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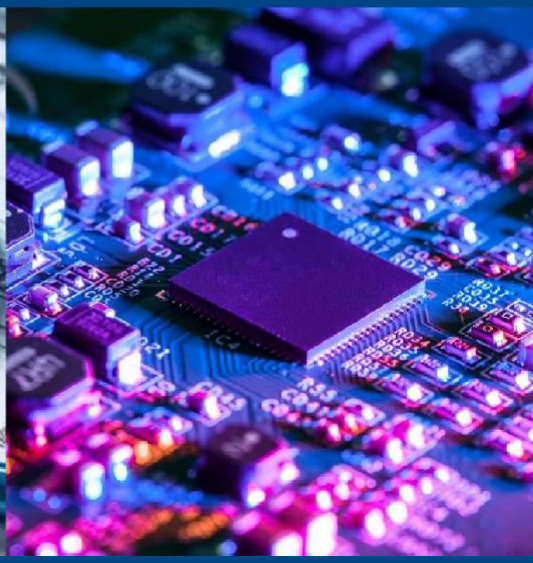

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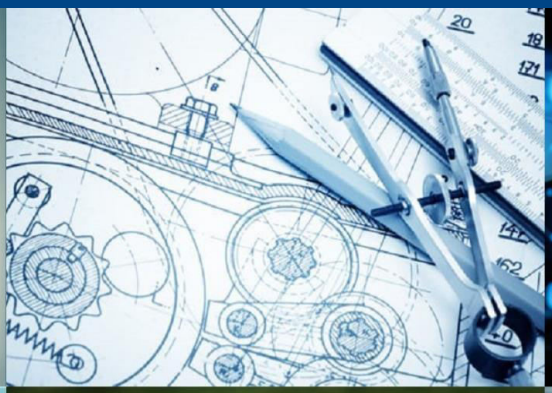

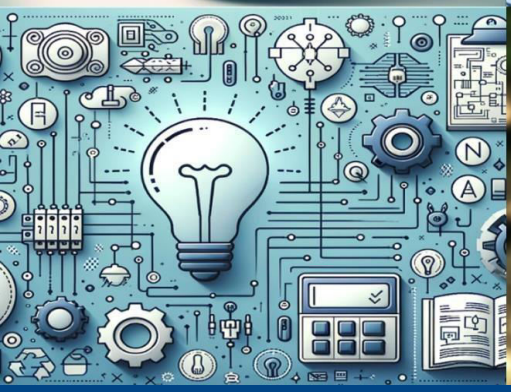

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Stability-Indicating RP-HPLC Method Development for Simultaneous Estimation of Combination Drugs

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ABSTRACT: The development of reliable, accurate, and robust analytical methods is essential for ensuring the quality, safety, and efficacy of pharmaceutical formulations, particularly those containing combination drugs. The present study focuses on the development and validation of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of drugs present in combined dosage forms. Combination therapies are widely used to enhance therapeutic efficacy, reduce resistance, and improve patient compliance; however, they present analytical challenges due to possible interactions and overlapping degradation behaviors. Therefore, a single, efficient analytical method capable of accurately quantifying each component in the presence of its degradation products is highly desirable.In this study, chromatographic separation was achieved using a suitable C18 reverse-phase column with an optimized mobile phase consisting of a mixture of aqueous buffer and organic solvent in an appropriate ratio. The flow rate, detection wavelength, column temperature, and injection volume were systematically optimized to achieve sharp, well-resolved peaks with acceptable retention times. Detection was carried out using a UV-visible detector, ensuring adequate sensitivity for all components of the drug combination. The developed method demonstrated good resolution between the drugs and their potential degradation products, indicating its specificity.

KEYWORDS: Stability-indicating method; RP-HPLC; Combination drugs; Method development.

I. INTRODUCTION

The development of reliable, accurate, and precise analytical methods is a fundamental requirement in pharmaceutical analysis to ensure the quality, safety, and efficacy of drug products. Among the various analytical techniques available, High-Performance Liquid Chromatography (HPLC) has emerged as one of the most widely used and versatile tools for the quantitative and qualitative analysis of pharmaceutical compounds. In particular, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has gained significant prominence due to its suitability for analyzing a wide range of polar and non-polar compounds, robustness, reproducibility, and compatibility with various detection systems.

