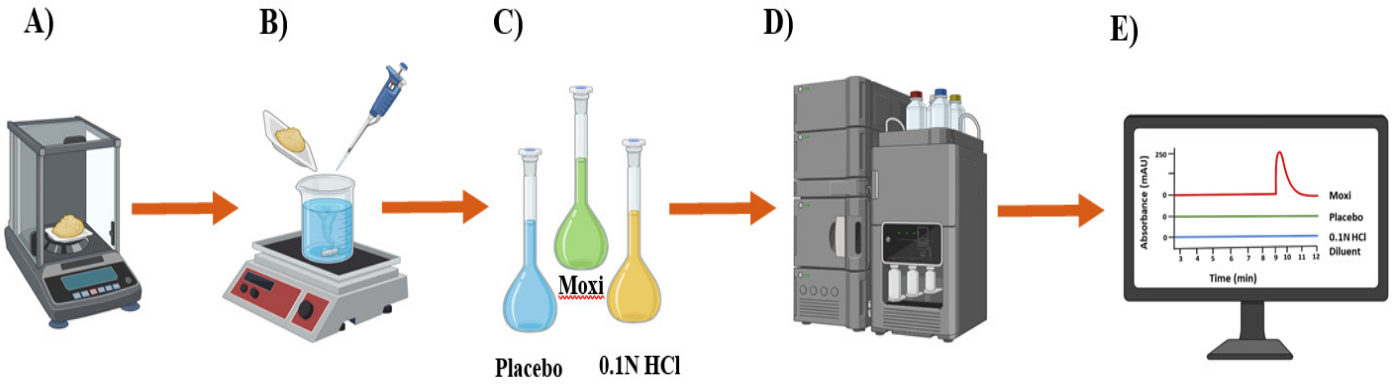


Figure 1: Schematic representation of method validation of Moxifloxacin for pharmaceutical formulation (A) Weighing balance used in the study (B) Sample preparation method (C) sample ready for the method validation study (D) HPLC used in the study (E) Data evaluation. (Created with BioRender.com).

prepared and two replicate of each concentration of the solution were injected. A working placebo solution is used to simulate the matrix in which the analyte is measured. The chromatogram of the working placebo solution showed no peak. This demonstrates that the method is not generating false positives from the placebo, indicating good specificity. Further, the chromatogram of the standard concentration (100 µg/mL) of moxifloxacin showing no other peaks as shown in Figure 3 and confirms that the method is accurately identifying and measuring moxifloxacin without interference from excipients. This is a critical aspect of specificity, ensuring that the method is specific to moxifloxacin. Moreover, the chromatogram of the blank (0.1 N HCl) did not show any peaks. The blank is a control to ensure that the solvent used does not contribute any interfering signals. The absence of peaks in the blank supports the cleanliness and selectivity of the method. Therefore, the moxifloxacin test method demonstrates good specificity and is suitable for its intended purposes. The absence of peaks in the placebo, standard concentration and blank chromatograms collectively indicates that the method is not affected by excipients that could lead to false results. The HPLC chromatograms for specificity are shown in Figures S3 and S4 of the SI.

Linearity and Range

The linearity of the analytical method for quantifying Moxifloxacin was assessed through the preparation of five different concentrations of standards as shown in Table 3. These concentrations included 80%, 90%, 100%, 110% and 120% of the target Moxifloxacin concentration. To establish the linearity, two replicates of each of the five dilutions, all of equal volume, were injected into the analytical system. The instrument response, measured in terms of average area, was utilized for graphical representation of the concentration versus instrument response (area) relationship as shown in graph as shown in Figure 4. The key parameters for evaluating linearity, namely slope, intercept, regression equation, correlation coefficient and coefficient of determination, were calculated from the resulting calibration curve as shown in Table 3. This comprehensive analysis aimed to provide insights into the accuracy, precision and reliability of the analytical method for Moxifloxacin quantification across a

range of concentrations. The HPLC chromatogram of range and linearity is shown in Figure S5 of the SI.

Limit of Detection and Limit of Quantification (LOD and LOQ)

The determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ) for moxifloxacin through serial dilution (1%, 2%, 3%, 4%, 5%, 10%, 100%) and duplicate injections has been carried out, resulting in a correlation coefficient of 0.9999 as shown in Figure 4. The high correlation coefficient 0.9999 indicates a strong linear relationship between the concentration of moxifloxacin and the response signal from the analytical method.[13,14] This suggests that the method used for analysis is reliable and provides consistent results across the concentration range tested. The LOD and LOQ were found to be at 0.029 µg/mL, 0.095 µg/mL respectively. The areas of the chromatograms for each concentration are provided in Table S1 of the SI. This

