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STABILITY INDICATING RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ANTI-ALLERGIC DRUGS IN BULK & TABLET DOSAGE FORM

Anamika Balasaheb Kakad[*1], Dr. Sagar Eknath Tambe[*2], Aishwarya Mukund Jadhav[*3], Radhika Sanjay Katpale

A. Nagar, Sangamner, Maharashtra, India, 422622.

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***Corresponding Author**
Anamika Balasaheb Kakad
A. Nagar, Sangamner, Maharashtra, India, 422622.

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ABSTRACT

A rapid, precise, and stability-indicating RP-UPLC method was developed and validated for the simultaneous estimation of selected anti-allergic drugs in bulk and tablet dosage forms. Separation was achieved using a suitable reverse-phase column with an optimized mobile phase composition to ensure sharp peak resolution and reduced run time. The method was validated in accordance with ICH guidelines, evaluating parameters such as linearity, accuracy, precision, robustness, specificity, and sensitivity. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed that the method can effectively distinguish the active pharmaceutical ingredients from their degradation products. The developed RP-UPLC method demonstrated high reproducibility and reliability for routine quality control analysis of anti-allergic formulations. Its sensitivity and stability-indicating capability make it suitable for application in both pharmaceutical research and industrial environments.

KEYWORDS: RP-UPLC, method development, method validation, anti-allergic drugs, stability-indicating method, simultaneous estimation, forced degradation studies, ICH guidelines, bulk drug, tablet dosage form.

INTRODUCTION

Analytical method development plays a crucial role in ensuring the quality, safety, and therapeutic effectiveness of pharmaceutical products. Anti-allergic drugs, which are widely prescribed for the management of allergic symptoms, require precise and reliable analytical techniques to monitor their potency and stability throughout manufacturing and storage. Since many anti-allergic formulations contain multiple active ingredients, a method capable of simultaneous estimation is essential for efficient quality control. Traditional chromatographic methods often require long analysis times or lack the sensitivity needed for modern pharmaceutical applications, making advanced techniques indispensable.[1]

Reverse Phase Ultra-Performance Liquid Chromatography (RP-UPLC) has emerged as a superior analytical tool due to its high resolution, faster analysis, reduced solvent consumption, and ability to detect low-level impurities. Its improved efficiency makes it suitable for routine analysis as well as detailed stability studies. A stability-indicating method is particularly important because it can differentiate the intact drug from its degradation products generated under various stress conditions such as heat, light, oxidation, or pH alterations. Establishing such a method ensures that the drug maintains its intended quality throughout its shelf life.

Method validation, guided by ICH regulatory requirements, further confirms that the developed analytical procedure is reliable and consistent. Parameters such as accuracy, precision, linearity, specificity, robustness, and sensitivity must be carefully evaluated to ensure suitability for routine quality control. Therefore, developing a validated stability-indicating RP-UPLC method for simultaneous estimation of anti-allergic drugs is essential for pharmaceutical industries to maintain compliance, guarantee product safety, and support ongoing formulation research.[2]

Objective of the Method

The primary objective of this study is to develop a rapid, precise, and robust **stability-indicating RP-UPLC method** for the **simultaneous estimation of selected anti-allergic drugs** in both bulk and tablet dosage forms. The method aims to achieve efficient separation of all active ingredients within a minimal analysis time while maintaining high resolution and sensitivity.[3]

Additional objectives include

- **To establish a chromatographic method capable of distinguishing the pure drugs from their degradation products** formed under various stress conditions, thereby confirming its stability-indicating nature.
- **To optimize chromatographic parameters**, including mobile phase composition, flow rate, wavelength selection, and column type, to ensure sharp, well-resolved peaks.
- **To validate the developed method according to ICH guidelines**, assessing critical parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ).
- **To demonstrate the applicability of the method for routine quality control**, ensuring reliable quantification of anti-allergic drugs in commercial tablet formulations.
- **To support pharmaceutical development and regulatory compliance** by providing a scientifically sound and reproducible analytical procedure.[5]

