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DEVELOPMENT AND VALIDATION OF A RELIABLE, PRECISE AND STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF OZENOXACIN IN PHARMACEUTICAL FORMULATIONS: A REVIEW

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Abstract:

Analytical method development and validation are essential components of pharmaceutical quality control to ensure the safety, efficacy, and stability of drug products. Ozenoxacin is a non-fluorinated quinolone antibiotic widely used in topical pharmaceutical formulations for the treatment of bacterial skin infections. Accurate estimation of this drug in dosage forms is necessary to maintain product quality and meet regulatory requirements. Reverse Phase High- Performance Liquid Chromatography (RP-HPLC) has emerged as a preferred analytical technique due to its high sensitivity, precision, and ability to separate active pharmaceutical ingredients from degradation products and excipients.This review presents a comprehensive overview of the development and validation of a reliable, precise, and stability-indicating RP-HPLC method for the estimation of Ozenoxacin in pharmaceutical formulations. The review discusses key aspects of method development, including selection of chromatographic conditions, mobile phase optimization, and detection parameters. Emphasis is placed on validation parameters such as linearity, accuracy, precision, specificity, robustness, and sensitivity, in accordance with international regulatory guidelines. Additionally, the importance of forced degradation studies in establishing the stability- indicating nature of the method is highlighted. Overall, this review aims to provide useful insights for researchers and analysts involved in pharmaceutical analysis and quality control of Ozenoxacin formulations.

Keywords: *Ozenoxacin; RP-HPLC; Stability-indicating method; Method validation; Pharmaceutical analysis*

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1. INTRODUCTION:

In the pharmaceutical industry, the quality, safety, and efficacy of drug products depend greatly on the use of accurate and reliable analytical methods. Analytical method development plays a vital role in drug discovery, formulation development, stability studies, and routine quality control testing. Among the various analytical techniques available, High-Performance Liquid Chromatography (HPLC) has gained widespread acceptance due to its high sensitivity, selectivity, precision, and reproducibility. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), in particular, is extensively employed for the quantitative estimation of drugs in bulk materials as well as finished pharmaceutical formulations.[1,2]

Ozenoxacin is a relatively newer non-fluorinated quinolone antibiotic used mainly for topical treatment of bacterial skin infections. Due to its therapeutic importance and increasing clinical use, there is a strong need for well-validated analytical methods that can accurately quantify the drug in pharmaceutical dosage forms. Reliable estimation of Ozenoxacin is essential not only for routine assay testing but also for monitoring its stability during manufacturing, storage, and shelf-life evaluation.[3]

Stability-indicating analytical methods are especially important because they can distinguish the active pharmaceutical ingredient from its degradation products, impurities, and formulation excipients. According to regulatory requirements, a stability-indicating method must be capable of detecting changes in drug content under various stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments. RP-HPLC is considered one of the most suitable techniques for developing stability-indicating methods due to its ability to provide efficient separation and accurate quantification in complex matrices.[4,5]

Method validation is another critical aspect of analytical method development. Validation ensures that the developed method is suitable for its intended purpose and consistently produces reliable results. Parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection, and limit of quantification are evaluated as per international regulatory guidelines. A properly validated RP-HPLC method enhances confidence in analytical results and supports regulatory approval and quality assurance activities.[6]

This review paper focuses on the development and validation of a reliable, precise, and stability-indicating RP-HPLC method for the estimation of Ozenoxacin in pharmaceutical formulations. It summarizes reported analytical approaches, discusses method development strategies, validation requirements, and stability studies, and highlights the significance of RP- HPLC in pharmaceutical analysis.[7]

2. Drug Profile of Ozenoxacin

Ozenoxacin is a non-fluorinated quinolone antibiotic developed for topical use in the management of bacterial skin infections. It has gained clinical importance due to its strong antibacterial activity combined with minimal systemic absorption, which reduces the risk of systemic side effects. The drug is mainly indicated for superficial skin infections such as impetigo and is formulated specifically for dermatological application. Owing to its increasing therapeutic use, accurate analytical evaluation of Ozenoxacin in pharmaceutical formulations has become essential.[7,8]