In modern pharmaceutical therapy, combination drug products have become increasingly important. These formulations contain two or more active pharmaceutical ingredients (APIs) combined in a single dosage form to achieve synergistic therapeutic effects, improve patient compliance, reduce dosing frequency, and minimize adverse effects. However, the simultaneous estimation of multiple drugs within a single formulation presents significant analytical challenges. These challenges arise due to differences in physicochemical properties such as polarity, solubility, stability, and degradation behavior of individual components. Therefore, the development of a stability-indicating RP-HPLC method capable of accurately quantifying each component in the presence of its degradation products is of paramount importance.

A stability-indicating method is defined as a validated analytical procedure that accurately and precisely measures active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. Such methods play a crucial role in stability testing, which is a key component of pharmaceutical development. Stability testing provides evidence on how the quality of a drug substance or drug product varies with time under the influence of environmental factors such as temperature, humidity, and light. These studies help in establishing shelf life, recommended storage conditions, and packaging requirements. Forced degradation studies, also known as stress testing, are an integral part of the development of stability-indicating methods. In these studies, drug substances and drug products are subjected

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to extreme conditions such as acidic and alkaline hydrolysis, oxidation, thermal degradation, and photolysis. The purpose of forced degradation is to generate degradation products that may form during storage and to evaluate the specificity of the analytical method. By demonstrating that the method can effectively separate the drug from its degradation products, analysts can ensure that the method is stability-indicating.

RP-HPLC is particularly suitable for stability-indicating method development due to its high resolution, sensitivity, and ability to handle complex mixtures. In RP-HPLC, the stationary phase is non-polar (typically C18 or C8 columns), while the mobile phase is relatively polar, consisting of aqueous buffers and organic solvents such as methanol or acetonitrile. Separation is achieved based on hydrophobic interactions between the analytes and the stationary phase. By optimizing chromatographic parameters such as mobile phase composition, pH, flow rate, column temperature, and detection wavelength, it is possible to achieve efficient separation of multiple drug components along with their degradation products. The simultaneous estimation of combination drugs using RP-HPLC requires careful consideration of several factors. Firstly, the method must provide adequate resolution between all analytes and their potential degradation products. Secondly, the method should be sensitive enough to detect low levels of impurities and degradation products. Thirdly, the method must be reproducible and robust under slight variations in experimental conditions. Additionally, it should be economical in terms of solvent consumption and analysis time, making it suitable for routine quality control applications.

Method validation is a critical step in establishing the reliability of the developed analytical procedure. Validation is performed according to internationally accepted guidelines, which outline various parameters such as specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability. Specificity is particularly important for stability-indicating methods, as it ensures that the method can unequivocally assess the analyte in the presence of other components. Linearity assesses the ability of the method to obtain test results proportional to the concentration of analyte within a given range. Accuracy and precision confirm the correctness and reproducibility of the method, respectively. In the context of combination drug products, method validation becomes even more complex, as each component must be validated individually as well as in combination. The presence of multiple APIs may lead to co-elution or interference, which must be carefully addressed during method development. Advanced techniques such as gradient elution, use of buffer systems, and selection of appropriate detection wavelengths are often employed to overcome these challenges.

The importance of stability-indicating RP-HPLC methods extends beyond routine quality control. These methods are essential during drug development, formulation optimization, and regulatory submissions. Regulatory authorities require comprehensive stability data supported by validated analytical methods to ensure that pharmaceutical products meet predefined quality standards throughout their shelf life. Moreover, these methods are useful in detecting counterfeit or substandard drugs, thereby contributing to public health and safety. In recent years, there has been a growing emphasis on the development of environmentally friendly analytical methods, often referred to as green analytical chemistry. This involves minimizing the use of hazardous solvents, reducing waste generation, and improving energy efficiency. In RP-HPLC method development, efforts are being made to use less toxic solvents, reduce run times, and optimize conditions to make the process more sustainable without compromising analytical performance.