Selection of Drugs

The selection of anti-allergic drugs for this study was based on their widespread therapeutic use, frequent combination in pharmaceutical formulations, and the need for a reliable analytical method capable of estimating them simultaneously. These drugs are commonly prescribed for managing allergic conditions such as rhinitis, urticaria, asthma-related symptoms, and seasonal allergies. Because they are often formulated together to enhance therapeutic effectiveness, there is a growing demand for analytical techniques that can assess their quality, stability, and potency in a single chromatographic run.

The chosen drugs represent different chemical classes with varying polarity, solubility, and stability characteristics, making them suitable candidates for the development of an advanced RP-UPLC method. Their diverse physicochemical properties also provide an opportunity to establish a robust chromatographic procedure that can effectively separate multiple analytes under optimized conditions. Furthermore, these drugs are prone to degradation under environmental stress such as heat, light, and pH changes, highlighting the need for a stability-indicating approach.[7]

By selecting these commonly used anti-allergic agents, the study aims to contribute to improved quality control practices in pharmaceutical industries, ensuring patient safety and regulatory compliance. The simultaneous estimation of these drugs through a single,

validated RP-UPLC method enhances analytical efficiency, reduces solvent usage, and supports rapid routine analysis compared to traditional chromatographic techniques.

Method Development Parameters

The development of a reliable RP-UPLC method requires systematic evaluation and optimization of several chromatographic parameters to ensure accurate, precise, and reproducible separation of the selected anti-allergic drugs. Each parameter is carefully studied to achieve sharp peak resolution, short analysis time, and stability-indicating capability.[11]

1. Selection of Mobile Phase

Choosing the right mobile phase is crucial for achieving optimum separation and peak symmetry. Various combinations of aqueous buffers (such as phosphate or acetate buffers) and organic solvents (acetonitrile or methanol) are tested. The pH, ionic strength, and ratio of organic solvents are adjusted to enhance analyte retention, resolution, and compatibility with UPLC conditions.

2. Selection of Stationary Phase (Column)

A reverse-phase C18 UPLC column is usually preferred due to its high efficiency, improved separation capability, and suitability for compounds with varying polarity. Different column lengths, particle sizes, and pore sizes are evaluated to achieve optimal theoretical plates and minimal tailing factors.[13]

3. Wavelength Selection

The detection wavelength is chosen based on the maximum absorbance (λmax) of each drug obtained from UV spectra. A common wavelength that provides adequate sensitivity for all selected drugs is used to ensure simultaneous quantification without compromising detection limits.

4. Flow Rate Optimization

The flow rate is optimized to balance resolution, analysis speed, and system backpressure. UPLC systems typically operate at lower flow rates compared to HPLC due to smaller particle sizes, allowing faster and more efficient separation while maintaining system stability.

5. Selection of Diluent

A suitable diluent is chosen to ensure complete solubility of all drugs and compatibility with the mobile phase. Usually, a mixture of organic solvent and buffer is used to maintain drug stability and prevent precipitation during sample preparation.[17]

6. Injection Volume

A minimal injection volume is selected to avoid peak distortion or overload. UPLC systems often require smaller injection volumes (1–5 µL) to ensure sharp, well-defined peaks and maintain column integrity.

7. Column Temperature

Temperature significantly influences retention time, peak shape, and overall reproducibility. Controlled column heating (e.g., 30–40°C) is used to reduce viscosity, improve mass transfer, and ensure consistent separation of all analytes.

8. Run Time and Elution Mode

Both isocratic and gradient elution modes are evaluated. Gradient elution is often preferred when analyzing multiple drugs with varying polarity, as it shortens run time while maintaining high resolution. The final method aims for minimal run time to enhance analytical throughput.[23]

9. System Suitability Parameters

Parameters such as retention time, USP plate count, resolution, tailing factor, and peak area reproducibility are monitored to ensure the system performs within acceptable limits before sample analysis.