Chemically, Ozenoxacin belongs to the quinolone class of antibacterial agents and possesses a quinolone core structure with specific substituents that enhance antibacterial potency. Unlike fluoroquinolones, it lacks a fluorine atom, which contributes to improved safety and reduced phototoxicity. The chemical structure significantly influences its chromatographic behavior, including retention time and interaction with the stationary phase in RP-HPLC systems.[9,10]

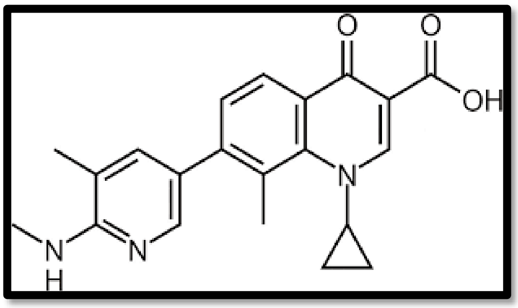

Figure 1: Chemical structure of Ozenoxacin

It provides a visual representation of the molecular framework relevant to analytical method development.

From a physicochemical perspective, Ozenoxacin appears as a crystalline solid with moderate lipophilicity. It exhibits limited aqueous solubility but shows better solubility in organic solvents such as methanol and acetonitrile, which are commonly employed in HPLC mobile phases. The drug demonstrates adequate UV absorbance, making it suitable for detection using UV or photodiode array detectors. These physicochemical properties play a critical role in selecting chromatographic conditions such as mobile phase composition, pH, and detection wavelength.[11,12]

Pharmacologically, Ozenoxacin exerts its antibacterial action by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes that are essential for bacterial DNA replication. This dual mechanism leads to effective bactericidal activity, particularly against Gram-positive organisms responsible for skin infections. Its localized topical action ensures high drug concentration at the site of infection while minimizing systemic exposure.[13] Ozenoxacin is commonly available in topical dosage forms such as creams and ointments, which contain multiple excipients including emulsifiers, preservatives, and stabilizing agents. These excipients may interfere with drug analysis, highlighting the need for a selective and specific analytical method. RP-HPLC is considered an ideal technique for this purpose due to its superior separation efficiency.[14]

Stability studies are crucial for ensuring product quality throughout its shelf life. Ozenoxacin may undergo degradation under stress conditions such as heat, light, and chemical exposure, necessitating the development of stability-indicating analytical methods.

Table 1: Physicochemical properties of Ozenoxacin relevant to RP-HPLC analysis[14,15]

Parameter	**Description / Value**
Chemical name	Ozenoxacin
Chemical class	Non-fluorinated quinolone antibiotic
Molecular formula	$C_{21}H_{18}FN_3O_4$
Molecular weight	~395.38 g/mol
Physical appearance	White to off-white crystalline powder
Solubility	Slightly soluble in water; freely soluble in methanol and acetonitrile
Log P (partition coefficient)	Approximately 2.5–3.0
pKa value	~6.5–7.0
UV absorption maximum (λmax)	Around 245–260 nm
Stability	Sensitive to light, heat, acidic, alkaline, and oxidative conditions
Common dosage forms	Topical cream and ointment
Analytical technique suitability	Suitable for RP-HPLC with UV/PDA detection

3. Regulatory Perspective on Analytical Method Validation

3.1 Importance of Regulatory Guidelines

Regulatory guidelines play a vital role in pharmaceutical analysis by ensuring that drug products meet required standards of quality, safety, and efficacy. Analytical methods used for drug estimation must be reliable and capable of producing accurate and reproducible results. Regulatory authorities require validated analytical procedures for product approval, routine quality control, and stability studies. Proper validation of analytical methods helps in minimizing errors, ensuring consistency, and maintaining compliance with regulatory expectations throughout the product life cycle.[16]

3.2 ICH Guidelines for Method Validation

The **International Council for Harmonisation** has established globally accepted guidelines for analytical method validation. According to ICH Q2 (R1), method validation involves the evaluation of parameters such as specificity, linearity, accuracy, precision, range, limit of detection, limit of quantification, and robustness. These parameters confirm that the analyticalmethod is suitable for its intended purpose. Following ICH guidelines improves the reliability of analytical results and supports regulatory submissions and approvals.[17]