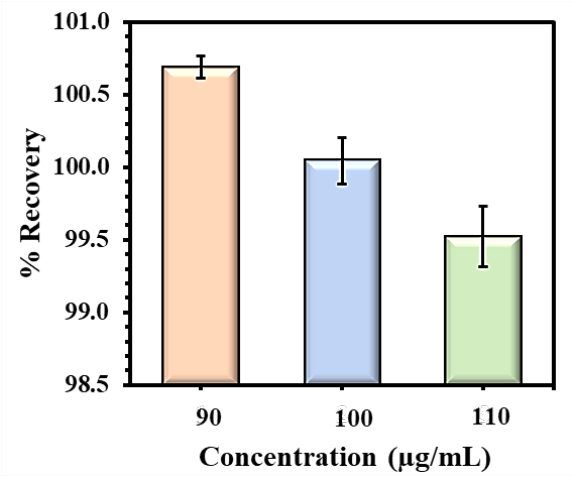
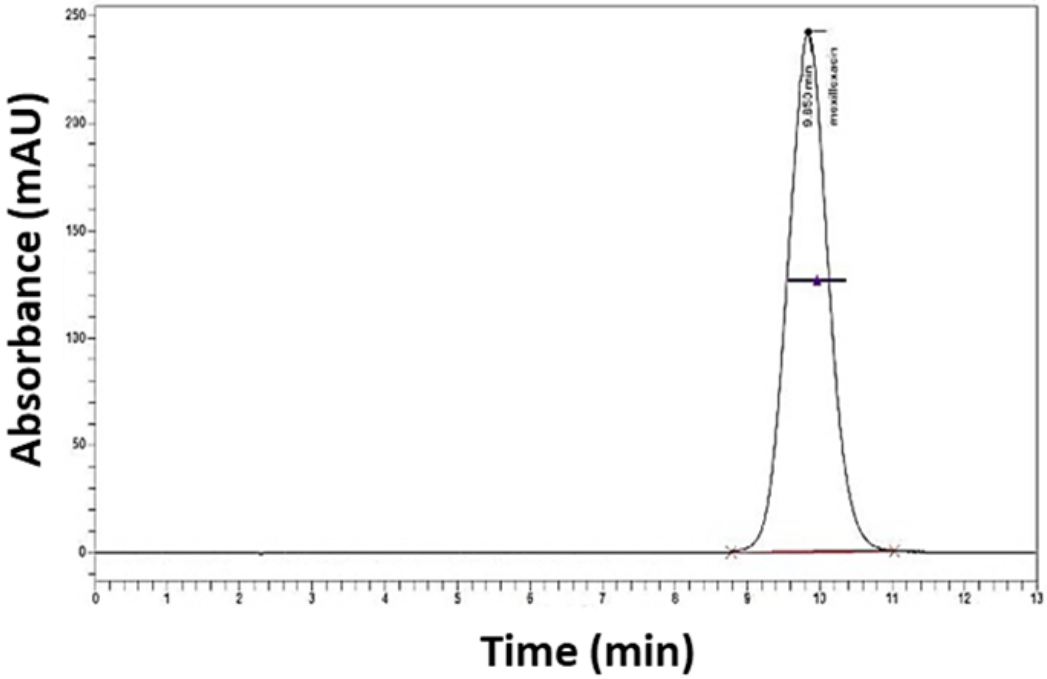


Figure 2: Three different concentration 90 µg/mL, 100 µg/mL, 110 µg/mL were used for the measurement of accuracy.

Table 2: Standard Area of Each Chromatogram for Precision.

Standard No.	Standard Concentration (µg/mL)	Standard Area (mAU)
STD-1A	100	9532676.9
STD-2A	100	9609090.7
STD-3A	100	9580064.3
STD-4A	100	9509240.8
STD-5A	100	9513493.1
	Average	9548913.2
	Standard Deviation	43828.7
	% RSD	0.46%



Peak #	Component Name	Time	Area	Height	Resolution	Tailing Factor	Plates (Tangent)
1	moxifloxacin	9.850	9,479,172.7	241,529.0		1.038	1,369
Total			9,479,172.7				

Figure 3: HPLC chromatogram of Moxifloxacin HCl sample (100 µg/mL) for specificity.

Table 3: Range and Linearity.

Theoretical Concentration (µg/mL)	Area of Standard-1 (mAU)	Area of Standard-2 (mAU)	Average Area (mAU)
80	7653500.2	7648017.9	7650759.1
90	8627001.2	8648766.1	8637883.7
100	9562795.4	9545840.9	9554318.2
110	10510615.0	10512304.8	10511459.9
120	11464664.1	11492191.8	11478428.0
		Correlation Coefficient	0.999
		Std. Deviation	95289.1
		Intercept	37655.7
		Regression equation	y = mx + c

Table 4: Standard Area of Each Concentration for LOD and LOQ.

Theoretical concentration (µg/mL)	Area of sample-1A (mAU)	Area of sample-2A (mAU)	Average area (mAU)
100	9636109.0	9671729.7	9653919.4
10	970988.3	964832.2	967910.3
5	493667.2	490600.8	492134.0
4	387449.0	393519.3	390484.2
3	289344.3	299760.2	294552.3
2	198233.9	198233.9	198233.9
1	108881.2	102395.1	105638.2