10. Forced Degradation Studies

Stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation are applied to evaluate the stability-indicating nature of the method. The goal is to ensure complete separation of degradation products from the active drugs without interference.

Forced Degradation Studies (Stability-Indicating Nature)

Forced degradation studies are an essential component in establishing the stability-indicating capability of an analytical method. These studies intentionally expose the selected anti-allergic drugs to stress conditions to generate potential degradation products. The purpose is to evaluate whether the developed RP-UPLC method can effectively separate the intact drugs

from their degraded forms without interference. A method that successfully resolves all degradation peaks from the main analytes is considered stability-indicating and suitable for routine stability testing.[29]

1. Acidic Degradation

The drugs are subjected to acidic conditions, commonly using hydrochloric acid (HCl) of appropriate strength. The sample is heated or allowed to react for a specific duration to induce hydrolytic breakdown. The study helps determine the susceptibility of the drug molecules to acid-catalyzed degradation, especially for compounds with ester or amide linkages.

2. Alkaline Degradation

Sodium hydroxide (NaOH) is used to initiate base-catalyzed hydrolysis. Many drugs, especially those with lactam, ester, or heterocyclic rings, show significant degradation under alkaline stress. The ability of the UPLC method to resolve basic degradation products from the parent peaks confirms specificity.

3. Oxidative Degradation

Oxidative stress is applied using hydrogen peroxide (H_2O_2) at suitable concentrations. This condition helps evaluate the vulnerability of phenolic, aromatic, or heteroatom-containing functional groups to oxidation. A stability-indicating method must distinctly separate oxidative degradants from the active ingredients.[30]

4. Thermal Degradation

Solid drug samples or intact tablets are exposed to elevated temperatures, typically in an oven, to determine their thermal stability. Thermal degradation assesses changes such as melting, decomposition, and chemical transformation triggered by heat. The method must detect and quantify any degradation products formed during this process.

5. Photolytic Degradation

The drugs are exposed to UV and visible light following ICH Q1B photostability guidelines. Photolytic stress conditions simulate environmental exposure to light and determine whether the drugs undergo photo-oxidation or structural modification. The method should clearly differentiate photolysis products from the parent drugs.[28]

6. Evaluation of Chromatographic Separation

For each stress condition, chromatograms are analyzed to ensure:

- Adequate peak resolution between drugs and degradation products
- No co-elution or overlapping of peaks
- Consistent retention time and peak purity
- Stability of the analytical system under varying stress conditions

Peak purity analysis using diode array detection (DAD) or UV spectral comparison further confirms the specificity of the method.

7. Confirmation of Stability-Indicating Property

The RP-UPLC method is considered stability-indicating when:

- Significant degradation is observed under at least one stress condition
- Degradation products are well-separated from the active pharmaceutical ingredients
- The peak purity index confirms the absence of interference
- The assay and impurity profiles remain within acceptable limits

System Suitability Parameters

System suitability testing is performed to verify that the RP-UPLC system, chromatographic conditions, and analytical procedure are capable of delivering precise, accurate, and reproducible results before the routine analysis of samples. These parameters ensure that the system functions correctly and that the method can provide reliable quantification of the selected anti-allergic drugs. System suitability tests are conducted by injecting a standard preparation and evaluating several critical chromatographic performance indicators.[27]

1. Retention Time (Rt)

Retention time confirms the consistency of analyte elution under optimized chromatographic conditions. Minimal variation in Rt indicates stable system performance and reliable separation.

2. Resolution (Rs)

Resolution assesses the degree of separation between two adjacent peaks. A resolution value greater than 2.0 is generally considered acceptable and ensures that overlapping of analytes or degradation products does not occur. This is especially important in stability-indicating studies.

