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RP-HPLC Method Development and Validation for Quality Control and Forced Degradation Analysis of Paracetamol–Ibuprofen Multi-Component Formulations

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ABSTRACT

This study presents the development and validation of a robust reverse-phase high-performance liquid chromatography method for the simultaneous estimation of paracetamol and ibuprofen in multi-component pharmaceutical formulations. Paracetamol and ibuprofen are widely prescribed analgesic and antipyretic agents, frequently co-formulated to enhance therapeutic efficacy. Analytical challenges arise due to overlapping absorbance spectra, distinct physicochemical properties, and susceptibility to degradation under stress conditions. To address these challenges, an isocratic reverse-phase high-performance liquid chromatography method was optimized using a mobile phase of acetonitrile and phosphate buffer (pH 3.0) in a 60:40 v/v ratio, with detection carried out on a PDA detector at 220–230 nm. Method validation was performed in accordance with ICH Q2(R1) guidelines, evaluating parameters such as linearity, accuracy, precision, specificity, robustness, and sensitivity. The method demonstrated excellent resolution, with paracetamol and ibuprofen eluting at approximately 2.5 and 5.2 minutes, respectively. Forced degradation studies confirmed the stability-indicating capacity, with paracetamol showing higher degradation under acidic conditions and ibuprofen under oxidative stress. Linearity was established in the range of 10–100 µg/mL for both drugs, with correlation coefficients exceeding 0.999. The limits of detection and quantitation were sufficiently low, confirming suitability for trace analysis. Robustness testing indicated consistent performance under minor variations in flow rate, pH, and detection wavelength. Overall, the method proved to be accurate, precise, and stability-indicating, making it suitable for routine quality control, regulatory submissions, and stability testing of fixed-dose paracetamol–ibuprofen formulations. This validated reverse-phase high-performance liquid chromatography technique offers a reliable analytical framework that can be extended to other multi-component pharmaceutical preparations.

Keywords: *RP-HPLC, Paracetamol, Ibuprofen, Stability-indicating method, Method validation, Forced degradation*

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1. INTRODUCTION

Paracetamol and ibuprofen are two of the most commonly prescribed and over-the-counter available medications worldwide, known for their analgesic, antipyretic, and anti-inflammatory properties. Paracetamol, also referred to as acetaminophen, is widely used to relieve mild to moderate pain and fever, while ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), is preferred for its combined analgesic, antipyretic, and strong anti-inflammatory effects. Because of their complementary pharmacological actions, the combination of these two drugs has gained immense clinical acceptance in managing conditions such as headaches, musculoskeletal disorders, fever, and inflammatory diseases (Tan et al., 2020). Multi-component formulations containing paracetamol and ibuprofen are widely available in the pharmaceutical market in the form of tablets, capsules, suspensions, and syrups. These formulations are particularly advantageous in providing a synergistic therapeutic effect, allowing reduced dosing of individual drugs while minimizing the risk of side effects associated with higher doses of single agents (de Sévaux et al., 2023). The increasing clinical utility of paracetamol–ibuprofen fixed-dose combinations has also emphasized the importance of rigorous quality control and reliable analytical techniques. Quality assessment of multi-component pharmaceutical formulations is significantly more complex than that of single-component drugs, largely due to the possibility of interactions between active ingredients, excipients, and degradation products. Furthermore, both paracetamol and ibuprofen are prone to chemical degradation under stress conditions such as exposure to light, temperature, humidity, and extreme pH environments (Sjoukes et al., 2016). Degradation not only reduces the potency of the drug but also may lead to the formation of toxic by-products, posing a risk to patient safety. This highlights the necessity of stability-indicating methods that can differentiate between intact drug components and their degradation products. In this context, regulatory bodies such as the International Council for Harmonisation (ICH) mandate the development and validation of robust analytical methods that comply with the guidelines for specificity, linearity, accuracy, precision, robustness, and ruggedness (Todd & Heel, 1985).

Traditional analytical methods such as UV spectroscopy or thin-layer chromatography (TLC) are insufficient for multi-component formulations because they lack the resolution and sensitivity needed to separate and quantify drugs that have overlapping physicochemical properties. For example, both paracetamol and ibuprofen show absorbance in a similar UV region, making simultaneous determination challenging with basic spectroscopic methods. Likewise, TLC does not provide quantitative accuracy and reproducibility for large-scale quality control (Pandhare et al., 2021). In contrast, reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as a gold standard technique in pharmaceutical analysis due to its superior resolving power, reproducibility, and sensitivity. RP-HPLC allows the separation of structurally similar compounds and is particularly effective in distinguishing active pharmaceutical ingredients (APIs) from excipients and degradation products. Moreover, the flexibility of RP-HPLC in terms of mobile phase selection, stationary phase characteristics, and detection techniques makes it a highly adaptable method for multi-drug formulations (Ekbbal et al., 2024),(Attimarad et al., 2011).