3.3 Regulatory Requirements for Stability-Indicating Methods

Stability-indicating analytical methods are mandatory for evaluating the stability of pharmaceutical products. Regulatory authorities require such methods to accurately measure the active pharmaceutical ingredient without interference from degradation products, impurities, or excipients. Forced degradation studies are conducted under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments. These studies help demonstrate the specificity of the method and its ability to separate the drug from its degradation products.[18]

3.4 Acceptance of RP-HPLC in Regulatory Analysis

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely accepted by regulatory agencies due to its high sensitivity, selectivity, and reproducibility. RP-HPLC methods are commonly used for assay determination, impurity profiling, and stability testing. For topical formulations like Ozenoxacin, RP-HPLC provides accurate drug estimation even in the presence of complex excipient matrices, making it a preferred technique in regulatory and quality control laboratories.[19]

4. Overview of RP-HPLC Technique

4.1 Principle of Reverse Phase HPLC

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis. In RP-HPLC, separation is achieved based on the differential partitioning of analytes between a non-polar stationary phase and a relatively polar mobile phase. The stationary phase commonly consists of silica particles chemically bonded with hydrophobic groups such as C18 or C8. Compounds with higher hydrophobicity exhibit greater retention on the column, allowing efficient separation of analytes from impurities and degradation products.[20]

4.2 Instrumentation of RP-HPLC

An RP-HPLC system consists of several essential components that work together to achieve accurate separation and detection. These include a solvent delivery system (pump), sample injector, chromatographic column, detector, and data acquisition system. The pump ensures a constant and precise flow of the mobile phase, while the injector introduces a fixed volume of sample into the system. The column is the core component where separation occurs, and detectors such as UV or photodiode array (PDA) detectors are commonly used for drug analysis due to their sensitivity and simplicity.[21]

4.3 Selection of Stationary and Mobile Phases

The selection of an appropriate stationary phase is crucial for achieving good resolution and peak symmetry. C18 columns are most commonly used due to their strong hydrophobic interactions and wide applicability. The mobile phase generally consists of a mixture of aqueous buffer and organic solvents such as methanol or acetonitrile. Mobile phase composition, pH, and flow rate significantly influence retention time, peak shape, and resolution, and therefore require careful optimization during method development.[22]

4.4 Detection Techniques in RP-HPLC

Detection in RP-HPLC is typically performed using UV or PDA detectors, which are suitable for compounds with UV-absorbing functional groups. The selection of detection wavelength is based on the maximum absorbance of the analyte, ensuring high sensitivity and accuracy. RP- HPLC combined with suitable detection techniques provides reliable quantification of drugs in complex pharmaceutical formulations.[23]

5. Method Development Strategy for Estimation of Ozenoxacin by RP-HPLC

5.1 Importance of Method Development

Method development is a systematic and critical step in establishing a reliable RP-HPLC procedure for the estimation of pharmaceutical drugs. The objective of method development is to achieve accurate quantification of the analyte with acceptable resolution, peak symmetry, sensitivity, and reproducibility. For Ozenoxacin, method development must also ensure adequate separation from formulation excipients and potential degradation products to support stability-indicating analysis. A well-developed method forms the foundation for successful validation and routine quality control testing.[24]

5.2 Selection of Chromatographic Conditions

The selection of chromatographic conditions is guided by the physicochemical properties of Ozenoxacin. Reverse phase columns, particularly C18 columns, are commonly preferred due to their strong hydrophobic interactions and broad applicability. Column dimensions and particle size significantly influence separation efficiency and analysis time. Shorter columns with smaller particle sizes provide faster analysis, whereas longer columns offer improved resolution.[25]

The choice of mobile phase plays a crucial role in achieving optimal retention time and peak shape. A combination of aqueous buffer and organic solvent such as acetonitrile or methanol is typically employed. The pH of the aqueous phase is carefully adjusted to maintain the drug in a suitable ionization state, minimizing peak tailing and improving reproducibility. Flow rate and column temperature are also optimized to balance resolution and run time.

5.3 Optimization of Mobile Phase Composition

Mobile phase optimization involves systematic variation of solvent ratio, pH, and buffer strength. Acetonitrile is often preferred over methanol due to its lower viscosity and better peak symmetry. Buffer systems such as phosphate buffer are widely used because of their good buffering capacity and compatibility with UV detection. Proper pH control is essential to ensure consistent retention and avoid variability during routine analysis.

