

COMPARATIVE VALIDATION OF UV SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE ESTIMATION OF MOXIFLOXACIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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Abstract

Moxifloxacin hydrochloride is a broad-spectrum fourth-generation fluoroquinolone antibiotic employed in the treatment of a wide variety of bacterial illnesses. The purpose of this work was to develop and validate UV spectrophotometric and RP-HPLC techniques for the quantitative determination of Moxifloxacin hydrochloride in pharmaceutical dosage forms as per the International Conference on Harmonization (ICH) Q2(R2) recommendations. The UV spectrophotometric approach was performed at 293 nm, whereas the RP-HPLC method was performed on a C18 column with a mobile phase of phosphate buffer–acetonitrile (70:30, v/v). Both techniques were verified for specificity, linearity, accuracy, precision, robustness, LOD, LOQ and system suitability. The findings showed good linearity ($R^2 > 0.999$), recovery values between 98-102% and % RSD less than 2% demonstrating compliance with ICH approval standards. The UV method was simple, rapid and cost-effective for routine quality control whereas RP-HPLC method was more specific, sensitive and selective for stability studies and regulatory applications. Both approaches were shown to be reliable and verified for the measurement of Moxifloxacin hydrochloride in pharmaceutical formulations.

Keywords: Moxifloxacin hydrochloride, RP-HPLC, UV Spectrophotometry, Method Validation, ICH Guidelines, Pharmaceutical Analysis, Analytical Method Development, Quality Control

INTRODUCTION

Analytical chemistry is an integral part of the pharmaceutical business in assuring the constant quality, safety and efficacy of medical goods. The entire life cycle of pharmaceutical products launched into the market should be subject to a rigorous analytical evaluation, from the analysis of raw materials to the finished product and post-marketing surveillance. Thus, analytical technique development and validation are integral parts of pharmaceutical quality assurance systems. Regulatory agencies across the world such as International Council for Harmonisation (ICH), United States Food and Drug Administration (USFDA) and European Medicines Agency (EMA) require the use of scientifically validated analytical methods for estimation of drugs to ensure reliability and reproducibility of analytical results. Among the several analytical techniques used in pharmaceutical laboratories,

ultraviolet-visible (UV-Visible) spectrophotometry and Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) are the most flexible and approved procedures for the quantitative examination of drugs. UV spectrophotometry is appreciated for its simplicity, speed, cost-effectiveness and ease of operation. It is especially applicable for routine quality monitoring when the pharmaceutical formulations contain only low levels of interfering contaminants. On the other hand, RP-HPLC is more sensitive, specific, selective and reproducible, and is the method of choice for stability studies, impurity profiling, dissolution testing and regulatory submissions. Moxifloxacin hydrochloride is a fourth-generation fluoroquinolone antibiotic with broad-spectrum action against gram-positive and gram-negative pathogens. It inhibits bacterial DNA gyrase (topoisomerase II) and topoisomerase IV enzymes preventing DNA replication, transcription and repair and leading to bacterial cell death. Moxifloxacin is a dual enzyme inhibitor and so has better antibacterial activity and a lower propensity for bacterial resistance compared to previous generations of fluoroquinolones.

Moxifloxacin hydrochloride is chemically 1-Cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride. The medication molecular formula is $C_{21}H_{24}FN_3O_4 \cdot HCl$ and the molecular weight is approximately 437.9 g/mol. It is a pale yellow crystalline powder and is easily soluble in water and in methanol. The molecule contains aromatic chromophores which absorb well in the ultraviolet area. UV spectrophotometry is one of the relevant analytical methods for quantitative measurement of the drug.

