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"Development and Validation of a Green RP-HPLC Method for Stability-Indicating Estimation of Suzetrigine in Bulk and Pharmaceutical Dosage Forms."

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1. Abstract

Analytical methods play a crucial role in pharmaceutical analysis for ensuring the quality, safety, and therapeutic efficacy of drug substances and pharmaceutical dosage forms. Among the various analytical techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has emerged as one of the most widely employed methods due to its high sensitivity, precision, reproducibility, and reliability in the quantitative estimation of pharmaceutical compounds.

In pharmaceutical research and quality control, the development of stability-indicating analytical methods is particularly important. These methods are capable of accurately determining the active pharmaceutical ingredient (API) in the presence of its degradation products, impurities, and formulation excipients, thereby providing essential information about the stability profile of the drug.

In recent years, the principles of Green Analytical Chemistry have gained increasing importance in chromatographic analysis. The aim of green analytical approaches is to reduce the consumption of hazardous organic solvents, minimize chemical waste generation, and promote environmentally sustainable laboratory practices without compromising analytical performance. Consequently, green RP-HPLC methods emphasize the use of safer solvents, optimized chromatographic conditions, and efficient analytical procedures.

Suzetrigine is an emerging pharmaceutical compound that requires the development of reliable and validated analytical methods for its quantitative estimation in both bulk drug substances and pharmaceutical formulations. This review article discusses the strategies involved in the development of green RP-HPLC methods, with particular emphasis on stability-indicating approaches and the method validation parameters recommended by regulatory guidelines.

Keywords:

Suzetrigine; Green Analytical Chemistry; RP-HPLC; Stability-Indicating Method; Method Development; Method Validation.

2. Introduction

2.1 Pharmaceutical Analytical Methods

Analytical methods play a fundamental role in the pharmaceutical field by ensuring that medicines are safe, effective, and of acceptable quality before they reach patients. During drug development and manufacturing, a variety of analytical techniques are employed to evaluate the identity, purity, potency, and stability of pharmaceutical substances. [1] These evaluations are performed at multiple stages of the pharmaceutical process, including raw material testing, formulation development, quality control, and stability studies. Therefore, reliable and precise analytical methods are essential to confirm that pharmaceutical products comply with established regulatory and quality standards. [2]

A wide range of analytical techniques is used in pharmaceutical analysis, mainly including spectroscopic and chromatographic methods. Among these, chromatographic techniques have gained significant importance because of their ability to separate complex mixtures into individual components with high efficiency. [3] These methods offer excellent sensitivity, selectivity, and accuracy, enabling the detection and quantification of drugs even at very low concentrations. Consequently, chromatographic techniques are extensively applied in the pharmaceutical industry for drug estimation, impurity profiling, and stability evaluation. [4]

2.2 RP-HPLC in Drug Analysis

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most widely utilized chromatographic techniques for pharmaceutical analysis. In RP-HPLC, separation of compounds occurs through the interaction between a non-polar stationary phase, commonly a C18 column, and a polar mobile phase. The components present in a sample are separated based on their relative affinities toward these phases, allowing efficient resolution of different substances within a mixture. [5]

RP-HPLC offers several advantages that make it highly suitable for drug analysis. The technique provides high precision, excellent resolution, rapid analysis, and reproducible results. It is also capable of analyzing complex pharmaceutical formulations and detecting compounds present in trace quantities. [6] Due to these benefits, RP-HPLC has become a preferred analytical method for various pharmaceutical applications, including quantitative drug estimation, impurity analysis, dissolution testing, and stability studies. As a result, it is routinely employed in pharmaceutical research laboratories as well as in quality control settings. [7]

2.3 Need for Green Analytical Chemistry

In recent years, increasing attention has been given to the environmental impact of analytical laboratory practices. Conventional analytical methods often require large volumes of organic solvents such as methanol and acetonitrile, which may pose potential risks to both environmental safety and laboratory personnel. [8] Furthermore, the disposal of these solvents contributes to chemical waste generation and environmental pollution.