Gradient elution is generally not required for single-component analysis like Ozenoxacin; therefore, isocratic elution is preferred due to simplicity and reproducibility. Optimization studies focus on achieving a sharp, symmetric peak with adequate theoretical plates and minimal interference.[26]

5.4 Selection of Detection Wavelength

Ozenoxacin exhibits sufficient UV absorbance due to its chromophoric functional groups. The detection wavelength is selected based on the drug's maximum absorbance to ensure high sensitivity. UV detection is widely used in routine analysis because it is cost-effective, robust, and suitable for quality control laboratories. Photodiode array detection further supports peak purity assessment, which is important for stability-indicating methods.[27]

5.5 System Suitability Considerations

Before sample analysis, system suitability parameters such as retention time, theoretical plate count, tailing factor, and % relative standard deviation (%RSD) of peak area are evaluated. These parameters ensure that the chromatographic system is performing adequately and capable of producing reliable results. Meeting system suitability criteria is a regulatory expectation and confirms the readiness of the analytical system.[28]

5.6 Application to Pharmaceutical Formulations

Topical formulations of Ozenoxacin contain various excipients that may interfere with drug analysis. Therefore, sample preparation procedures such as dilution, filtration, and sonication are optimized to extract the drug efficiently without affecting chromatographic performance. The developed RP-HPLC method must demonstrate specificity by clearly resolving the Ozenoxacin peak from excipient peaks.[29]

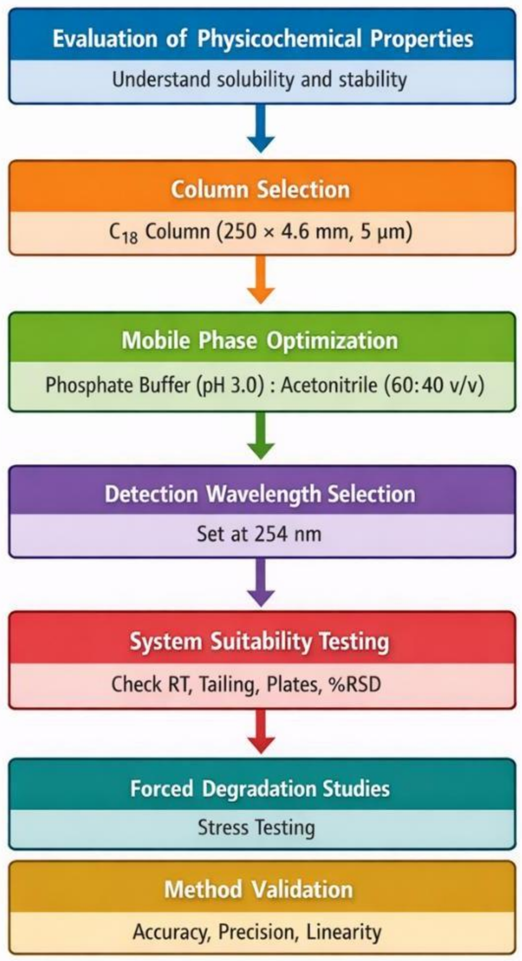

Figure 2: General strategy for RP-HPLC method development for Ozenoxacin, illustrating selection of column,

mobile phase optimization, detection wavelength selection, and system suitability evaluation.[30]

Table 2: Optimized RP-HPLC chromatographic conditions for estimation of Ozenoxacin

Parameter	Optimized Condition
Column	C18 column (250 × 4.6 mm, 5 µm)
Mobile phase	Phosphate buffer (pH 3.0) : Acetonitrile (60:40 v/v)
Buffer	0.02 M potassium dihydrogen phosphate
Flow rate	1.0 mL/min
Detection wavelength	254 nm
Injection volume	20 µL
Column temperature	Ambient (25 ± 2 °C)
Elution mode	Isocratic
Run time	10 minutes
Retention time	~4.5 minutes