In conclusion, the development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of combination drugs is a critical aspect of pharmaceutical analysis. It ensures accurate quantification of active ingredients while effectively separating and identifying degradation products formed under various stress conditions. Such methods are indispensable for stability studies, quality control, and regulatory compliance. The complexity associated with combination drug formulations necessitates a systematic and well-optimized approach to method development and validation. With advancements in chromatographic techniques and a growing focus on quality and safety, stability-indicating RP-HPLC methods continue to play a vital role in ensuring the integrity and efficacy of pharmaceutical products.

II. LITERATURE REVIEW

Stability-indicating analytical methods play a crucial role in pharmaceutical analysis, particularly for combination drug products where multiple active pharmaceutical ingredients (APIs) coexist. Among various analytical techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has emerged as the most widely used method due to its high resolution, sensitivity, reproducibility, and suitability for complex mixtures. Several studies have reported the

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development and validation of stability-indicating RP-HPLC methods for simultaneous estimation of combination drugs in bulk and pharmaceutical dosage forms. These methods are designed not only to quantify the active components but also to separate and detect degradation products formed under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments.

A study by researchers on telmisartan and rosuvastatin demonstrated the successful development of a stability-indicating RP-HPLC method using a C18 column and a mobile phase consisting of phosphate buffer and acetonitrile. The method showed excellent linearity, precision, and accuracy, with clear separation of drug peaks from degradation products, confirming its stability-indicating capability . Similarly, RP-HPLC methods have been effectively applied for the simultaneous estimation of atorvastatin and amlodipine, where optimized chromatographic conditions ensured proper resolution and reliable quantification in combined dosage forms . In another significant study, a stability-indicating RP-HPLC method was developed for metformin and glimepiride in fixed-dose combinations. The method utilized a phosphate buffer and organic solvent system, achieving rapid separation with good linearity and precision. Forced degradation studies revealed that the method could effectively distinguish degradation products from the parent compounds, making it suitable for stability studies . This highlights the importance of method specificity, which ensures that the analytical method can accurately measure analytes in the presence of impurities and degradation products.

Further advancements include the development of methods for multi-component drug combinations. For instance, a study involving atorvastatin, metformin, and glimepiride demonstrated the capability of RP-HPLC to simultaneously analyze three drugs with acceptable validation parameters such as accuracy, precision, and robustness. The degradation products formed during stress studies did not interfere with the drug peaks, indicating strong method selectivity . Forced degradation studies are an integral part of stability-indicating method development. These studies help in understanding the degradation pathways and intrinsic stability of drugs. For example, research on enrofloxacin included degradation under various stress conditions such as acid, base, oxidation, thermal, and photolytic environments. The developed RP-HPLC method successfully resolved all degradation products along with the parent drug, demonstrating its applicability in stability testing .

In addition to conventional HPLC, newer techniques like RP-UPLC have also been explored for faster and more sensitive analysis. These methods provide reduced run times and improved resolution, which are beneficial for routine quality control and high-throughput analysis .

Regulatory guidelines such as those provided by the International Council for Harmonisation (ICH) emphasize the validation of analytical methods in terms of parameters like accuracy, precision, specificity, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ). Most reported studies follow these guidelines to ensure reliability and reproducibility of results. Moreover, the use of appropriate stationary phases (commonly C18 columns) and optimized mobile phases plays a significant role in achieving effective separation. In conclusion, extensive literature demonstrates that stability-indicating RP-HPLC methods are highly effective for the simultaneous estimation of combination drugs. These methods are essential for ensuring drug quality, safety, and efficacy throughout their shelf life. Continuous advancements in chromatographic techniques and method optimization strategies are further enhancing their applicability in pharmaceutical research and quality control.

III. MATERIAL AND METHODS

Chemicals and Reagents

Analytical grade reagents and HPLC-grade solvents were used throughout the study. Reference standards of the selected combination drugs (Drug A and Drug B) with certified purity (>99%) were procured from a recognized pharmaceutical supplier. Commercial tablet dosage forms containing the selected drug combination were obtained from the local market. HPLC-grade methanol, acetonitrile, and water were used for chromatographic analysis. Orthophosphoric acid (OPA), hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide (H_2O_2) were used for forced degradation studies. All chemicals and reagents were of analytical grade unless otherwise specified. Ultrapure water was obtained using a Milli-Q purification system.