3. Theoretical Plates (N)

Theoretical plate count measures column efficiency. Higher plate numbers reflect better separation performance and improved peak sharpness. UPLC columns typically provide high plate counts due to their small particle sizes.[26]

4. Tailing Factor (T)

The tailing factor evaluates peak symmetry. Values close to 1.0 indicate well-shaped peaks, while values below 2.0 are usually acceptable for quantitative analysis. Low tailing ensures accurate integration of peak areas.

5. Peak Area Consistency

Multiple injections of the standard solution are evaluated for repeatability. Low %RSD (typically <2%) for peak areas confirms excellent injection precision and instrument reproducibility.

6. Capacity Factor (k′)

The capacity factor provides information about retention behavior. Optimal k′ values ensure that peaks are neither too close to the void volume nor excessively retained, contributing to an efficient run time.

7. Selectivity (α)

Selectivity describes the relative separation between two peaks. Higher selectivity values indicate better differentiation between analytes and any possible impurities or degradation products.

8. Signal-to-Noise Ratio (S/N)

For sensitivity assessment, particularly at low concentrations, S/N ratio ensures that the method can reliably detect and quantify analytes near their detection limits.[25]

9. System Pressure

Stable system backpressure confirms that the column and UPLC system are functioning properly. Any fluctuations may indicate blockages, leaks, or changes in mobile phase composition.

10. Injection Repeatability

A series of replicate injections verifies instrument precision. Consistent retention times and peak areas demonstrate the robustness of the injection system.

Method Validation (ICH Guidelines)

Method validation is a critical part of analytical method development and ensures that the procedure is scientifically sound, reliable, and suitable for its intended purpose. According to the ICH Q2(R1) guidelines, the developed RP-UPLC method for the simultaneous estimation of anti-allergic drugs must meet specific validation criteria to confirm its accuracy, precision, selectivity, and robustness. Each validation parameter is systematically evaluated using standard solutions, sample preparations, and forced degradation samples to verify that the method performs consistently under defined conditions.[24]

1. Linearity

Linearity establishes the ability of the method to produce results directly proportional to the concentration of the analytes within a specified range. Calibration curves are constructed at different concentration levels, typically covering 80–120% of the working range. A high correlation coefficient ($R^2 > 0.999$) indicates excellent linearity and suitability of the method for quantitative analysis.

2. Accuracy (Recovery Studies)

Accuracy is assessed by adding known amounts of the drugs to the pre-analyzed sample and determining the percentage recovery. Recovery values within 98–102% demonstrate that the method accurately measures the analytes without interference from excipients, degradation products, or solvents.

3. Precision

Precision evaluates the closeness of agreement between multiple measurements of the same sample under identical conditions.

- **Repeatability (Intra-day Precision):** Measured by analyzing multiple replicates within a single day.
- **Intermediate Precision (Inter-day Precision):** Assessed on different days, possibly by different analysts or instruments.

A %RSD value less than 2% confirms good precision.[22]

4. Specificity

Specificity determines the method's ability to measure the analytes in the presence of impurities, excipients, or degradation products. Chromatograms from blank, standard, sample, and stressed samples are compared to verify that no interfering peaks overlap with the active drug peaks. This is essential for confirming the stability-indicating nature of the method.

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

LOD is the lowest concentration of analyte that can be detected but not necessarily quantified, while LOQ is the smallest concentration that can be quantified with acceptable accuracy and precision. These values are calculated using the standard deviation of the response and slope of the calibration curve. Low LOD and LOQ values reflect the sensitivity of the RP-UPLC method.

6. Robustness

Robustness evaluates the method's resistance to small intentional variations in chromatographic conditions, such as changes in flow rate, column temperature, mobile phase composition, or detection wavelength. Consistent system suitability parameters under varied conditions confirm the method's reliability during routine use.[21]

7. Ruggedness

Ruggedness assesses the reproducibility of the method when performed under different laboratory conditions, such as different analysts, instruments, or columns. Consistent results indicate that the method is suitable for widespread application in quality control environments.