5.7 Significance of the Developed Method

The optimized RP-HPLC method provides a balance between simplicity, sensitivity, and robustness. It is suitable for routine quality control analysis as well as stability studies. The use of commonly available solvents and columns enhances method reproducibility and regulatory acceptability. This method development strategy ensures reliable estimation of Ozenoxacin in pharmaceutical formulations and supports subsequent validation and stability-indicating studies.[31,32]

6. Stability-Indicating RP-HPLC Method for Estimation of Ozenoxacin

A stability-indicating analytical method is an essential requirement in pharmaceutical analysis, as it ensures accurate estimation of the active pharmaceutical ingredient in the presence of degradation products, impurities, and formulation excipients. For drugs like Ozenoxacin, which are used in topical formulations and exposed to various environmental conditions, the development of a stability-indicating RP-HPLC method is particularly important. Regulatory agencies require such methods to demonstrate the stability of the drug throughout its shelf life and under different stress conditions.[33]

The primary objective of a stability-indicating RP-HPLC method is to achieve clear separation between the drug peak and any peaks arising from degradation products. This is achieved through forced degradation studies, where the drug substance or formulation is subjected to stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments. These studies help in understanding degradation pathways and confirm the specificity of the developed analytical method.[34]

Forced degradation under acidic and alkaline conditions is usually performed by exposing the drug to dilute hydrochloric acid or sodium hydroxide solutions. These conditions simulate pH-related degradation that may occur during storage or formulation. Oxidative degradation is carried out using hydrogen peroxide to evaluate the drug's susceptibility to oxidation. Thermal degradation studies involve exposing the drug to elevated temperatures, while photolytic degradation assesses the effect of light exposure. The stressed samples are then analyzed using the developed RP-HPLC method to evaluate separation efficiency.

A stability-indicating method must demonstrate specificity, meaning that the Ozenoxacin peak should remain well resolved from degradation peaks with acceptable peak purity. Photodiode array detection is often employed to assess peak purity and confirm the absence of co-eluting impurities. Adequate resolution and symmetrical peak shape indicate that the method is suitable for stability analysis.[35]

The extent of degradation observed under different stress conditions provides valuable information about the chemical stability of Ozenoxacin. Generally, moderate degradation under extreme conditions is desirable to prove the discriminatory power of the method. Excessive degradation is avoided to prevent complete loss of the parent drug peak.[36]

Table 3: Typical forced degradation conditions for stability-indicating RP-HPLC method

Stress condition	Degradation agent	Exposure conditions
Acidic	0.1 N HCl	60 °C for 2–4 hours
Alkaline	0.1 N NaOH	60 °C for 2–4 hours
Oxidative	3% H_2O_2	Room temperature for 24 hours
Thermal	Dry heat	80 °C for 24 hours
Photolytic	UV light	As per ICH guidelines

Following forced degradation, chromatograms are examined to ensure that degradation products do not interfere with the retention time of Ozenoxacin. The method is considered stability-indicating only if the drug peak remains pure and well separated under all stress conditions. The retention time consistency and peak symmetry further support method reliability.[37]

Validation of the stability-indicating RP-HPLC method is carried out in accordance with regulatory guidelines. Parameters such as specificity, precision, accuracy, and robustness are particularly important in stability studies. Robustness testing involves deliberate variation in method conditions, such as slight changes in mobile phase composition, pH, or flow rate, to evaluate method performance.[38]

Table 4: Key system suitability parameters for stability-indicating RP-HPLC method

Parameter	Acceptance criteria	Typical value
Retention time	Consistent	~4.5 min
Tailing factor	≤ 2.0	1.2–1.4
Theoretical plates	≥ 2000	> 5000
% RSD of peak area	≤ 2.0	< 1.0

The stability-indicating RP-HPLC method developed for Ozenoxacin offers reliable quantification in pharmaceutical formulations and supports regulatory compliance. Its ability to separate degradation products from the active drug ensures accurate stability assessment during shelf-life studies. Such methods are indispensable for quality control laboratories and play a critical role in ensuring the safety and efficacy of pharmaceutical products.[39]

7. Validation of RP-HPLC Method for Estimation of Ozenoxacin

Validation of the developed RP-HPLC method is a critical step to ensure that the analytical procedure is reliable, accurate, and suitable for its intended purpose. Method validation confirms that the RP-HPLC method consistently produces reproducible results when applied to the estimation of Ozenoxacin in pharmaceutical formulations. Validation parameters are evaluated in accordance with internationally accepted regulatory guidelines and include specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness.