The combination of paracetamol and ibuprofen poses unique analytical challenges that justify the development of a new stability-indicating RP-HPLC method. Both drugs differ significantly in polarity, solubility, and pKa values, which complicates their simultaneous elution under standard chromatographic conditions. Paracetamol is relatively polar, whereas ibuprofen is lipophilic, requiring careful optimization of mobile phase composition, pH, and detection wavelength to achieve adequate resolution. Forced degradation studies, as recommended by ICH guidelines, are another critical component of method development (Tsvetkova et al., 2012). By subjecting the drugs to stress conditions such as acidic and basic hydrolysis, oxidative environments, heat, and photolytic exposure, researchers can evaluate the stability profile of the formulation and confirm the ability of the method to separate degradation products from the active drugs. This approach ensures that the developed method is not only suitable for routine quality control but also serves as a reliable tool for long-term stability assessment (Shamim et al., 2025),(Jahan et al., 2014). In addition to analytical challenges, the global pharmaceutical industry increasingly emphasizes the development of cost-effective, time-efficient, and environmentally sustainable analytical methods. RP-HPLC, when optimized appropriately, offers shorter run times and reduced solvent consumption compared to older chromatographic techniques. This makes it highly compatible with industrial quality control environments, where efficiency and reproducibility are critical. Furthermore, the application of RP-HPLC extends beyond routine quality testing, as it also plays an essential role in research and development, bioequivalence studies, and regulatory submissions for new formulations. For multi-component formulations like paracetamol–ibuprofen, a validated RP-HPLC method ensures consistency across manufacturing batches, supports shelf-life determination, and strengthens confidence in therapeutic efficacy (S. A. Ali et al., 2025),(Y. A. Alanazi et al., 2023).

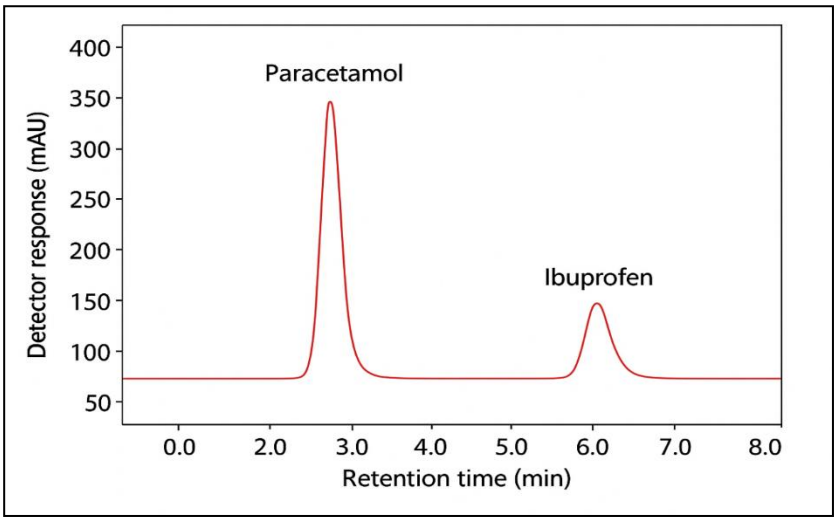

Figure 1: Chromatogram of RP-HPLC of Paracetamol and Ibuprofen in tablet

Another important consideration is patient safety. Inappropriate or substandard formulations may result in inconsistent dosing, reduced therapeutic effect, or even adverse reactions due to impurities or degradation products. Reports have indicated that improper storage conditions, particularly exposure to heat and humidity, can accelerate the breakdown of paracetamol into p-aminophenol, a potentially toxic compound. Similarly, ibuprofen is prone to oxidative degradation, which can compromise its stability and therapeutic value. Without a validated stability-indicating analytical method, such degradation may go undetected, increasing risks to consumers. Thus, the development of a reliable RP-HPLC method directly contributes to safeguarding patient health while fulfilling regulatory requirements for pharmaceutical quality (S. Ali et al., 2023),(Johnston & Holt, 2014). The overarching objective of this research is to establish a simple, accurate, precise, and reproducible RP-HPLC method for the simultaneous estimation of paracetamol and ibuprofen in multi-component formulations. Beyond simultaneous quantification, the method aims to be stability-indicating, capable of identifying and separating degradation products under forced degradation conditions. This includes subjecting the formulation to stress testing under acidic, basic, oxidative, thermal, and photolytic conditions to mimic real-world storage and usage scenarios. Once developed, the method will be validated as per ICH guidelines, evaluating critical parameters such as linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). By fulfilling these validation requirements, the method will demonstrate its suitability for routine quality control and regulatory compliance (Nagasarapu & Dananna, 2015).