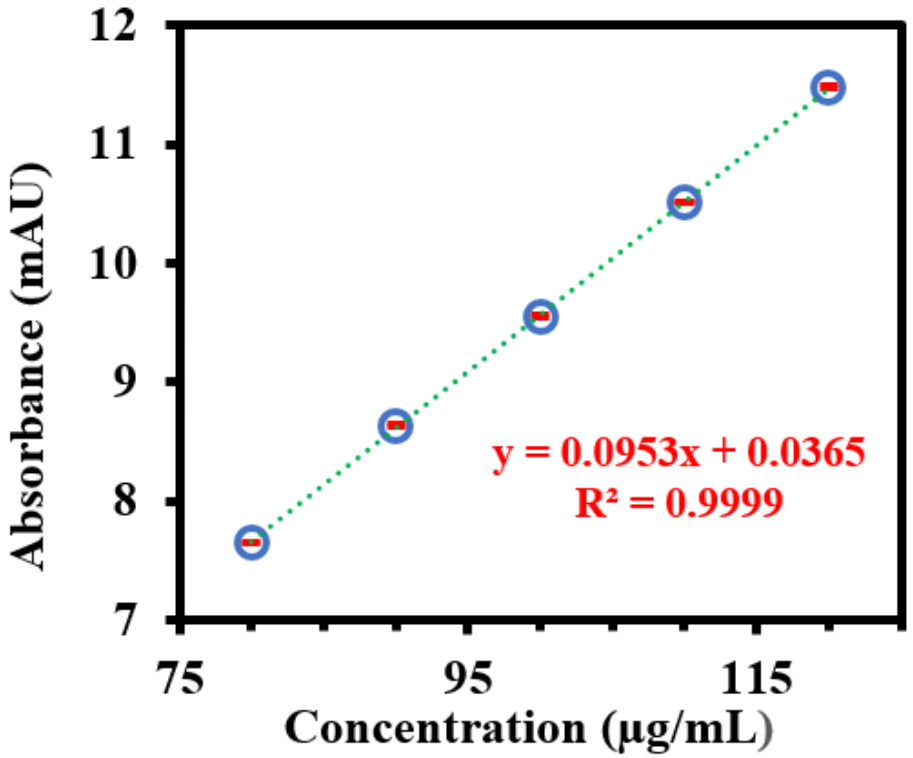


Figure 4: Five different concentration 80 µg/mL, 90 µg/mL, 100 µg/mL, 110 µg/mL, 120 µg/mL were used for the measurement of range and linearity of moxifloxacin method. Legends keep hollow in order to show the error bar.

indicates that the analytical method is sensitive enough to accurately measure moxifloxacin at relatively low concentrations. Moreover, the calculation of LOD and LOQ is included in Moreover, the calculation of LOD and LOQ is included in Figure S1 of the SI.

DISCUSSION

The analytical method of moxifloxacin was validated in accordance with the International Conference on Harmonization (ICH) guidelines. A simple, specific and isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method with UV detection at 293 nm and column Agela Technology, Venosil XBP C18, 10 um, 4.6 mm×250 mm was developed and validated for analysis of Moxifloxacin hydrochloride (MOXI) in presence of its degradation products. The composition of the mobile phase was phosphate buffer and methanol (18:7 v/v) at flow rate of 1.3 mL/min, temperature 50°C maintained by using column oven was employed throughout the study. While 0.1 N HCl was used as diluent. Initially, a System Suitability Test (SST) was conducted to assess the suitability of the chromatographic system. Five replicates of the working solution of moxifloxacin (100 µg/mL) were used and the RSD was calculated. For the specificity test, three solutions were prepared: a working placebo, a standard moxifloxacin solution at 100 µg/mL and a 0.1N diluent. Two replicates of each solution were injected. The retention time of the moxifloxacin peak was found to be 9.99 min from the moxifloxacin standard stock solution. The placebo and the 0.1 N diluent showed no peaks that could interfere with the moxifloxacin Peaks (API), ensuring the specificity of the moxifloxacin method.

For accuracy, three replicates of each concentration (90 µg/mL, 100 µg/mL and 110 µg/mL) were used, with all concentrations prepared in 0.1N HCl. A stock placebo was also prepared, along with secondary placebo solutions. The purpose of the placebo solution was to check for any interference of the excipients with the Active Pharmaceutical Ingredient (API), moxifloxacin. The placebo is essential to account for any interference with excipient and it helps to establish a baseline for accuracy.[15-17] The obtained results, expressed as the % recovery of moxifloxacin, were found to be within the range of 99-101% across all concentration levels tested. These results ensure the reliable quantification of moxifloxacin.

Precision is measured in terms of Relative Standard Deviation (RSD) and is commonly used to assess the variability or reproducibility of analytical data.[18,19] For the precision test, six replicates of the same concentration (100 µg/mL of moxifloxacin) were injected. The results were expressed as the % Relative Standard Deviation (RSD). The low RSD value indicates that the method is capable of producing consistent results under the same operating conditions, ensuring the method's reliability for routine analysis.