Specificity is the ability of the method to measure the analyte response in the presence of potential interferences such as excipients, impurities, and degradation products. In the developed method, specificity is demonstrated by analyzing blank samples, placebo samples, and stressed samples. The chromatograms show no interfering peaks at the retention time of Ozenoxacin, confirming that the method is specific and suitable for routine analysis and stability studies.[40,41]

Linearity evaluates the method's ability to obtain test results that are directly proportional to the concentration of analyte within a given range. Linearity is assessed by analyzing standard solutions of Ozenoxacin at different concentration levels. A calibration curve is constructed by plotting peak area versus concentration, and the correlation coefficient is calculated. The method demonstrates excellent linearity over the selected concentration range, indicating its suitability for quantitative analysis.[42]

Table 5: Linearity data for RP-HPLC estimation of Ozenoxacin

Concentration (µg/mL)	Mean peak area
5	128945
10	256781
15	384912
20	512603

25	640428

The correlation coefficient (r^2) obtained is greater than 0.999, confirming good linearity.

Accuracy expresses the closeness of agreement between the measured value and the true value. It is determined by recovery studies using the standard addition method. Known amounts of Ozenoxacin are added to the pre-analyzed sample at different levels, and the percentage recovery is calculated. The results indicate that the method is accurate and free from interference by formulation excipients.[43]

Table 6: Accuracy (recovery) studies for Ozenoxacin

Recovery level (%)	Amount added (µg/mL)	% Recovery
80	8	99.2
100	10	100.1
120	12	99.6

Precision indicates the degree of repeatability of the analytical method under normal operating conditions. Precision is evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). The results are expressed as percentage relative standard deviation (%RSD), which remains within acceptable limits, demonstrating good precision of the method.[44]

Table 7: Precision data for RP-HPLC method

Parameter	%RSD
Intra-day precision	0.68
Inter-day precision	0.82

Sensitivity of the method is assessed by determining the limit of detection (LOD) and limit of quantification (LOQ). These parameters indicate the lowest amount of drug that can be detected and quantified with acceptable accuracy and precision. LOD and LOQ are calculated based on the standard deviation of the response and the slope of the calibration curve.

Table 8: Sensitivity parameters

Parameter	Value
LOD	0.5 µg/mL
	1.5 µg/mL

Robustness evaluates the reliability of the method under small, deliberate variations in analytical conditions such as flow rate, mobile phase composition, and detection wavelength. Minor changes in these parameters do not significantly affect the retention time or peak area, indicating that the method is robust.[45]

Ruggedness assesses the reproducibility of the method under normal but variable laboratory conditions, such as different analysts or instruments. The method shows consistent results, confirming its rugged nature.

Overall, the validated RP-HPLC method demonstrates excellent specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness. These validation results confirm that the method is suitable for routine quality control analysis and stability testing of Ozenoxacin in pharmaceutical formulations, meeting regulatory requirements and ensuring product quality and safety.[46]

8. Application of the RP-HPLC Method to Pharmaceutical Formulations

The practical applicability of a developed and validated RP-HPLC method is demonstrated through its successful use in the analysis of pharmaceutical dosage forms. For topical antibiotic products, accurate estimation of the active drug in the presence of excipients is essential to ensure dose uniformity, therapeutic efficacy, and regulatory compliance. The validated RP- HPLC method was applied to the quantitative estimation of Ozenoxacin in marketed pharmaceutical formulations to confirm its suitability for routine quality control and stability testing.[47]

Topical formulations such as creams and ointments contain a complex mixture of excipients including emulsifiers, preservatives, stabilizers, and viscosity-enhancing agents. These components may interfere with analytical measurements if the method lacks sufficient specificity. Therefore, appropriate sample preparation is a critical step. The formulation sample is accurately weighed, dissolved in a suitable solvent such as methanol, sonicated to ensure complete extraction of the drug, and filtered to remove insoluble excipients. The resulting solution is suitably diluted with the mobile phase before injection into the HPLC system.