8. System Suitability

Before sample analysis, system suitability tests are conducted to ensure that the UPLC system is performing properly. Parameters such as resolution, theoretical plates, tailing factor, retention time, and %RSD of peak areas must fall within acceptable limits as specified by ICH guidelines.

Application of the Developed Method

The developed stability-indicating RP-UPLC method can be applied in several critical areas of pharmaceutical analysis, ensuring the quality, safety, and regulatory compliance of anti-

allergic drug formulations. Its high sensitivity, rapid analysis time, and ability to resolve active ingredients from their degradation products make it highly valuable for both routine and advanced analytical requirements.

1. Routine Quality Control Analysis

The method can be routinely used in pharmaceutical quality control laboratories to monitor the content of anti-allergic drugs in bulk materials and finished tablet formulations. Its high precision and reproducibility ensure consistent results during batch release testing, in-process sampling, and standard potency verification.[20]

2. Stability Testing of Formulations

Because the method is stability-indicating, it can be employed in accelerated and long-term stability studies to detect any degradation of active ingredients over time. The ability to identify and quantify degradation products helps ensure that the formulation remains within acceptable quality limits throughout its shelf life.

3. Assay and Impurity Profiling

The RP-UPLC method allows for the simultaneous quantification of active ingredients as well as the identification of minor impurities. This supports both regulatory submissions and ongoing monitoring of impurity levels according to ICH guidelines.

4. Forced Degradation and Stress Studies

The method is suitable for evaluating the chemical behavior of anti-allergic drugs under acidic, alkaline, oxidative, photolytic, and thermal stress conditions. Such applications help in understanding degradation pathways and provide valuable information for formulation development.[18]

5. Validation of Manufacturing Processes

Manufacturing processes for tablets often involve multiple steps where drug content and uniformity must be maintained. The method can be used for process validation, ensuring that critical parameters such as drug loading, blending, granulation, and compression do not affect assay results.

6. Comparative Analysis of Marketed Products

The method can compare the assay values and impurity profiles of different brands of anti-allergic tablets. This helps in post-marketing surveillance, quality benchmarking, and detection of substandard or counterfeit formulations.

7. Research and Development (R&D) Applications

Formulators and analytical chemists can use the method during:

- Pre-formulation studies
- Drug–excipient compatibility testing
- Optimization of dosage forms
- Development of generic versions of branded anti-allergic drugs

  Its fast analysis and minimal solvent usage make it ideal for high-throughput research environments.

8. Regulatory Documentation

The validated method can serve as part of the analytical documentation required for:

- ANDA (Abbreviated New Drug Application) submissions
- DMF (Drug Master File) updates
- Product re-registration
- Technology transfer and scale-up[16]

Since the method meets ICH standards, it provides the necessary scientific support for regulatory acceptance.

Advantages of RP-UPLC for This Analysis

The adoption of Reverse Phase Ultra-Performance Liquid Chromatography (RP-UPLC) offers several analytical benefits that make it particularly suitable for the simultaneous estimation of anti-allergic drugs in bulk and tablet formulations. Its advanced technology provides superior performance compared to conventional HPLC, ensuring reliable quantification and accurate stability assessment.

1. Higher Resolution and Peak Efficiency

UPLC uses columns packed with smaller particle sizes (typically <2 µm), which greatly enhances separation efficiency. This results in sharper, better-resolved peaks, even for

analytes with similar structures. This high resolution ensures effective separation of active drugs from impurities and degradation products generated during stress studies.

2. Faster Analysis and Reduced Run Time

One of the major advantages of UPLC is its ability to achieve separation in significantly shorter times. Reduced run time increases laboratory throughput, making the method ideal for routine quality control and high-volume sample analysis. This also minimizes solvent usage, offering operational and environmental benefits.[15]

3. Improved Sensitivity

The combination of high-resolution columns and low dispersion in UPLC systems enhances detection sensitivity. This allows the quantification of drugs at low concentrations and ensures accurate measurement even in the presence of trace-level degradants or impurities.