For the range and linearity assessment, four solutions of different concentrations were prepared i.e. 80 µg/mL, 90 µg/mL, 100 µg/mL and 110 µg/mL. Two replicates of each concentration were injected for the range and linearity analysis. A graph was plotted with instrument response versus concentration. The results were obtained using the regression equation, with a correlation coefficient (R^2) of 0.999. Key parameters for evaluating linearity, including slope, intercept, regression equation, correlation coefficient and coefficient of determination, were calculated from the resulting calibration curve, as shown in Table 3. This comprehensive analysis aimed to provide insights into the accuracy, precision and reliability of the analytical method for moxifloxacin quantification across a range of concentrations. The outcomes of these calculations are essential for ensuring the robustness and suitability of the method for subsequent quantitative analyses in pharmaceutical or clinical settings.[20,21]

For the determination of LOD (Limit of Detection) and LOQ (Limit of Quantification), different concentrations were prepared: 100 µg/mL, 10 µg/mL, 5 µg/mL, 4 µg/mL, 3 µg/mL, 2 µg/mL and 1 µg/mL. Two replicates of each concentration were injected. The LOD and LOQ were found to be 0.029 µg/mL and 0.095 µg/mL, respectively. These low values indicate that the analytical method is sensitive enough to accurately measure moxifloxacin at relatively low concentrations. The calculations for LOD and LOQ are included in Figure S1 of the SI.

Therefore, the proposed analytical method was found to be sensitive, specific and was successfully applied for the estimation of moxifloxacin in pharmaceutical formulations

CONCLUSION

In this study, a simple, specific and isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was successfully developed and validated for the analysis of Moxifloxacin hydrochloride (MOXI) in the presence of its degradation products. The Agela Technology Venosil XBP C18 column, with dimensions 4.6mm×250mm and particle size of 10µm, was employed for chromatographic separation, resulting in a sharp and well-defined moxifloxacin peak at a retention time of 9.99 min. The optimized mobile phase composition of phosphate buffer and methanol (18:7 v/v) at a flow rate of 1.3 mL/min, coupled with a column oven maintained at 50°C, provided efficient and reproducible separation throughout the study. The use of 0.1N HCl as the diluent further supported the sensitivity and specificity of the method. The calibration curve demonstrated excellent linearity, with a correlation coefficient (R^2) of 0.999, meeting the criteria outlined by the ICH guidelines. The detection and quantification limit were found as 0.029 µg mL^{-1} and 0.095 µg mL^{-1} respectively. The developed RP-HPLC method with UV detection at 293 nm showcased its sensitivity and specificity in successfully quantifying moxifloxacin in pharmaceutical formulations, even in the presence of potential

degradation products. The diluent used in the analytical method of moxifloxacin determination is very low cost and easily available that will provide provision for low-cost medicine. Overall, the results indicate that the proposed method holds promise for routine analysis in pharmaceutical quality control laboratories, providing a valuable tool for accurate and efficient moxifloxacin quantification.

ACKNOWLEDGEMENT

Zahid thanks Dr. Muhammad Haji and Dr. Zaheer Masood (FUUAST) for helpful conversations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RSD: Relative standard deviation; **API:** Active pharmaceutical ingredient; **RP-HPLC:** Reversed phase-high performance liquid chromatography; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **SST:** System Suitability; **HPLC:** Test High pressure liquid chromatography; **ICH:** International conference on harmonization.

SUMMARY

A specific, simple and time-saving isocratic RP-HPLC method was developed and validated for the estimation of moxifloxacin in the presence of its degradation products, following ICH guidelines. An Agela Technology C18 column (Venosil XBP, dimensions 4.6mm×250mm, particle size 10µm) was used for chromatographic separation. The mobile phase consisted of phosphate buffer and methanol in a ratio of 18:7 v/v. The column oven temperature was maintained at 50°C and 0.1N HCl was used as the diluent. This method demonstrated promising results, with a correlation coefficient (R^2) of 0.999. The LOD and LOQ were found to be 0.029 µg/mL and 0.095 µg/mL, respectively. The use of 0.1N HCl as the diluent and the low-cost mobile phase make this method both economical and highly sensitive and accurate. Therefore, numerous analyses can be performed using this method for the estimation of moxifloxacin, potentially leading to significant cost savings in drug product.