To address these concerns, the concept of Green Analytical Chemistry (GAC) has been introduced. The primary objective of green analytical chemistry is to design and develop analytical methods that are environmentally sustainable and safer to perform. [9] This approach emphasizes the use of less hazardous solvents, reduction in solvent consumption, and minimization of chemical waste. In chromatographic analysis, green practices may involve selecting eco-friendly solvents, optimizing chromatographic

conditions to shorten analysis time, and improving overall method efficiency. [10] Implementing these strategies helps ensure that analytical procedures remain both scientifically reliable and environmentally responsible. [11]

2.4 Stability-Indicating Methods

Stability-indicating methods are specialized analytical procedures designed to accurately determine the active pharmaceutical ingredient (API) in the presence of its potential degradation products, impurities, and formulation excipients. Such methods are crucial for understanding the stability behavior of pharmaceutical compounds under different environmental conditions. [12]

During stability studies, drug substances are subjected to various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic environments, in order to evaluate possible degradation pathways. [13] An effective stability-indicating method must be capable of clearly separating the intact drug from its degradation products, allowing precise assessment of the drug's stability profile. [14] Regulatory authorities, particularly the International Council for Harmonisation (ICH), strongly recommend the development and validation of stability-indicating analytical methods to ensure that pharmaceutical products maintain their quality, safety, and efficacy throughout their shelf life. [15]

2.5 Suzetrigine

Suzetrigine is a recently developed pharmaceutical compound that has attracted significant attention due to its potential application in the management of pain-related disorders. It is classified as a non-opioid analgesic agent that acts by selectively targeting specific voltage-gated sodium channels involved in the transmission of pain signals within the nervous system. By inhibiting these channels, Suzetrigine reduces the propagation of pain impulses from peripheral sensory neurons to the central nervous system, thereby contributing to effective pain relief. [16]

In contrast to traditional opioid analgesics, Suzetrigine provides an alternative approach to pain management with a reduced risk of adverse effects commonly associated with opioid therapy, such as drug dependence, tolerance, and respiratory depression. The selective mechanism of action of Suzetrigine mainly influences nociceptive neurons, allowing the drug to exert its therapeutic effects in a more targeted manner. [17]

Recent pharmacological studies have explored the potential use of Suzetrigine in the treatment of various pain conditions, including acute pain, postoperative pain, and certain forms of neuropathic pain. Because of its promising pharmacological profile and improved safety characteristics, Suzetrigine has become an area of growing interest in pharmaceutical research and drug development.

For the development and quality assurance of pharmaceutical formulations containing Suzetrigine, the availability of reliable and validated analytical methods is essential. Accurate quantification of the drug in bulk materials and pharmaceutical dosage forms is necessary to ensure product quality, stability, and compliance with regulatory requirements. Consequently, the development of sensitive, robust, and environmentally sustainable analytical techniques, such as green RP-HPLC methods, is important for the routine analysis and stability assessment of Suzetrigine in pharmaceutical preparations. [18]

Table:1. Drug Profile of Suzetrigine

Parameter	Description
Drug Name	Suzetrigine
Code Name	VX-548
Drug Class	Non-opioid analgesic
Pharmacological Class	Selective voltage-gated sodium channel (NaV1.8) inhibitor

Molecular Formula	$C_{21}H_{21}ClF_2N_4O_2$
Molecular Weight	~434.87 g/mol
Mechanism of Action	Selectively blocks NaV1.8 sodium channels in peripheral sensory neurons, reducing transmission of pain signals to the central nervous system
Therapeutic Indication	Management of acute pain and neuropathic pain
Route of Administration	Oral
Dosage Form	Tablets / Oral formulations (under development and clinical evaluation)
Pharmacological Activity	Analgesic activity
Advantages	Non-opioid pain relief with lower risk of addiction and respiratory depression
Solubility	Slightly soluble in water; soluble in organic solvents
Analytical Methods Reported	RP-HPLC, LC-MS, and other chromatographic techniques
Application in Pharmaceutical Analysis	Quantification in bulk drug substances and pharmaceutical dosage forms; stability studies

3. Green Analytical Chemistry in Chromatography

3.1 Principles of Green Analytical Chemistry

Green Analytical Chemistry (GAC) has emerged as an important concept in modern analytical science with the aim of minimizing the environmental impact of analytical procedures. Traditional chromatographic methods often require large volumes of organic solvents, which can be hazardous to both the environment and laboratory personnel. Therefore, the principles of green analytical chemistry focus on developing analytical methods that are safer, more sustainable, and resource-efficient. [19]