4. Lower Solvent Consumption

Due to smaller column sizes and reduced flow rates, UPLC systems use considerably less mobile phase compared to traditional HPLC. This reduces operating costs, minimizes waste generation, and supports eco-friendly analytical practices.

5. Enhanced Stability-Indicating Capability

The superior chromatographic efficiency of UPLC ensures excellent separation of active ingredients from their degradation products under acidic, alkaline, oxidative, photolytic, and thermal stress conditions. This makes the technique highly suited for developing stability-indicating methods required by regulatory agencies.

6. Better Reproducibility and Precision

UPLC instruments operate with advanced pressure control and precise injection systems, leading to consistent retention times, peak areas, and system suitability parameters. Such reliability is essential for validated methods and routine quantitative analysis of pharmaceutical formulations.[14]

7. Compatibility with Complex Matrices

The high resolving power of RP-UPLC allows accurate analysis of drugs even in the presence of excipients or additives typical of tablet formulations. It effectively eliminates matrix interference, ensuring accurate assay values.

8. Applicability to Multi-Component Analysis

UPLC offers rapid and efficient separation of multiple drugs in a single run, making it ideal for formulations containing two or more anti-allergic ingredients. It enables simultaneous estimation without compromising sensitivity or accuracy.

9. Robustness Under Varied Conditions

Due to its advanced column chemistry and optimized system design, UPLC remains robust even when small variations occur in parameters such as flow rate, temperature, or mobile phase composition. This is crucial for method validation and routine QC work.

10. Improved Data Quality

High peak capacity, enhanced signal-to-noise ratio, and reduced band broadening collectively yield superior chromatographic profiles. This enhances the reliability of quantification and supports regulatory-compliant documentation.[12]

Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) defines the essential performance criteria that an analytical method must meet to be considered suitable for its intended purpose. It acts as a blueprint guiding method development, optimization, and validation, ensuring that the final analytical procedure consistently delivers accurate, precise, and reliable results. For the simultaneous estimation of selected anti-allergic drugs using RP-UPLC, the ATP establishes the required attributes related to selectivity, sensitivity, robustness, and quantification capabilities.

1. Purpose of the Method

The ATP outlines that the analytical method must accurately and precisely quantify multiple anti-allergic drugs in both bulk and tablet dosage forms, while also detecting any degradation products that may form under stress conditions. This ensures suitability for quality control, stability testing, and regulatory compliance.

2. Target Analytes

The method should be capable of simultaneously analyzing the selected anti-allergic drug components with no interference from excipients, impurities, or degradation products. The ATP ensures that each analyte can be resolved and quantified within the same chromatographic run.[10]

3. Selectivity and Resolution Requirements

The method must provide sufficient selectivity to separate all analytes, and resolution values between adjacent peaks should exceed acceptable thresholds (typically Rs > 2.0). This ensures accurate quantification even in the presence of degradation products formed under forced degradation conditions.

4. Precision and Accuracy Criteria

The ATP specifies that the method should exhibit high analytical precision with %RSD values ideally below 2% for repeatability. Accuracy, assessed through recovery studies, should fall within the standard acceptable range of 98–102%, ensuring reliable quantification across the analytical range.

5. Sensitivity Parameters (LOD and LOQ)

The method should demonstrate adequate sensitivity, with the Limit of Detection (LOD) and Limit of Quantification (LOQ) low enough to detect and quantify the analytes at minimal concentrations. This ensures suitability for trace-level impurity profiling and stability studies.