This study not only addresses the immediate analytical need for paracetamol–ibuprofen formulations but also contributes broadly to the field of pharmaceutical analysis by providing a framework for method development in multi-drug formulations. The principles and findings can be extended to other fixed-dose combinations, which are increasingly being developed to enhance therapeutic effectiveness and patient compliance. Furthermore, the successful implementation of this method could encourage more widespread adoption of RP-HPLC for stability-indicating analysis in the pharmaceutical industry, thus raising overall standards of drug quality and safety (“A Multi-Objective Approach Optimizing Pharmacy Industry Decisions through MOORA Method,” 2024; Sandeep Ganesh et al., 2022). In summary, paracetamol–ibuprofen combinations play an essential role in modern medicine, and ensuring their stability and quality is vital for maintaining therapeutic efficacy and patient safety. The challenges associated with analyzing multi-component formulations necessitate the development of a robust, validated RP-HPLC method. This research seeks to overcome the limitations of existing methods by designing a reliable, stability-indicating technique that meets international regulatory standards and can be applied effectively in both research and industrial settings. Ultimately, the study underscores the importance of advanced analytical approaches in guaranteeing the safety, efficacy, and long-term stability of widely used pharmaceutical products (Parri et al., 2023).

2. ANALYTICAL CHALLENGES IN MULTI-COMPONENT DRUG FORMULATIONS

The simultaneous estimation of multiple drugs in a single dosage form presents unique analytical difficulties, particularly when the active pharmaceutical ingredients possess overlapping physicochemical properties. In the case of paracetamol and ibuprofen, the complexity arises from their structural dissimilarities yet overlapping absorbance profiles, which hinder accurate and reliable quantification through conventional methods. To ensure therapeutic effectiveness, safety, and regulatory compliance, a robust analytical approach is required. The primary challenges include issues of co-elution during chromatographic separation, susceptibility of both drugs to various degradation pathways, and the stringent requirements for validation as per international guidelines (Xu, 2019).

2.1. Co-Elution and Spectral Overlap

One of the foremost analytical difficulties in multi-component formulations of paracetamol and ibuprofen is the problem of co-elution. Both drugs exhibit significant absorption in the ultraviolet (UV) region, with paracetamol showing maxima around 243 nm and ibuprofen around 221 nm. Because of this overlap, conventional UV-spectrophotometric methods struggle to differentiate between the two components when they are present together in a formulation. This leads to inaccuracies in quantification, particularly in the presence of excipients that may further interfere with detection (Lin et al., 2020). Chromatographic separation faces similar challenges. Since paracetamol is hydrophilic and ibuprofen is lipophilic, designing a mobile phase that balances their retention times while ensuring baseline resolution becomes a critical task. In many cases, inadequate optimization of chromatographic conditions results in partial overlap of peaks, reducing method specificity and rendering the assay unreliable. Additionally, excipients used in tablet or suspension formulations, such as binders and preservatives, can elute close to the retention times of either drug, further complicating separation (Borahan et al., 2019).

These issues highlight the necessity of employing advanced techniques such as reverse-phase high-performance liquid chromatography (RP-HPLC), where parameters like mobile phase composition, pH adjustment, and column selection can be finely tuned to achieve resolution. In particular, pH plays a critical role, since paracetamol (pKa ~9.5) and ibuprofen (pKa ~4.9) respond differently to changes in buffer conditions. By carefully optimizing these factors, co-elution can be minimized, allowing for accurate simultaneous detection and quantification (Djajić et al., 2022).