REFERENCES

1. Gore J, Bryant Z, Stone MD, Nollmann M, Cozzarelli NR, Bustamante C. Mechanochemical analysis of DNA gyrase using rotor bead tracking. Nature. 2006;439(7072):100-4.
2. Hernandez-Arteseros JA, Barbosa J, Compano R, Prat MD. Analysis of quinolone residues in edible animal products. J Chromatogr A. 2002;945(1-2):1-24.
3. xInderlied CB, Nash K. Antibiotics in laboratory medicine. Lippincott Williams and Wilkins, Philadelphia. 1996:127-9.
4. Samanidou VF, Evaggelopoulou EN, Papadoyannis IN. Development of a validated HPLC method for the determination of four penicillin antibiotics in pharmaceuticals and human biological fluids. Journal of separation science. 2006;29(11):1550-60.
5. Balfour JA, Wiseman LR. Moxifloxacin. Drugs. 1999;57(3):363-73; discussion 74.
6. Burkhardt O, Welte T. 10 years' experience with the pneumococcal quinolone moxifloxacin. Expert Review of Anti-infective Therapy. 2009;7(6):645-68.
7. Motwani SK, Chopra S, Ahmad FJ, Khar RK. Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations. Spectrochim Acta A Mol Biomol Spectrosc. 2007;68(2):250-6.
8. Vishwanathan K, Bartlett MG, Stewart JT. Determination of moxifloxacin in human plasma by liquid chromatography electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal. 2002;30(4):961-8.
9. ICH Q2 guideline on validation of analytical procedures: European Medicnes Agency; [Available from: https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline. Bose A. HPLC calibration process parameters in terms of system suitability test. Austin Chromatogr. 2014;1(2):1-4.
10. Food U, Administration D, editors. Center for Drug Evaluation and Research, US Food and Drug Administration. Eightieth Meeting of the Cardiovascular and Renal Drugs Advisory Committee; 1997: US Department of Health and Human Services Washington, DC.Mukherjee A, Bera A. A detailed study of validation parameters and system suitability test in HPLC. Res J Pharm Bio Chem Sci. 2012;3:426-47.
11. Vial J, Jardy A. Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. Analytical Chemistry. 1999;71(14):2672-7.
12. Alquadeib BT. Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets. Saudi pharmaceutical journal. 2019;27(1):66-70.
13. Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR J Pharm. 2015;5(10):7-19.
14. Bruce P, Minkkinen P, Riekkola M-L. Practical method validation: validation sufficient for an analysis method. Microchimica Acta. 1998;128:93-106.
15. Araujo P. Key aspects of analytical method validation and linearity evaluation. Journal of chromatography B. 2009;877(23):2224-34.
16. González AG, Herrador MÁ. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. TrAC Trends in Analytical Chemistry. 2007;26(3):227-38.
17. Green JM. Peer reviewed: a practical guide to analytical method validation. Analytical chemistry. 1996;68(9):305A-9A.
18. Ermer J, Miller JHM. Method validation in pharmaceutical analysis: A guide to best practice: John Wiley and Sons; 2006.
19. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R. Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods. Química Nova. 2020;43:1190-203.
20. Ermer J, Miller JHM. Method validation in pharmaceutical analysis: A guide to best practice: John Wiley & Sons; 2006.
21. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R. Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods. Química Nova. 2020;43:1190-203.

Cite this article: Siraj Z, Adil O, Amin B, Tahiri IA. Development and Validation of Assay Method for the Estimation of Moxifloxacin in Bulk and Pharmaceutical Formulations by RP-HPLC. Indian J Pharmacy Practice. 2025;18(1):64-70.

Supplementary Table 1: Standard Area of Each Concentration for LOD and LOQ.

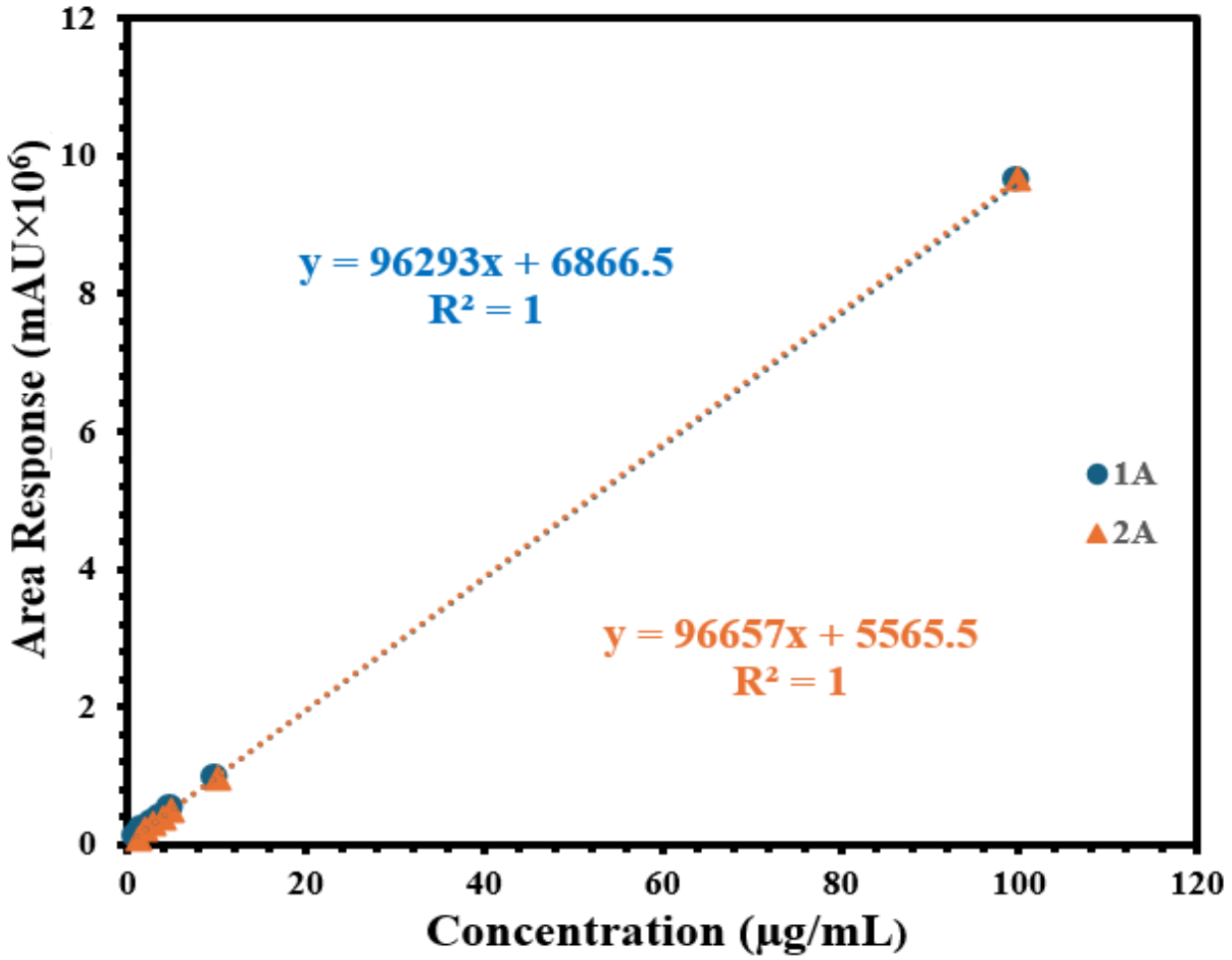
Theoretical concentration (µg/mL)	Area of sample-1A (mAU)	Area of sample-2A (mAU)	Average area (mAU)	Standard Deviation
100	9636109.0	9671729.7	9653919.4	25187.6
10	970988.3	964832.2	967910.3	4353.0
5	493667.2	490600.8	492134.0	2168.3
4	387449.0	393519.3	390484.2	4292.4
3	289344.3	299760.2	294552.3	7365.2
2	198233.9	198233.9	198233.9	0.0
1	108881.2	102395.1	105638.2	4586.4