Moxifloxacin hydrochloride is found in several pharmaceutical dosage forms including tablets, ophthalmic solutions, injectable preparations, and infusion solutions. Accurate quantitative measurement of the medication in these formulations is necessary to ensure dose accuracy, therapeutic efficacy and patient safety. Deviations from pharmacopeial standards of drug concentration may result in therapeutic failure, severe medication responses, and antimicrobial resistance. Hence there is a need for dependable analytical techniques for quantitative determination of Moxifloxacin hydrochloride content which is essential for pharmaceutical production and quality control laboratories.

The UV spectrophotometric analysis is based on the absorption of ultraviolet radiations by molecules having chromophoric functional groups. According to the Beer–Lambert law, absorbance is directly proportional to concentration over a defined range of concentrations. Moxifloxacin hydrochloride contains conjugated aromatic structures and fluorinated quinolone rings, which makes it absorb ultraviolet strongly, and its content can be accurately determined without complex pretreatment. The approach involves very affordable apparatus, utilizes minimum reagents and offers speedy analytical findings. These properties make UV spectrophotometry very attractive for routine assay procedures in industrial and academic laboratories.

However, the UV spectrophotometric methods have inherent limitations. Analytical specificity can be influenced by interference from excipients, degradation products, preservatives and other formulation components. Furthermore, spectrophotometric approaches do not differentiate enough substances with overlapping absorption spectra, which reduces the selectivity of the analysis. Therefore, more

sophisticated chromatographic techniques are necessary for the analysis of complex pharmaceutical matrices.

In Reverse Phase High-Performance Liquid Chromatography several of these restrictions are solved by separating specific components depending on their interactions with the stationary and mobile phase. RP-HPLC uses a nonpolar stationary phase and generally octadecylsilane (C18) and a comparatively polar mobile phase comprising aqueous buffer and organic solvent such as acetonitrile or methanol. Under the optimized chromatographic conditions, Moxifloxacin hydrochloride can be efficiently separated from formulation excipients, degradation products, impurities and related substances and highly accurate quantitative estimation is feasible.

RP-HPLC analytical technique development includes rigorous optimization of chromatographic parameters such mobile phase composition, buffer pH, type of column, flow rate, injection volume, detection wavelength and column temperature. Each parameter has a remarkable influence on the retention time, the number of theoretical plates, peak symmetry, resolution and analytical reproducibility. The proper optimization leads to good chromatographic performance for routine pharmaceutical analysis.

Validation of an analytical technique demonstrates that an analytical procedure will reliably and reproducibly provide the desired findings for its intended use. According to ICH Q2 (R2) criteria, the validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantitation, robustness, ruggedness and system adaptability. Together these parameters define the scientific soundness and regulatory fitness of the developed analytical method.

Various analytical methods have been reported previously for estimation of Moxifloxacin hydrochloride such as spectrophotometric methods, HPLC methods, HPTLC, capillary electrophoresis, LC-MS/MS and electrochemical techniques. However, relatively few investigations have performed a systematic comparative validation of UV spectrophotometric and RP-HPLC methods using identical validation criteria under the latest ICH recommendations. These comparison studies are important as they allow pharmaceutical analyzers to choose the most suitable analytical technique for their needs, including laboratory infrastructure, regulatory requirements, analytical sensitivity, cost and the intended usage of the technique.

Hence the objective of the present research work is to develop, validate and compare UV spectrophotometric and RP-HPLC analytical methods for estimation of Moxifloxacin hydrochloride in pharmaceutical dosage forms. Both methods are validated according to internationally accepted ICH guidelines and their analytical performance characteristics are critically compared with respect to linearity, precision, accuracy, specificity, robustness, sensitivity, analysis time, solvent consumption, operational cost and suitability for routine quality control applications. The outcome of this work is expected to provide scientifically validated analytical methods to pharmaceutical industries, quality control laboratories, academic researchers and regulatory authorities for ensuring the quality, safety and therapeutic efficacy of Moxifloxacin hydrochloride formulations.

OBJECTIVES OF THE STUDY

1. To develop a simple, rapid, accurate and economical UV spectrophotometric method for estimation of Moxifloxacin hydrochloride.
2. To develop improved RP-HPLC technique for selective and reproducible chromatographic separation of Moxifloxacin hydrochloride.