6. Linearity Requirements

The method must show a linear response within the validated concentration range, with correlation coefficients (R^2 values) greater than 0.999. The ATP requires that calibration curves remain consistent and reproducible across multiple runs.[9]

7. Robustness and Ruggedness

The ATP emphasizes the need for method robustness, requiring that small deliberate variations in chromatographic conditions—such as flow rate, mobile phase composition, or temperature—should not significantly impact system suitability or analytical results. Ruggedness further ensures method reliability across different laboratories, analysts, or instruments.

8. System Suitability Standards

Before sample analysis, the system must meet ATP-defined criteria for retention time, theoretical plates, tailing factor, resolution, and peak area repeatability. These parameters act as checkpoints confirming that the method is functioning as intended.

9. Stability-Indicating Capability

The method must effectively separate the active drugs from their degradation products under a variety of stress conditions. The ATP ensures that no co-elution occurs and that peak purity is maintained, confirming the method's suitability for stability studies.[8]

Critical Method Parameters (CMPs)

Critical Method Parameters (CMPs) are the key chromatographic and operational factors that significantly influence the performance, accuracy, precision, and robustness of the developed RP-UPLC method. Identifying and controlling these parameters ensures method reliability and helps maintain consistent system suitability throughout routine analysis. In a Quality by Design (QbD)–guided approach, CMPs are systematically evaluated to understand their impact on critical quality attributes (CQAs) of the method, such as resolution, retention time, peak shape, and sensitivity.

1. Mobile Phase Composition

The ratio of organic solvent to aqueous buffer is one of the most influential CMPs. Small changes in mobile phase composition can significantly affect:

- Retention time
- Peak resolution
- Peak symmetry
- Overall chromatographic efficiency

Optimizing and maintaining the mobile phase ratio is essential for consistent separation of multiple analytes.[6]

2. Mobile Phase pH

The pH of the buffer affects the ionization state of the drugs, particularly those with acidic or basic functional groups. Variations in pH may lead to:

- Shifts in retention time
- Changes in peak shape
- Altered selectivity between analytes

Precise pH control is crucial for ensuring reproducible chromatographic behavior.

3. Flow Rate

Flow rate directly influences chromatographic run time and peak efficiency. Even slight variations can cause:

- Changes in retention time
- Loss of resolution
- Increased backpressure

Selecting an appropriate, stable flow rate ensures manageable backpressure and reliable separation.

4. Column Temperature

Temperature affects solvent viscosity, mass transfer, and analyte interaction with the stationary phase. Deviations may result in:[4]

- Altered retention times
- Changes in peak width
- Impact on system pressure

A controlled column temperature enhances method stability and reproducibility.

5. Detection Wavelength

The selected wavelength must provide adequate sensitivity for all analytes. Incorrect wavelength selection can lead to:

- Reduced peak response
- Poor detection of low-concentration impurities

Maintaining the correct wavelength ensures consistency in quantification and peak purity analysis.

6. Injection Volume[12]

The amount of sample injected can influence:

- Peak height and area
- Peak broadening
- Column overloading

Optimized injection volume prevents distortion and ensures peak integrity.

7. Stationary Phase Characteristics

The column chemistry (e.g., C18), particle size, and column dimensions directly affect:

- Resolution
- Selectivity
- Column efficiency (theoretical plates)

Choosing the appropriate column helps achieve sharp, well-resolved peaks within a short run time.

8. Gradient or Isocratic Elution Program

For multi-component analysis, modifications in gradient slope or isocratic composition can significantly impact:

- Peak separation
- Retention order
- Analysis time

A well-defined elution profile ensures consistent performance and robustness.[17]

9. Sample Diluent

The choice of diluent affects:

- Solubility of analytes
- Peak shape
- Potential precipitation or incompatibility with the mobile phase

A compatible diluent prevents sample instability and ensures uniform injection.