Supplementary Table 2: Standard Area of Each Chromatogram for System Suitability.

Standard - No.	Standard Concentration (µg/mL)	Standard Area (mAU)
STD -1A	100	9572958.2
STD -2A	100	9546991.8
STD -3A	100	9565046.8
STD -4A	100	9535157.5
STD -5A	100	9557547.0
	Average	9555540.3
	Standard Deviation	13314.05
	% RSD	0.14

Supplementary Table 3: Standard Area of Each Chromatogram for Accuracy.

Theoretical Concentration (µg/mL)	Area of Standard-1 (mAU)	Area of Standard-2 (mAU)	Area of Standard-3 (mAU)
90	8652747.1	8665043.4	8659236.5
100	9551372.8	9577645.4	9549924.6
110	10436612.3	10479008.6	10467986.6

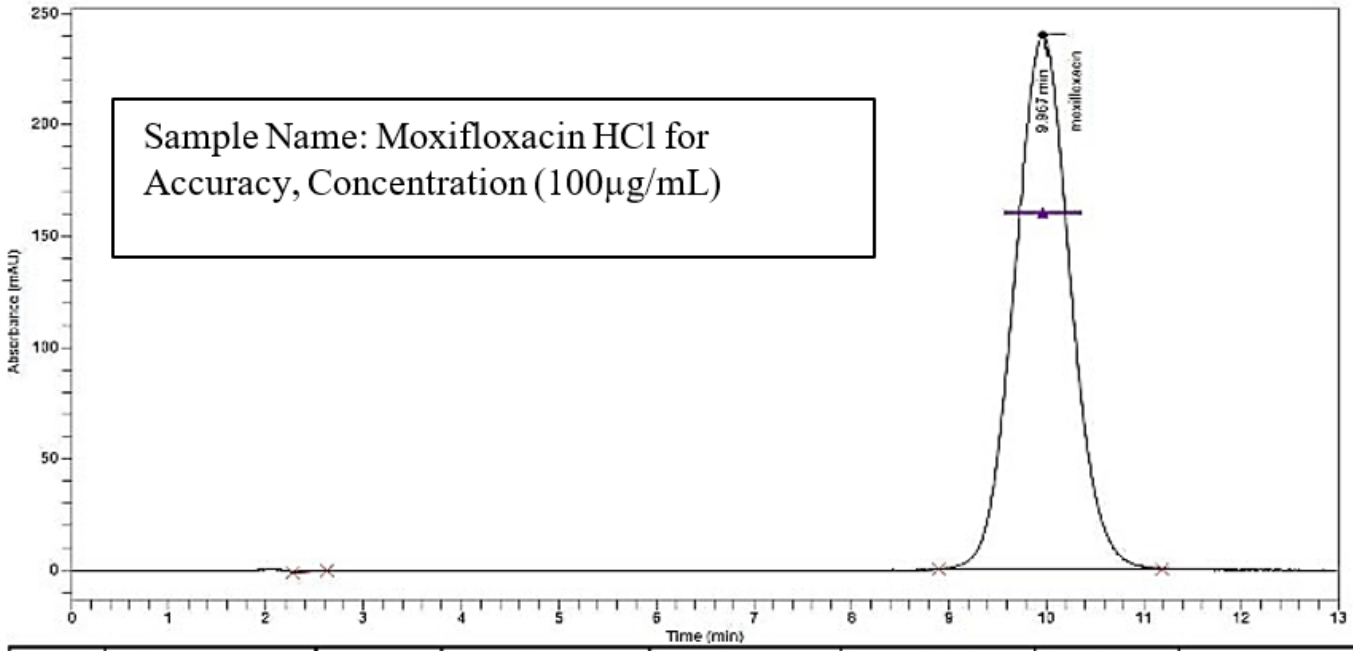


S. No	Intercept	Slope
1	6866.5	96293
2	5565.5	96657
Average	6216	96475
St. Deviation	919.9	257.4

$$\text{LOD} = \frac{3\,\sigma}{S} = \frac{3\times 919.9}{96475} = 0.029\ \mu\text{g/mL}$$