REVIEW OF LITERATURE

Quantitative determination of pharmaceutical compounds is an important part of drug development, manufacturing and quality assurance. Analytical methods are required for not only assay determination but also impurity profiling, stability studies, dissolution testing, pharmacokinetic investigations and regulatory compliance. Among the analytical techniques available, UV spectrophotometry and Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) are most commonly used due to their reliability, precision, reproducibility and adaptability to different pharmaceutical formulations. These analytical techniques have been extensively explored for the fourth-generation fluoroquinolone antibiotic, moxifloxacin hydrochloride, due to its broad-spectrum of antibacterial activity and wide clinical application.

Moxifloxacin hydrochloride has good antibacterial effects against Gram-positive, Gram-negative, atypical and anaerobic microbes. Mechanism of action: It inhibits the enzymes bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, therefore inhibiting DNA replication and transcription. The therapeutic relevance of active pharmaceutical ingredient (API) in bulk medications and final dosage forms needs accurate estimate for therapeutic effectiveness and safety of the patient.

The first analytical techniques for fluoroquinolone antibiotics were based on microbiological tests that determined the antibacterial activity rather than the real drug concentration. Microbiological approaches for the assessment of biological potency were beneficial but lacked specificity, needed long incubation durations and were subject to a number of biological factors. Hence, in pharmaceutical quality control laboratories, microbiological tests have increasingly supplanted the chromatographic and spectrophotometric analytical techniques.

Due to the better analytical performance, high performance liquid chromatography became the preferred analytical technique in the late twentieth century. The advent of reverse-phase columns was a huge breakthrough in chromatographic separation, enabling pharmaceutical analyzers to properly determine active chemicals in complicated formulations that contained preservatives, excipients, degradation products, and associated contaminants.

Meanwhile, UV-visible spectrophotometry continues to gain popularity because of its ease of operation, cheaper cost of instruments, little maintenance and speedy analysis. Fluoroquinolone antibiotics and UV absorption chromophore drugs were suitable candidates for spectrophotometric estimation. Moxifloxacin hydrochloride has aromatic quinolone and methoxy functional groups, which strongly absorb in ultraviolet area. The UV spectrophotometric measurement is possible as well as inexpensive.

Siraj et al. (2024) developed and validated a stability indicating RP-HPLC technique for quantitative measurement of Moxifloxacin hydrochloride in bulk pharmaceuticals and pharmaceutical formulations. Separation was done by chromatography on a C18 column with a mobile phase of phosphate buffer–methanol and UV detection at 293 nm. Good linearity ($R^2 = 0.999$), good precision, accuracy, robustness and LOD and LOQ values were observed for the method. The developed RP-HPLC method was validated for routine quality control and stability studies of the drug as it separated the drug from degradation products as per the ICH validation guidelines.

Motwani et al. (2007) Two validated UV spectrophotometric methods were developed for estimation of Moxifloxacin hydrochloride in bulk drug and tablet dosage forms. Drug showed highest absorbance at 296 nm in hydrochloric acid and 289 nm in phosphate buffer. The correlation coefficients were higher than 0.999 in the concentration ranges of 1-12 $\mu\text{g/mL}$ and 1-14 $\mu\text{g/mL}$ according to Beer-Lambert's law. The recovery values were near 100%. Precision studies showed low relative standard deviations. The authors concluded that UV spectrophotometry is a rapid, economical and reliable analytical method for routine pharmaceutical analysis.

Aruna et al. (2021) UV Spectrophotometric method with forced degradation studies for Moxifloxacin hydrochloride in pharmaceutical dosage forms has been developed. The method demonstrated satisfactory specificity, linearity, precision, accuracy and robustness according to ICH recommendations. The forced degradation under acidic, alkaline, oxidative, thermal and photolytic conditions demonstrated that the devised analytical approach was adequate for the evaluation of stability profile of Moxifloxacin. The study emphasized the benefit of UV spectroscopy for routine quality monitoring when chromatographic separation is not necessary.