One of the key principles of green analytical chemistry is the reduction of solvent consumption during analysis. By optimizing chromatographic conditions such as flow rate, column dimensions, and run time, the amount of solvent used in each analysis can be significantly decreased. This not only reduces chemical waste but also lowers operational costs. [20]

Another important principle is the use of eco-friendly solvents in place of toxic organic solvents whenever possible. Solvents such as ethanol, water, or other less hazardous alternatives are preferred because they are safer to handle and generate less environmental pollution. The selection of appropriate solvents plays a major role in improving the overall greenness of chromatographic methods. [21]

Energy efficiency is also an important aspect of green analytical chemistry. Analytical techniques should be designed in a way that reduces energy consumption and analysis time while maintaining reliable results. Shorter run times and efficient instrumentation help improve laboratory productivity and contribute to sustainable analytical practices. [22]

Figure No. 1. Principles Of Green Analytical Chemistry

3.2 Green Chromatographic Techniques

The application of green principles in chromatography has led to the development of several environmentally friendly analytical approaches. Among these, green HPLC methods have gained significant attention in pharmaceutical analysis. Green HPLC focuses on reducing the amount of hazardous solvents, minimizing waste generation, and improving the efficiency of chromatographic procedures without compromising analytical performance. [23]

Another important advancement is Ultra-Performance Liquid Chromatography (UPLC). Compared to conventional HPLC, UPLC operates with smaller particle size columns and higher pressure systems, which allows faster separation and improved resolution. As a result, the technique requires less solvent and shorter analysis time, making it a more environmentally friendly option. [24]

The use of biodegradable and less toxic solvents is also encouraged in green chromatographic techniques. Solvents such as ethanol and water-based mobile phases are often preferred because they are safer and have a lower environmental impact. These approaches support the development of sustainable analytical methods in pharmaceutical research and quality control laboratories. [25]

3.3 Assessment Tools for Greenness

To evaluate the environmental friendliness of analytical methods, several assessment tools have been developed. These tools help researchers measure how well an analytical procedure follows the principles of green analytical chemistry. [26]

One commonly used tool is the Analytical Eco-Scale, which evaluates analytical methods based on factors such as the amount of chemicals used, hazards associated with reagents, energy consumption, and waste production. The method is assigned a score, where a higher score indicates a greener analytical procedure. [27]

Another widely used assessment approach is the Green Analytical Procedure Index (GAPI). This tool provides a visual representation of the environmental impact of analytical methods by considering different stages of the analytical process, including sample preparation, reagent use, and waste generation. The results

are presented in a colored diagram that allows easy comparison between different analytical methods. [28]

The AGREE metric is another modern tool used to assess the greenness of analytical methods. It evaluates analytical procedures based on the twelve principles of green analytical chemistry and provides a numerical score along with a graphical representation. These tools help researchers design and improve analytical methods that are both effective and environmentally responsible.
[29]

4. RP-HPLC Method Development

4.1 Selection of Chromatographic Conditions

The development of an RP-HPLC method begins with the proper selection of chromatographic conditions to achieve accurate and reliable separation of analytes. One of the first considerations is the selection of the chromatographic column, which plays a critical role in the separation process. In most pharmaceutical analyses, C18 columns are commonly used because they provide good retention and separation of many drug molecules. [30]

The composition of the mobile phase is another important factor in method development. The mobile phase generally consists of a mixture of aqueous buffers and organic solvents such as methanol or acetonitrile. The ratio of these components must be optimized to achieve good peak shape, appropriate retention time, and efficient separation. [31]

The flow rate of the mobile phase also affects chromatographic performance. An appropriate flow rate ensures proper interaction between the analyte and the stationary phase while maintaining reasonable analysis time. Additionally, the detection wavelength must be selected based on the maximum absorbance of the analyte so that accurate and sensitive detection can be achieved. [32]