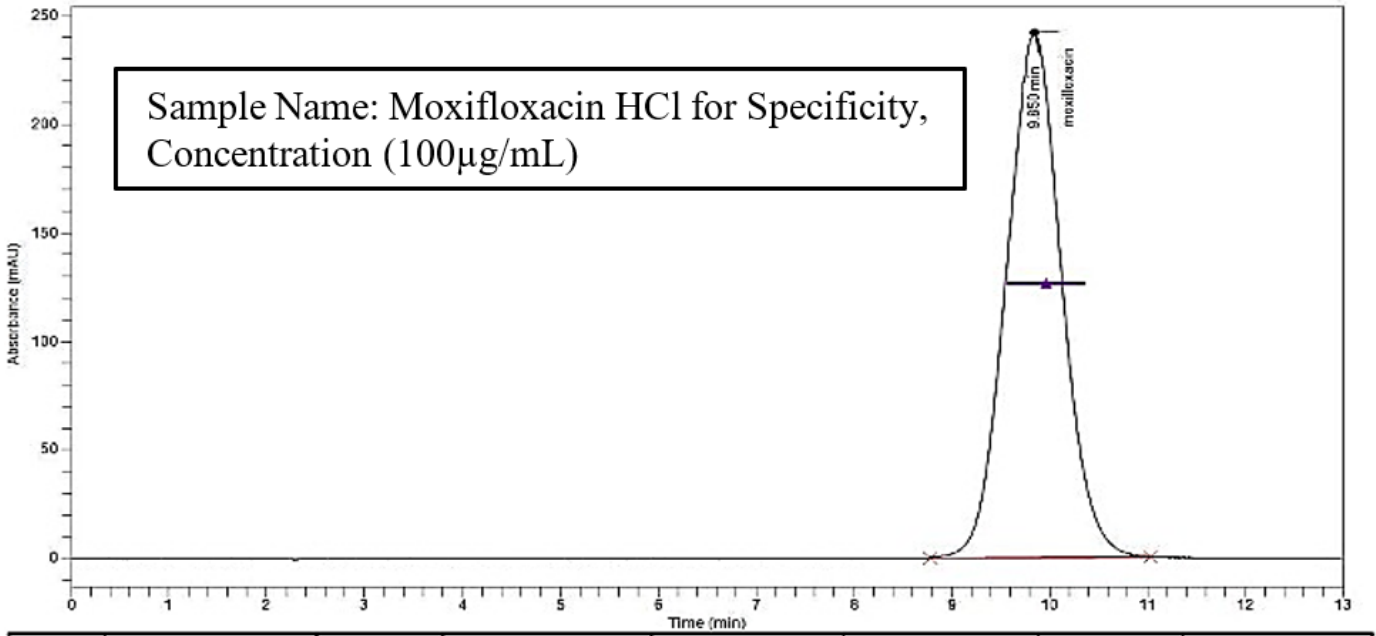
$$\text{LOQ} = \frac{10\,\sigma}{S} = \frac{10\times 919.9}{96475} = 0.095\ \mu\text{g/mL}$$

Supplementary Figure 1: LOD and LOQ of Moxifloxacin.



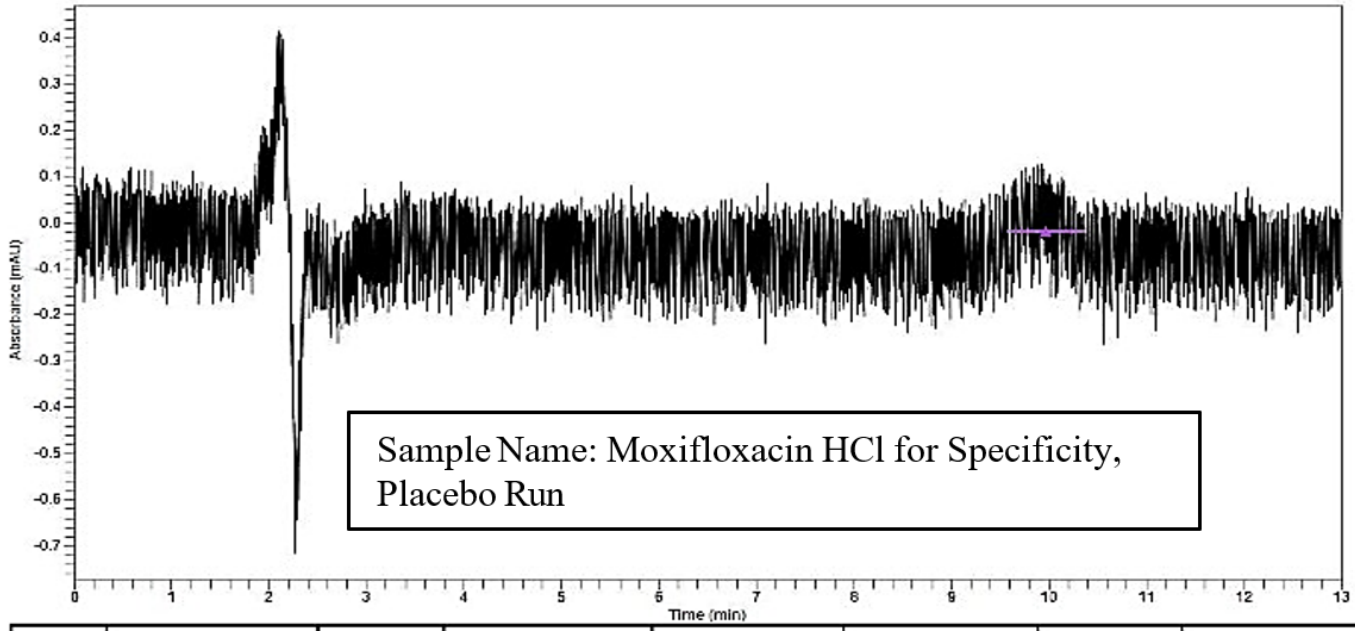
Peak #	Component Name	Time	Area	Height	Resolution	Tailing Factor	Plates (Tangent)
1		2.472	9,715.3	630.4		0.887	191
2	moxifloxacin	9.967	9,551,372.8	239,509.0	8.32	1.043	1,347
Total			9,561,088.1				