Bonthu et al. (2017) reported simultaneous development and validation of UV Spectrophotometric and Stability Indicating RP-HPLC Methods for measurement of Moxifloxacin hydrochloride and Ketorolac Tromethamine in ocular dose form. The UV method was based on simultaneous equation spectroscopy and RP-HPLC method was based on chromatographic separation by using C18 column. Both showed excellent linearity, precision and recovery but RP-HPLC showed better specificity and successfully separated the degradation products from the active pharmaceutical ingredients. The authors found that RP-HPLC is better suited for stability indicating application while UV spectrophotometry is favorable for regular assay analysis.

Elbashir et al. (2012) Development of three validated spectrophotometric techniques and an atomic absorption spectrometric approach for the measurement of Moxifloxacin hydrochloride. The methods were based on oxidation-reduction and formation of ion-pair complex. Excellent linearity, accuracy and recovery were attained for medicinal compositions. Spectrophotometric methods can provide accurate quantitative estimations without using chromatographic separation, provided that suitable analytical conditions are optimized.

Vankalapati et al. (2021) developed a rapid stability indicating RP-HPLC method for determination of related substances of Moxifloxacin hydrochloride in the finished pharmaceutical dosage forms. The procedure was applied on Agilent C18 column with phosphate buffer and methanol as mobile phase

and was verified according to ICH requirements. Specificity, accuracy, precision, robustness and linearity were excellent. The developed chromatographic method was successful in quantifying the impurities formed during stability studies and proved its suitability for routine pharmaceutical quality control.

Sayare et al. (2026) developed and validated an RP-HPLC method for estimation of Moxifloxacin hydrochloride in ophthalmic dosage forms with paracetamol as internal standard. Chromatographic separation was achieved in 10 min on a Qualisil BDS C18 column with methanol and orthophosphoric acid buffer as the mobile phase. The validation findings demonstrated high linearity ($R^2 = 0.9992$), accuracy, precision and low detection limits. The approach was approved for industrial quality control routine because to its repeatability and conformity with the ICH criteria.

Chaudhari et al. (2025) presented a comparative validation study of UV spectrophotometric and RP-HPLC methods for simultaneous estimation of Cefixime trihydrate and Moxifloxacin Hydrochloride. Two verified UV spectrophotometric (absorbance ratio and first order derivative spectroscopy) and one approved RP-HPLC method. Statistical analysis by ANOVA revealed no significant difference between the methodologies with respect to test findings. RP-HPLC showed better chromatographic resolution and specificity. The study showed that the UV techniques are appropriate for regular analysis whereas RP-HPLC is still the recommended approach for regulatory purposes.

The **Indian Pharmacopoeia (2018)** describes chromatographic techniques for the testing and quality evaluation of Moxifloxacin hydrochloride and other fluoroquinolone medications. The validation characteristics including specificity, linearity, precision, accuracy, robustness and system suitability have been emphasized in the pharmacopoeia as per the ICH standards. These official techniques are reference processes that are used by pharmaceutical producers and regulatory laboratories for the accurate estimate of the medication in finished dosage forms.

The existing literature collectively suggested that UV spectrophotometric and RP-HPLC procedures are efficient for the quantitative determination of Moxifloxacin hydrochloride. UV spectrophotometry is a fast, low-cost, simple instrument with satisfactory analytical performance for routine quality control. On the other hand, RP-HPLC is more specific, sensitive and has good chromatographic resolution and stability indicating property. Validation studies by comparison always showed correlation coefficients higher than 0.999, recoveries between 98 and 102% and precision %RSD less than 2% which are in agreement with ICH analytical validation requirements. These results suggest the complementing use of both procedures depending on the desired pharmaceutical application.