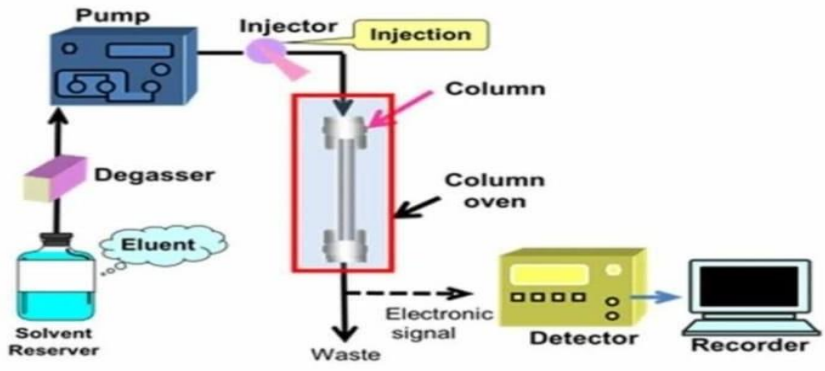

Figure no. 2 RP-HPLC Method Development

4.2 Optimization Strategies

During RP-HPLC method development, various strategies are used to optimize chromatographic conditions. One commonly used approach is the trial-and-error method, where different mobile phase compositions, pH values, and flow rates are tested until satisfactory separation is obtained. Although this method is simple, it may require considerable time and resources. [34]

A more systematic approach is the Design of Experiments (DoE) method. In this strategy, multiple experimental factors are studied simultaneously to understand their effect on chromatographic performance. This approach helps identify the most influential parameters and allows efficient optimization of the analytical method. [35]

Another advanced approach is the Quality by Design (QbD) concept. QbD focuses on understanding the relationship between process variables and method performance. By identifying critical method parameters and establishing a design space, QbD helps in developing robust and reliable analytical methods with improved reproducibility.

Table 2. RP-HPLC Method Development Parameters

Parameter	Importance
Column type	Affects separation efficiency
Mobile phase	Determines polarity and retention
Flow rate	Influences analysis time
Detection wavelength	Determines sensitivity
Injection volume	Affects peak shape

4.3 Factors Affecting RP-HPLC Performance

Several factors influence the performance and reliability of RP-HPLC analysis. One important factor is the pH of the mobile phase, which can affect the ionization state of the analyte and ultimately influence its retention and separation. Therefore, selecting an appropriate pH is essential for achieving consistent chromatographic results. [36]

The temperature of the chromatographic system can also impact separation efficiency. Changes in temperature may affect the viscosity of the mobile phase and the interaction between the analyte and stationary phase. Maintaining a controlled temperature helps improve reproducibility and peak shape.

Another factor is the injection volume of the sample. Injecting excessive sample volume may lead to peak broadening and reduced resolution, while very small injection volumes may decrease sensitivity. Therefore, an optimal injection volume should be selected to ensure accurate and reliable analysis. [37]

5. Stability-Indicating Method Development

5.1 Forced Degradation Studies

Forced degradation studies are an important part of stability-indicating method development. These studies are performed to evaluate the stability of a drug under various stress conditions and to identify possible

degradation products. By understanding the degradation behavior of the drug, researchers can develop analytical methods capable of separating the drug from its degradation products. [38]

Common stress conditions used in forced degradation studies include acidic and alkaline hydrolysis, where the drug is exposed to acidic or basic environments to determine its stability. Oxidative degradation is performed using oxidizing agents such as hydrogen peroxide to study the effect of oxidation on the drug molecule.

Other stress conditions include thermal degradation, where the drug is exposed to elevated temperatures, and photolytic degradation, where the drug is exposed to light. These studies help in identifying degradation pathways and provide valuable information for the development of stability-indicating analytical methods. [39]

5.2 Identification of Degradation Products

After performing forced degradation studies, it is necessary to identify and separate the degradation products from the main drug compound. Chromatographic techniques such as RP-HPLC are widely used for this purpose because they provide efficient separation of different components present in the sample. [40]

Proper chromatographic separation ensures that the peak corresponding to the drug is well resolved from peaks representing degradation products or impurities. In addition, peak purity analysis is often performed using diode-array detection or other advanced detection techniques to confirm that the drug peak is free from interference by other compounds. [41]

5.3 Importance in Regulatory Submissions

Stability-indicating methods are an essential requirement for regulatory approval of pharmaceutical products. Regulatory authorities require comprehensive stability data to ensure that a drug remains safe and effective throughout its shelf life. Analytical methods used in stability studies must therefore be capable of accurately detecting the drug in the presence of degradation products. [42]