Supplementary Figure 2: Moxifloxacin run for accuracy in case of 100 µg/mL concentration.



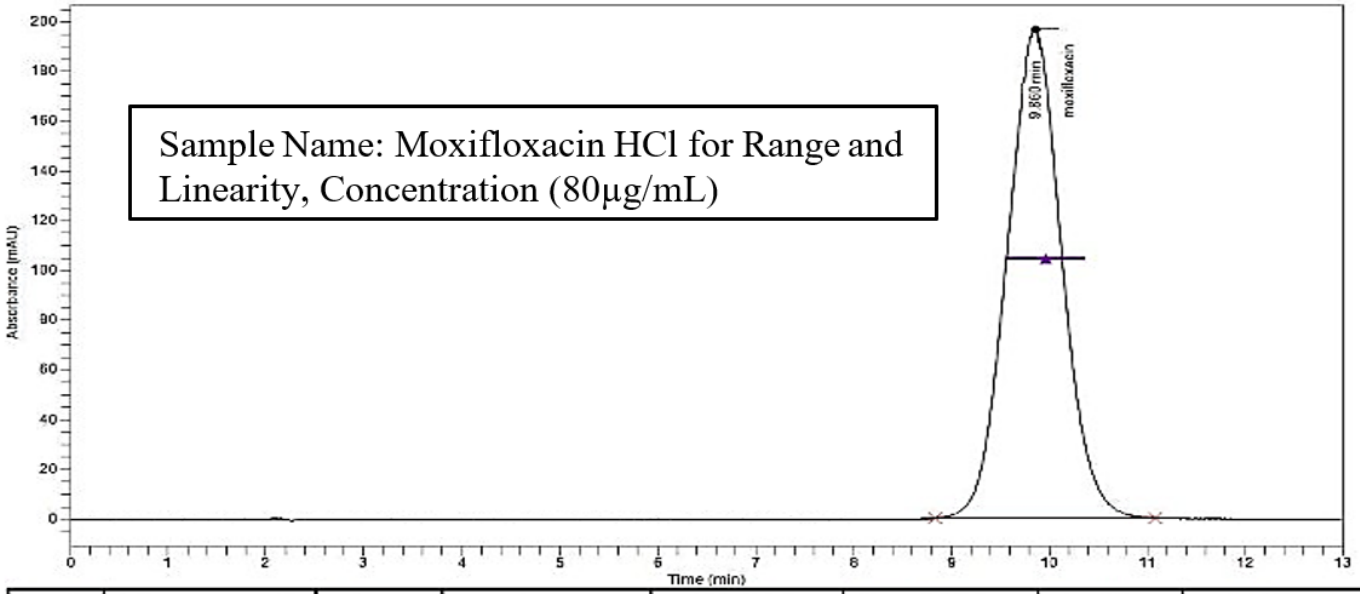
Peak #	Component Name	Time	Area	Height	Resolution	Tailing Factor	Plates (Tangent)
1	moxifloxacin	9.850	9,479,172.7	241,529.0		1.038	1,369
Total			9,479,172.7				

Supplementary Figure 3: Moxifloxacin run for specificity in case of 100 µg/mL concentration.



Peak #	Component Name	Time	Area	Height	Resolution	Tailing Factor	Plates (Tangent)
Total							

Supplementary Figure 4: Placebo run for the determination of specificity.



Peak #	Component Name	Time	Area	Height	Resolution	Tailing Factor	Plates (Tangent)
1	moxifloxacin	9.860	7,653,500.2	196,378.5		1.033	1,394
Total			7,653,500.2				

Supplementary Figure 5: Moxifloxacin run for range and linearity in case of 80 µg/mL concentration.