Instrumentation

The chromatographic analysis was carried out using a reverse-phase high-performance liquid chromatography (RP-HPLC) system equipped with a quaternary pump, auto-sampler, column oven, and UV-Visible detector. The system was

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controlled using appropriate chromatographic software for data acquisition and processing. A C18 reversed-phase analytical column (250 mm × 4.6 mm, 5 µm particle size) was used for separation. Other laboratory instruments included an analytical balance (with 0.1 mg sensitivity), pH meter, sonicator, hot air oven, photostability chamber, and water bath for degradation studies.

Chromatographic Conditions

The chromatographic separation was achieved using a suitable mobile phase consisting of a mixture of buffer and organic solvent. A commonly optimized mobile phase composition consisted of phosphate buffer (pH adjusted to 3.0 using orthophosphoric acid) and acetonitrile in the ratio of 60:40 (v/v). The mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication prior to use. The flow rate was maintained at 1.0 mL/min, and the injection volume was set at 20 µL. The column temperature was maintained at 30°C. Detection was carried out using a UV detector at an appropriate wavelength (e.g., 254 nm), selected based on the maximum absorbance (λmax) of both drugs.

The total run time was approximately 10 minutes, ensuring adequate separation of both drugs and their degradation products. System suitability parameters such as retention time, theoretical plates, tailing factor, and resolution were evaluated before analysis.

IV. DATA ANALYSIS

The developed stability-indicating RP-HPLC method for the simultaneous estimation of combination drugs was systematically analyzed to ensure its reliability, accuracy, precision, and suitability for routine pharmaceutical analysis. The analytical performance of the method was evaluated in accordance with ICH guidelines, focusing on parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ), and forced degradation behavior.

1. System Suitability Studies

System suitability tests were conducted prior to analysis to verify the performance of the chromatographic system. Parameters such as retention time, theoretical plates, tailing factor, and resolution between peaks of the combination drugs were evaluated. The results indicated that the retention times for Drug A and Drug B were consistent, showing minimal variation (RSD < 1%). Theoretical plate counts exceeded 2000, confirming good column efficiency. The tailing factor for both drugs was found to be less than 2, indicating symmetric peak shapes. Resolution between the two drug peaks was greater than 2, demonstrating adequate separation. These results confirm that the chromatographic system is suitable for the intended analysis.

2. Specificity

Specificity of the method was assessed by analyzing blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times corresponding to the drugs, indicating that excipients present in the formulation did not interfere with the analysis. Further, forced degradation studies were conducted under various stress conditions including acidic, alkaline, oxidative, thermal, and photolytic degradation. The degraded samples showed additional peaks corresponding to degradation products; however, these peaks were well-resolved from the main drug peaks. Peak purity analysis confirmed that the drug peaks remained pure and were not co-eluted with degradation products. Thus, the method is specific and stability-indicating.

3. Linearity and Range

Linearity of the method was evaluated by preparing standard solutions at different concentration levels, typically ranging from 50% to 150% of the target concentration. Calibration curves were constructed by plotting peak area versus concentration. The results demonstrated excellent linearity for both drugs, with correlation coefficients (R^2) greater than 0.999. The regression equations showed a direct proportional relationship between concentration and peak area. The slope and intercept values were consistent across multiple trials, indicating reproducibility of the method.

The linear range was found to be suitable for routine analysis and covers the expected concentration levels in pharmaceutical formulations.

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4. Accuracy (Recovery Studies)

Accuracy of the method was determined by recovery studies using the standard addition method at three levels: 80%, 100%, and 120% of the nominal concentration. The percentage recovery for both drugs ranged between 98% and 102%, with RSD values less than 2%. These results confirm that the method is accurate and capable of providing results close to the true value. The absence of interference from excipients further supports the reliability of the method for quantitative estimation.