Validated stability-indicating methods provide reliable information about the degradation behavior of pharmaceutical compounds and help establish appropriate storage conditions and expiration dates. As a result, these methods play a crucial role in pharmaceutical quality assurance and regulatory submissions. [43]

6. Method Validation According to ICH Guidelines

Analytical method validation is an essential step in pharmaceutical analysis to ensure that an analytical method is suitable for its intended purpose. Validation confirms that the method produces reliable, accurate, and consistent results when used for the analysis of pharmaceutical substances and formulations. The International Council for Harmonisation (ICH) provides widely accepted guidelines for analytical method validation, particularly in document ICH Q2(R1). According to these guidelines, several validation parameters must be evaluated to demonstrate the performance of an analytical method. [44]

6.1 Specificity

Specificity refers to the ability of an analytical method to accurately measure the analyte in the presence of other components such as impurities, degradation products, and excipients present in the formulation. In chromatographic methods like RP-HPLC, specificity is demonstrated by obtaining well-resolved peaks for the analyte without interference from other substances. This ensures that the method can selectively identify and quantify the drug even in complex sample matrices. [45]

6.2 Linearity

Linearity describes the ability of the analytical method to produce results that are directly proportional to the concentration of the analyte within a given range. It is typically evaluated by preparing standard solutions at different concentration levels and constructing a calibration curve. A linear relationship between concentration and detector response is usually expressed by the **correlation coefficient (R^2)**, which should be close to 1 for a valid method. [46]

6.3 Accuracy

Accuracy represents the closeness of the measured value to the true value of the analyte. It indicates how correct the analytical results are. Accuracy is generally evaluated through recovery studies by adding known amounts of the analyte to the sample matrix and determining the percentage recovery. Acceptable recovery values confirm that the analytical method can provide reliable results. [47]

6.4 Precision

Precision refers to the degree of agreement among repeated measurements of the same sample under specified conditions. It indicates the reproducibility of the analytical method. Precision is usually evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). The results are commonly expressed as percentage relative standard deviation (%RSD). [48]

6.5 Limit of Detection (LOD)

The limit of detection is the lowest amount of analyte that can be detected by the analytical method but not necessarily quantified with acceptable accuracy. It indicates the sensitivity of the method and is typically calculated based on the standard deviation of the response and the slope of the calibration curve. [49]

6.6 Limit of Quantification (LOQ)

The limit of quantification represents the lowest concentration of the analyte that can be quantitatively determined with acceptable precision and accuracy. LOQ is generally higher than the limit of detection and is an important parameter when analyzing low concentrations of pharmaceutical compounds. [50]

6.7 Robustness

Robustness refers to the ability of the analytical method to remain unaffected by small but deliberate variations in method parameters. These variations may include changes in flow rate, mobile phase composition, pH, temperature, or detection wavelength. A robust method ensures consistent results even when minor changes occur during routine analysis. [51]

6.8 System Suitability Testing

System suitability tests are performed to verify that the chromatographic system is functioning properly before sample analysis. These tests evaluate parameters such as retention time, theoretical plates, tailing factor, and resolution. System suitability ensures that the analytical system is capable of producing reliable and reproducible results during routine analysis. [52]

Table 3. ICH Method Validation Parameters

Parameter	Purpose
Specificity	Ability to measure analyte in presence of impurities
Linearity	Proportional relationship between concentration and response
Accuracy	Closeness of measured value to true value
Precision	Repeatability of results
LOD	Lowest detectable concentration
LOQ	Lowest quantifiable concentration
Robustness	Stability of method under small variations

7. Applications of Green RP-HPLC in Pharmaceutical Analysis

Green RP-HPLC has gained considerable importance in pharmaceutical analysis because it combines the efficiency of chromatographic techniques with environmentally friendly analytical practices. By reducing solvent consumption, minimizing hazardous chemicals, and improving method efficiency, green RP-HPLC provides a sustainable approach for routine pharmaceutical analysis. [53]

Estimation of Drugs in Bulk

Green RP-HPLC methods are widely used for the quantitative estimation of pharmaceutical compounds in bulk drug substances. These methods allow accurate determination of the active pharmaceutical ingredient with high sensitivity and precision while reducing the environmental impact of analytical procedures. [54]