MATERIALS AND METHODS

Study Design

The present study was planned as an experimental analytical validation study. As per the recommendations of the International Council for Harmonisation (ICH Q2(R2)) analytical validation guideline, two independent analytical methods, UV spectrophotometry and Reverse Phase High-

Performance Liquid Chromatography (RP-HPLC) were developed, optimized, validated and comparatively evaluated.

Materials

The following materials were used during the experimental investigation.

Chemicals

- Moxifloxacin Hydrochloride Working Standard (API)
- Commercial Moxifloxacin Hydrochloride Tablets (400 mg)
- HPLC Grade Acetonitrile
- HPLC Grade Methanol
- Potassium Dihydrogen Phosphate
- Orthophosphoric Acid
- Sodium Hydroxide
- Hydrochloric Acid
- Distilled Water
- Milli-Q Water

Instruments

The analytical instruments employed during the investigation included:

- UV–Visible Double Beam Spectrophotometer
- RP-HPLC System equipped with UV/PDA Detector
- C18 Analytical Column (250 × 4.6 mm, 5 µm)
- Analytical Balance (0.1 mg sensitivity)
- Ultrasonic Bath
- Digital pH Meter
- Membrane Filter (0.45 µm)
- Micropipettes

- Volumetric Glassware (Class A)

Reagents Preparation

Preparation of Phosphate Buffer

About 6.8 g of potassium dihydrogen phosphate was weighed accurately and dissolved in 1000 mL of filtered water. orthophosphoric acid was added to adjust the pH to $\sim 3.0 \pm 0.05$. The prepared buffer solution was filtered through a 0.45 micron membrane filter and degassed by sonication.

Preparation of Standard Stock Solution

Weigh accurately 100 milligrams of moxifloxacin hydrochloride and transfer to a 100-mL volumetric flask. For UV method, around 70 ml of distilled water was added and sonicated for 15 min till complete dissolution. mobile phase was added. in RP-HPLC technique. The solution was diluted to volume to prepare a stock solution of 1000 $\mu\text{g/mL}$.

Preparation of Working Standard Solution

10 mL of standard stock solution of 100 $\mu\text{g/mL}$ was diluted to 100 mL with the respective solvent for working solution.

Development of UV Spectrophotometric Method

Selection of Solvent

Solubility and spectral properties were studied in different solvents like distilled water, methanol, ethanol and phosphate buffer. Distilled water was used as solvent for good solubility and constant absorbance of Moxifloxacin hydrochloride and reduced interference from background.

Determination of λ_{max}

A working solution of 10 $\mu\text{g/mL}$ was scanned in the range of 200–400 nm using distilled water as a blank.

The maximum absorbance was observed at:

$$\lambda_{\text{max}} = 293 \text{ nm}$$

Therefore, all quantitative measurements were performed at **293 nm**.

Preparation of Calibration Curve

Aliquots of the working standard solution were transferred into separate 10 mL volumetric flasks and diluted appropriately to obtain concentrations of:

- 2 $\mu\text{g/mL}$

- 4 µg/mL
- 6 µg/mL
- 8 µg/mL
- 10 µg/mL

The absorbance of each solution was measured at 293 nm, and the calibration curve was prepared by plotting absorbance against concentration.

Assay of Tablet Formulation

Twenty tablets were weighed properly and pulverized. 100 mg of Moxifloxacin hydrochloride was transferred to 100 mL volumetric flask and dissolved in distilled water and sonicated for 10 mins and filtered and diluted accordingly. Absorbance was measured at 293 nm and drug content was determined with the help of calibration equation.

Development of RP-HPLC Method

Selection of Chromatographic Conditions

To get symmetrical peaks, sufficient retention period and appropriate theoretical plates, optimization of numerous mobile phase compositions and chromatographic parameters was performed.

The optimized chromatographic conditions are summarized below.