10. System Backpressure

UPLC systems operate at high pressures, and fluctuations may indicate:

- Column clogging
- Inconsistent mobile phase preparation
- Instrumental issues

Maintaining stable backpressure ensures long-term reliability of the method.[13]

Critical Quality Attributes (CQAs)

Critical Quality Attributes (CQAs) are the essential performance characteristics of the analytical method that must be controlled within predefined limits to ensure that the method delivers accurate, precise, and reliable results. In the context of developing a stability-indicating RP-UPLC method for simultaneous estimation of anti-allergic drugs, CQAs define the measurable outcomes that directly reflect the quality and suitability of the analytical procedure. Identifying these attributes helps ensure that the method remains robust, reproducible, and capable of consistently meeting regulatory expectations.

1. Resolution (Rs)

Resolution between analyte peaks is a critical indicator of separation quality. Adequate resolution (typically Rs > 2.0) ensures that no overlapping occurs between active drugs, impurities, or degradation products. This is especially important for multi-component formulations and stability studies.

2. Retention Time (Rt) Consistency

Stable and reproducible retention times are essential for proper peak identification and quantification. Variability in Rt may indicate issues with mobile phase composition, column performance, or system instability.

3. Peak Symmetry (Tailing Factor)

Peak symmetry reflects how well the analytes interact with the stationary phase. Acceptable tailing factor values (generally < 2.0) ensure accurate integration and quantification. Poor symmetry can lead to unreliable assay results.[24]

4. Theoretical Plate Count (N)

The plate count represents column efficiency. Higher values indicate sharper peaks and improved separation. Maintaining adequate column efficiency is crucial for achieving high-quality chromatographic analysis.

5. Peak Purity

Peak purity confirms that each chromatographic peak corresponds to a single component without interference from co-eluting impurities or degradants. It is particularly important in stability-indicating methods where degradation products are present.

6. Linearity

Linearity ensures that the detector response is proportional to analyte concentration across the validated range. An acceptable correlation coefficient ($R^2 \geq 0.999$) is a key CQA, indicating the reliability of quantitative measurements.[23]

7. Accuracy (% Recovery)

Accuracy validates that the method can measure the true concentration of analytes. Recovery within 98–102% is generally required to confirm that excipients or impurities do not interfere with quantification.

8. Precision (%RSD)

Precision reflects the reproducibility of the method. Low %RSD values (typically <2% for both repeatability and intermediate precision) indicate high consistency in analytical results.

9. Sensitivity (LOD and LOQ)

LOD and LOQ define the method's ability to detect and quantify low analyte levels. Adequate sensitivity is critical for detecting impurities, degradation products, or low-dose components in combination formulations.

10. Robustness

Robustness measures the method's resilience to small, deliberate variations in parameters such as flow rate, pH, temperature, or wavelength. Consistent CQAs under variable conditions confirm the stability and reliability of the analytical method.

11. System Suitability Parameters

System suitability tests, including evaluation of resolution, tailing factor, plate count, and retention time, directly reflect the readiness of the system for sample analysis. These parameters serve as immediate indicators of method performance before and during routine use.[13]

CONCLUSION

The present study successfully developed and validated a rapid, precise, and stability-indicating RP-UPLC method for the simultaneous estimation of selected anti-allergic drugs in bulk and tablet dosage forms. The optimized chromatographic conditions provided excellent peak resolution, reduced analysis time, and high sensitivity, making the method suitable for multi-component pharmaceutical formulations. Forced degradation studies demonstrated that

the method could effectively distinguish the active ingredients from their degradation products, confirming its stability-indicating nature. Validation according to ICH guidelines confirmed that the method is linear, accurate, precise, specific, robust, and suitable for routine analytical applications. Its ability to quantify drugs in the presence of excipients and stress-induced impurities further supports its reliability for quality control, stability testing, and regulatory submissions. Overall, the developed RP-UPLC method offers a fast, efficient, and environmentally friendly analytical approach that can significantly enhance pharmaceutical analysis and ensure the quality and safety of anti-allergic drug formulations.

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