The assay of marketed formulations is performed by comparing the peak area of the sample solution with that of a standard solution of known concentration. The assay results provide information on the actual drug content present in the formulation relative to the labeled claim. The method demonstrates good selectivity, as no interfering peaks are observed at the retention time of Ozenoxacin, confirming that excipients do not affect drug quantification.[48]

Table 9: Assay results of Ozenoxacin in marketed pharmaceutical formulation

Formulation	Labeled claim	Amount found	% Assay
Topical cream	10 mg/g	9.92 mg/g	99.2

The assay results fall within the acceptable regulatory limits of 95–105%, indicating that the formulation meets quality standards. The low variability in results further demonstrates the precision and reliability of the developed RP-HPLC method.

Recovery studies are also performed as part of method application to evaluate the accuracy of drug estimation in the presence of formulation excipients. Known quantities of standard Ozenoxacin are added to the pre-analyzed formulation sample, and the total amount recovered is calculated. High recovery values indicate that the method is capable of accurately estimating the drug without interfernce from formulation components.[49]

Table 10: Recovery studies for Ozenoxacin in pharmaceutical formulation

Level (%)	Amount added (mg)	Amount recovered (mg)	% Recovery
80	8	7.94	99.3
100	10	10.02	100.2
120	12	11.90	99.1

The recovery values are close to 100%, confirming the accuracy of the method and its suitability for routine analysis. These results also support the specificity of the method, as excipients present in the formulation do not interfere with the drug peak.

In addition to assay and recovery studies, the method is suitable for use in stability testing of pharmaceutical formulations. Samples subjected to stability studies under different storage conditions can be analyzed using the same RP-HPLC method to monitor changes in drug content over time. The ability of the method to separate the drug from its degradation products ensures accurate assessment of formulation stability.[50]

The application of the validated RP-HPLC method to pharmaceutical formulations highlights its robustness and reproducibility. The use of commonly available solvents, standard chromatographic columns, and UV detection makes the method cost-effective and practical for quality control laboratories. Furthermore, the simplicity of the sample preparation procedure enhances its routine applicability in industrial and regulatory settings.[51]

9. Future Perspectives

The development of reliable and stability-indicating RP-HPLC methods for the estimation of Ozenoxacin has strengthened quality control and regulatory evaluation of pharmaceutical formulations. Despite the effectiveness of current methods, future advancements in analytical technologies offer significant scope for improvement.

One important future direction is the adoption of ultra-high-performance liquid chromatography (UHPLC), which enables faster analysis, improved resolution, and reduced solvent consumption. Such techniques can enhance efficiency in high-throughput quality control laboratories. Additionally, the application of green analytical chemistry principles is expected to gain importance, with emphasis on minimizing organic solvent usage and reducing environmental impact without compromising analytical performance.

Hyphenated techniques such as HPLC coupled with mass spectrometry (HPLC-MS) may further support detailed identification of degradation products and impurity profiling, providing deeper insight into the stability behavior of Ozenoxacin. Automation and software- assisted method optimization also represent promising areas, as they can improve reproducibility, reduce human error, and shorten method development time.

SUMMARY & CONCLUSION:

This review focuses on the development and validation of a reliable and stability-indicating RP-HPLC method for the estimation of Ozenoxacin in pharmaceutical formulations. RP-HPLC is widely used in pharmaceutical analysis because it provides accurate, precise, and reproducible results. The review discusses the importance of method development, optimization of chromatographic conditions, forced degradation studies, and method validation as per regulatory guidelines. Validation parameters such as linearity, accuracy, precision, specificity, robustness, and sensitivity confirm the suitability of the method. Application of the method to marketed formulations demonstrates its usefulness in routine quality control and stability testing. Overall, the review highlights the importance of stability-indicating analytical methods in ensuring drug quality and safety.

A validated stability-indicating RP-HPLC method is essential for reliable estimation of Ozenoxacin in pharmaceutical formulations. The discussed method provides accurate and consistent results even in the presence of degradation products and excipients. Its simplicity, robustness, and regulatory acceptability make it suitable for routine analysis and stability studies. Such methods play a key role in maintaining product quality, supporting regulatory compliance, and ensuring patient safety.

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