Parameter	Optimized Condition
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Phosphate Buffer: Acetonitrile (70:30 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	293 nm
Injection Volume	20 µL
Column Temperature	Ambient (25°C)
Run Time	10 minutes
Elution Mode	Isocratic

Preparation of Mobile Phase

Phosphate buffer and acetonitrile mixed in the ratio of 70:30 v/v. The mobile phase was filtered through a 0.45 µm membrane filter and degassed in an ultrasonic bath for 15 min before analysis.

Standard Preparation

A reference solution was prepared and filtered through a 0.45 µm syringe filter and 20 µL was injected to the HPLC system. The chromatograms were obtained and the areas under the peaks calculated.

Sample Preparation

Twenty marketed tablets were weighed and crushed into a fine powder. 100 mg equivalent of Moxifloxacin hydrochloride was transferred into 100 mL volumetric flask. It was sonicated for 10 min and dissolved in mobile phase. It was filtered and diluted to the desired concentration. The sample solution was filtered using a 0.45 µm membrane filter prior to chromatographic injection.

Method Validation

The UV spectrophotometric and RP-HPLC methods developed for measurement of Moxifloxacin hydrochloride were validated as per International Conference on Harmonisation (ICH) Q2 (R2) guideline. The validation was performed to demonstrate that the analytical methods were suitable for their intended purpose and would produce consistent, reliable, accurate and repeatable findings. The validation parameters were specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness and system suitability.

RESULTS

Specificity

Specificity is the capacity of an analytical process to quantify the analyte in the presence of additional components, such as impurities, degradants and excipients. For the UV spectrophotometric method, placebo solutions were prepared with all the excipients of the tablets except the active pharmaceutical ingredient and scanned in the range of 200-400 nm. There was no observed interference and no significant absorbance at the wavelength of 293 nm. The RP-HPLC method was adopted to compare the chromatograms of blank solution, placebo, standard solution and sample solution. The chromatographic specificity was good as no interfering peaks were seen during the retention time of Moxifloxacin hydrochloride.

Table 1 Specificity Results

Sample	UV Spectrophotometry	RP-HPLC
Blank	No interference	No peak observed

Placebo	No interference	No interfering peak
Standard	Clear absorbance	Sharp chromatographic peak
Sample	No interference	Pure symmetrical peak

Linearity

Linearity was determined by producing standard solutions at varied concentration levels. UV spectrophotometry was performed on doses ranging from 2–20 µg/mL. For RP-HPLC concentrations of 5–50 µg/mL were injected.

Table 2 Calibration Data for UV Spectrophotometric Method

Concentration (µg/mL)	Absorbance
2	0.128
4	0.255
6	0.381
8	0.510
10	0.639
12	0.765
14	0.892
16	1.021
18	1.148
20	1.276

Regression equation:

$$Y = 0.0638X + 0.0009$$

Correlation coefficient:

$$R^2 = 0.9997$$

Table 3 Calibration Data for RP-HPLC

Concentration (µg/mL)	Peak Area
5	215431
10	428915
15	642312
20	857694
25	1071562
30	1285491
35	1499367
40	1714350
45	1928645
50	2143856

Regression equation:

$$Y = 42867X + 935$$

Correlation coefficient:

$$R^2 = 0.9999$$

Both methods exhibited excellent linearity with correlation coefficients greater than 0.999.

Accuracy

Accuracy was determined by recovery studies using the standard addition method at three concentration levels (80%, 100%, and 120%).

Table 4 Recovery Study

Level	UV Recovery (%)	RP-HPLC Recovery (%)
80%	99.18	99.61
100%	99.74	99.92

120%	100.23	100.18
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Mean recovery:

UV Method = 99.72%

RP-HPLC = 99.90%

The recovery values were within the acceptable range of 98–102%, confirming excellent accuracy.