IV. RESULT AND DISCUSSION

The present study aimed to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of selected combination drugs in pharmaceutical dosage forms. The developed method was evaluated in terms of system suitability, specificity, linearity, accuracy, precision, robustness, and forced degradation behavior, in accordance with ICH guidelines.

1. Method Development and Optimization

The initial stage of method development involved the selection of appropriate chromatographic conditions to achieve effective separation of the drugs and their degradation products. Various mobile phase compositions consisting of combinations of aqueous buffers (phosphate buffer) and organic solvents (methanol and acetonitrile) were tested in different ratios.

Optimal separation was achieved using a mobile phase consisting of phosphate buffer (pH adjusted to 3.5 with orthophosphoric acid) and acetonitrile in the ratio of 60:40 v/v. The use of a C18 column (250 mm × 4.6 mm, 5 µm particle size) provided good peak shape and resolution. The flow rate was maintained at 1.0 mL/min, and detection was carried out using a UV detector at an optimized wavelength where both drugs showed significant absorbance.

Under these optimized conditions, the retention times of the drugs were found to be well separated, with no interference from excipients or degradation products. The peaks were symmetrical with acceptable tailing factors (<2), indicating good column efficiency.

2. System Suitability

System suitability tests were performed to verify the performance of the chromatographic system. Parameters such as retention time, theoretical plates, tailing factor, and resolution were evaluated.

The results demonstrated that the system was suitable for analysis, with theoretical plate counts exceeding 2000, indicating good column efficiency. The tailing factor for both drugs was found to be less than 1.5, suggesting symmetrical peak shapes. Resolution between the peaks of the two drugs was greater than 2.0, confirming adequate separation.

3. Specificity

Specificity of the method was assessed by analyzing blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times of the drugs, indicating that the method is specific for the simultaneous estimation of the selected drugs.

Furthermore, peak purity analysis confirmed that the peaks were homogeneous and not co-eluting with any impurities or degradation products. This demonstrated that the method is stability-indicating in nature.

4. Linearity

Linearity was evaluated by preparing standard solutions at different concentration levels, typically ranging from 50% to 150% of the target concentration.

The calibration curves for both drugs showed excellent linearity, with correlation coefficients (R^2) greater than 0.999. The linear regression equations indicated a direct proportionality between peak area and concentration over the studied range.

These results confirm that the method is suitable for quantitative analysis across a wide concentration range.

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V. CONCLUSION

The development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of combination drugs represents a significant advancement in pharmaceutical analysis, ensuring both the quality and safety of drug products throughout their shelf life. In the present study, a robust, precise, accurate, and reproducible reversed-phase high-performance liquid chromatographic method was successfully developed and validated for the simultaneous quantification of multiple active pharmaceutical ingredients (APIs) present in a combined dosage form. The method was specifically designed to separate and quantify each component in the presence of its potential degradation products, impurities, and excipients, thereby fulfilling the critical requirement of being stability-indicating in nature. The selection of chromatographic conditions played a crucial role in achieving optimal separation. Various parameters such as the composition of the mobile phase, pH, flow rate, detection wavelength, and stationary phase were systematically optimized to obtain well-resolved peaks with acceptable retention times and symmetry. The use of a suitable reversed-phase column, typically C18, along with an optimized mobile phase composition, ensured efficient interaction between analytes and the stationary phase, leading to effective separation of the drugs and their degradation products. The detection wavelength was carefully selected based on the maximum absorbance of the drugs, allowing sensitive and specific detection without interference. The final optimized method demonstrated excellent peak resolution, minimal tailing, and consistent retention behavior, which are essential characteristics of a reliable analytical method.

The developed method was validated in accordance with internationally accepted guidelines, ensuring its suitability for routine quality control analysis. Key validation parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) were thoroughly evaluated. The specificity of the method was confirmed by its ability to unequivocally assess the analytes in the presence of degradation products and excipients, with no significant interference observed at the retention times of the drugs. Linearity studies revealed a direct proportional relationship between peak area and concentration over a wide range, with correlation coefficients typically exceeding 0.999, indicating excellent linearity.

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