Analysis in Pharmaceutical Dosage Forms

In addition to bulk drug analysis, green RP-HPLC techniques are commonly applied for the analysis of drugs in various pharmaceutical dosage forms such as tablets, capsules, and suspensions. These methods help ensure that the amount of active ingredient present in the formulation meets the required quality standards. [55]

Stability Studies

Green chromatographic methods are also used in stability studies to evaluate the behavior of drugs under different storage conditions. Stability-indicating RP-HPLC methods can effectively separate the drug from its degradation products, allowing accurate monitoring of drug stability over time.
[56]

Quality Control Laboratories

In pharmaceutical quality control laboratories, green RP-HPLC methods are increasingly being adopted because they provide reliable analytical results while supporting sustainable laboratory practices. The use of environmentally friendly solvents and reduced solvent consumption makes these methods suitable for routine analysis and regulatory compliance in pharmaceutical industries.[57]

8. Challenges in Green HPLC Method Development

Although green analytical chemistry has gained considerable importance in pharmaceutical analysis, the development of green HPLC methods still faces several challenges. One of the major difficulties is related to solvent selection. Traditional HPLC methods often rely on organic solvents such as acetonitrile and methanol, which provide excellent chromatographic performance. However, replacing these solvents with environmentally friendly alternatives may sometimes affect separation efficiency, peak shape, or analysis time. Therefore, selecting suitable green solvents while maintaining good chromatographic performance remains a significant challenge.
[58]

Another challenge is instrument compatibility. Some green solvents or modified mobile phases may not always be compatible with conventional HPLC systems or column materials. In certain cases, adjustments in system pressure, column type, or detector settings may be required to maintain optimal performance. This can make the transition from traditional analytical methods to greener approaches more complex.

Cost considerations also play an important role in the adoption of green analytical methods. While green chemistry aims to reduce long-term environmental impact and operational costs, the initial implementation may require investment in advanced instrumentation, new chromatographic columns, or method optimization procedures. For some laboratories, particularly those with limited resources, these costs may present a practical challenge during the early stages of adopting green analytical techniques. [59]

9. Future Perspectives

With increasing emphasis on sustainable laboratory practices, the future of pharmaceutical analysis is expected to focus strongly on the development of greener chromatographic techniques. Researchers are continuously exploring new mobile phase systems, environmentally friendly solvents, and improved column technologies that can reduce solvent consumption and analytical waste while maintaining high analytical performance.

Another promising direction is the integration of nano-analytical technologies with chromatographic techniques. Nanomaterials and nano-based stationary phases have the potential to improve separation efficiency, sensitivity, and selectivity in chromatographic analysis. These advancements may help reduce analysis time and solvent usage, thereby supporting greener analytical approaches. [60]

The application of artificial intelligence (AI) and chemometric tools is also expected to play an important role in future analytical method development. AI-based models and statistical optimization techniques can help predict optimal chromatographic conditions, reduce experimental trials, and improve method robustness. The use of such advanced computational tools may significantly accelerate method development while supporting sustainable analytical practices.

10. Conclusion

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) remains one of the most widely used analytical techniques in pharmaceutical analysis due to its high sensitivity, accuracy, and reliability. The development of RP-HPLC methods involves careful optimization of chromatographic conditions, including column selection, mobile phase composition, flow rate, and detection parameters. In addition, proper validation of the analytical method according to ICH guidelines is essential to ensure reliable and reproducible results.

The incorporation of green analytical chemistry principles into chromatographic method development has become increasingly important in recent years. Green RP-HPLC methods aim to reduce solvent consumption, minimize hazardous chemicals, and decrease environmental impact while maintaining analytical performance. Such approaches contribute to more sustainable pharmaceutical analysis.

Furthermore, the development of validated stability-indicating analytical methods plays a critical role in ensuring the quality, safety, and stability of pharmaceutical products. These methods enable accurate detection of the active pharmaceutical ingredient in the presence of degradation products and impurities, thereby supporting regulatory compliance and effective quality control. Overall, the adoption of green and validated analytical methods will continue to play an essential role in modern pharmaceutical research and drug quality assurance.

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