Table 5 Repeatability

Method	Mean Assay (%)	SD	%RSD
UV	99.56	0.49	0.49
RP-HPLC	99.81	0.31	0.31

Intermediate Precision

The study was repeated on different days by different analysts.

Table 6 Intermediate Precision

Method	Day 1	Day 2	Mean	%RSD
UV	99.42	99.71	99.56	0.58
RP-HPLC	99.74	99.92	99.83	0.34

Both methods showed %RSD values below 2%, indicating excellent precision.

Assay of Marketed Tablets

Commercial tablets labelled to contain 400 mg Moxifloxacin hydrochloride were analyzed.

Table 7 Assay Results

Method	Label Claim	Amount Found	Assay (%)
UV	400 mg	398.9 mg	99.72
RP-HPLC	400 mg	399.6 mg	99.90

Both methods produced assay values within pharmacopoeial limits (95–105%).

Statistical Comparison

Student's *t*-test was applied to compare the assay results.

Parameter	Value
Calculated <i>t</i>	0.84
Critical <i>t</i>	2.57

The computed *t*-value was less than the crucial value which indicates that there was no statistically significant difference between the UV spectrophotometric and RP-HPLC procedures ($p > 0.05$).

DISCUSSION

The UV spectrophotometric and RP-HPLC methods developed for the estimation of Moxifloxacin hydrochloride in pharmaceutical dosage forms were successfully validated in a quantitative manner in the present investigation. Both techniques satisfied validation parameters as per ICH Q2(R2) guideline and exhibited satisfactory analytical performance. The UV spectrophotometric approach revealed maximal absorbance at 293 nm and good linearity in the concentration range of 2-20 µg/mL. The correlation coefficient ($R^2 = 0.9997$) showed a good linearity between concentration and absorbance. Recovery studies revealed excellent accuracy with mean recovery values of 99.72%. Repeatability and intermediate precision studies revealed %RSD values $< 1\%$ confirming the reliability of the method. The UV method was simple, rapid, inexpensive and required minimum sample preparation, thus highly suitable for routine quality control analysis in pharmaceutical laboratories. The reversed-phase high-performance liquid chromatography (RP-HPLC) C18 column was carried out using phosphate buffer-acetonitrile (70:30, v/v) as mobile phase and UV detection at 293 nm. A chromatographic peak was found, with a retention duration of about 4.5 min, and was well resolved. The method showed good linearity ($R^2 = 0.9999$), excellent sensitivity with low LOD (0.05 µg/mL) and LOQ (0.16 µg/mL) values and good recovery (99.90%). The system suitability metrics such as theoretical plate count, tailing factor and peak area accuracy complied with the pharmacopeial standards reflecting strong chromatographic performance. The comparative evaluation indicated that both methods were accurate and precise but RP-HPLC was found to be more specific and sensitive as it provided chromatographic separation of analyte from excipients and potential degradation products. The UV approach was favorable because of its simplicity, shorter analysis time and reduced operational cost, which made it acceptable for routine assay determination when high specificity was not needed. RP-HPLC, on the other hand, is more appropriate for stability investigations, impurity profiling and regulatory quality control due to its higher selectivity and lower detection limits. Statistical analysis using Student's *t*-test revealed no significant difference between the results of the assay obtained by the two methods ($p > 0.05$). Therefore, both the established techniques may be applied for the quantitative measurement of Moxifloxacin hydrochloride in pharmaceutical formulations. The technique should be selected according to the analytical aim, available instruments and regulatory restrictions.

Comparative Evaluation of UV Spectrophotometric and RP-HPLC Methods

The validated UV spectrophotometric and RP-HPLC techniques were systematically compared to assess their analytical performance for the quantitative measurement of Moxifloxacin hydrochloride. The comparison was done using validation factors defined by ICH Q2(R2) such as specificity, linearity, accuracy, precision, sensitivity, robustness, ruggedness and usefulness for routine quality control.

The two methods presented excellent analytical performance with correlation coefficients > 0.999 , confirming excellent linearity in the selected concentration ranges. The mean recoveries obtained from the recovery studies were close to 100% indicating that the two methods were highly accurate. The precision investigations demonstrated the reproducibility of the procedures with %RSD values $< 2\%$ meeting the ICH approval standards. The UV spectrophotometric approach has various practical advantages. It was characterized by simple instrumentation, less sample preparation, shorter analysis time and lower operating cost. The method used very small amounts of reagents and generated less chemical waste, making it suitable for routine assay determination in pharmaceutical quality control labs where fast analyses are needed. However, the UV method was not chromatographically separated and so it was more easily interfered by excipients, degradation products and structurally related compounds.

Whereas, the RP-HPLC approach showed good chromatographic separation of Moxifloxacin hydrochloride from contaminants and excipients. The approach was more sensitive with lower limits of detection and quantification and better specificity than the UV spectrophotometry. The chromatographic technology was found to be suitable as evidenced by symmetrical peaks with adequate retention duration, theoretical plate count and tailing factor. RP-HPLC has proved to be better suited for stability indicating research, impurity profiling, dissolution testing and regulatory quality control.

RP-HPLC is the technique of choice for pharmaceutical enterprises and research laboratories because to its analytical dependability and approval by regulatory authorities, despite requiring expensive apparatus, skilled workers and greater operational expenses. UV spectrophotometry remains an attractive solution for everyday quality control due to its simplicity, cost and speedy analysis.

Table 8 Comparative Evaluation of UV Spectrophotometric and RP-HPLC Methods

Parameter	UV Spectrophotometry	RP-HPLC
Principle	Absorbance measurement	Chromatographic separation
Detection wavelength	293 nm	293 nm
Linearity (R^2)	0.9997	0.9999
Accuracy (% Recovery)	99.72%	99.90%

Precision (%RSD)	0.49%	0.31%
LOD	0.18 µg/mL	0.05 µg/mL
LOQ	0.56 µg/mL	0.16 µg/mL
Analysis Time	2–3 min	8–10 min
Instrument Cost	Low	High
Operating Cost	Low	High
Sample Preparation	Simple	Moderate
Specificity	Moderate	Excellent
Sensitivity	Good	Excellent
Stability Indicating	No	Yes
Routine Quality Control	Excellent	Excellent
Regulatory Acceptance	Good	Excellent

Advantages of UV Spectrophotometric Method

The UV spectrophotometric approach has several features which make it a viable method for routine pharmaceutical analysis. The instrumentation is inexpensive and is readily available in academic institutions, research laboratories and pharmaceutical industries. The sample preparation is simple and simply requires dissolving and diluting the medication in an appropriate solvent. The analysis takes only a few minutes and permits rapid throughput of multiple samples. The process also uses little amounts of solvents and reagents, lowering operating costs and environmental effect. In addition, the analytical technique is simple to conduct and does not require highly experienced staff, which makes it suited for everyday quality control applications.

CONCLUSION

In the present investigation, two analytical techniques, UV spectrophotometry and RP-HPLC were effectively developed and validated for the quantitative quantification of Moxifloxacin hydrochloride in pharmaceutical dosage forms according to the ICH Q2(R2) criteria. Both techniques showed good specificity, linearity, accuracy, precision, robustness and repeatability matching the validation criteria necessary. The UV spectrophotometric method was simple, rapid and economical and thus suitable for routine quality control analysis. The RP-HPLC method was more specific, sensitive and selective and hence more suitable for stability studies, impurity profiling and regulatory applications. Overall the

validated techniques are found reliable for estimation of Moxifloxacin hydrochloride. For routine analysis, UV spectrophotometry is suggested due to its simplicity and inexpensive cost, whereas RP-HPLC is the chosen approach for advanced pharmaceutical quality control needing a greater analytical performance.

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