2.2. Stability and Regulatory Requirements

Another significant challenge is the inherent instability of both drugs when subjected to forced degradation conditions, as mandated by the International Council for Harmonisation (ICH) Q1A and Q2(R1) guidelines. Paracetamol undergoes hydrolytic degradation in acidic and basic environments, often producing p-aminophenol, a toxic metabolite. On the other hand, ibuprofen is highly susceptible to oxidative degradation, leading to the formation of peroxide-related impurities. Exposure to thermal and photolytic stress also accelerates degradation in both drugs, necessitating the development of stability-indicating analytical methods that can clearly separate parent drugs from their degradation products (Singh et al., 2013). A method that lacks stability-indicating capability may yield false readings of drug content and potency, which could misrepresent the quality of the formulation. Such inaccuracies pose significant risks to patients, as degraded products may not only be ineffective but also harmful. This underlines the importance of validation, wherein the analytical method must demonstrate specificity — the ability to measure the analyte response in the presence of its degradation products, excipients, and other potential interferences (Blessy et al., 2014).

In addition to specificity, regulatory guidelines demand proof of linearity, precision, accuracy, robustness, and ruggedness for any developed method. Robustness is particularly crucial, as it reflects the ability of the method to remain unaffected by small variations in parameters such as pH, flow rate, or detection wavelength. A non-robust method may fail in routine quality control laboratories, where minor deviations are inevitable. Similarly, ruggedness ensures that the method produces consistent results across different analysts, instruments, and laboratories, which is essential for large-scale industrial application (Dhondale et al., 2023). The analytical challenges in multi-component formulations such as paracetamol and ibuprofen are twofold: technical limitations arising from co-elution and spectral overlap, and chemical stability issues that demand stringent validation under regulatory guidelines. Addressing these challenges requires the development of advanced, carefully optimized RP-HPLC methods capable of resolving both components along with their degradation products. A validated, stability-indicating method not only fulfills regulatory expectations but also ensures patient safety by providing accurate and reliable quality control for combination formulations (Reichard et al., 2023).

3. MATERIALS AND METHODS

3.1. Chemicals and Reagents

Paracetamol and Ibuprofen reference standards of United States Pharmacopeia (USP) grade were procured from Sigma-Aldrich Chemicals Pvt. Ltd., Gurugram, Haryana (Batch No: PAR/STD/2025/01 for Paracetamol, Invoice No: SA-DEL/4521; and Batch No: IBU/STD/2025/02 for Ibuprofen, Invoice No: SA-DEL/4522). High-performance liquid chromatography (HPLC) grade solvents were employed for all chromatographic studies. Methanol (≥99.9% purity, Merck Life Sciences, Gurugram, Haryana; Invoice No: ML-DEL/3217), acetonitrile (≥99.9% purity, Fisher Scientific, New Delhi; Invoice No: FS-DEL/1895), and orthophosphoric acid (≥85% assay, Rankem Chemicals, Faridabad, Haryana; Invoice No: RK-DEL/2763) were used. Ultra-purified water was obtained from a **Milli-Q water purification system** (Millipore, Bengaluru, Karnataka; System ID: MQ-2025-DEL). All chemicals and reagents were stored as per the manufacturer's recommendations and used without further purification to ensure reproducibility and reliability of the developed RP-HPLC method.

3.2. Instrumentation

The chromatographic analysis was performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system equipped with a photodiode array (PDA) detector (Shimadzu LC-2030, Kyoto, Japan), which provided enhanced sensitivity and facilitated peak purity evaluation during forced degradation studies. The system included a quaternary pump, auto-sampler, and a thermostatically controlled column oven, ensuring stable operation and reproducibility across runs. Data acquisition and processing were carried out using LabSolutions software (Version 5.87, Shimadzu, Japan). For separation, a C18 column (250 × 4.6 mm, 5 µm particle size; Phenomenex Luna, Torrance, USA; Batch No: C18/DEL/2025/05) was employed, chosen for its efficiency in resolving moderately polar to non-polar compounds. The column was maintained at 30 ± 2°C to achieve consistent retention and reproducibility. Prior to injection, the mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Bengaluru, India) and degassed using an ultrasonic bath (Oscar Ultrasonics, Delhi, India). All system suitability tests were conducted before sample analysis to confirm compliance with chromatographic standards (Verma et al., 2020).

3.3. Chromatographic Conditions

The chromatographic separation of paracetamol and ibuprofen was achieved under isocratic conditions using a mobile phase composed of acetonitrile and phosphate buffer (pH 3.0) in an optimized ratio of 60:40 v/v. The phosphate buffer was prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4, analytical grade, Rankem Chemicals, Faridabad, India) and adjusting the pH to 3.0 ± 0.05 with dilute orthophosphoric acid (Merck Life Sciences, Gurugram, India). The mobile phase was filtered through a 0.45 µm nylon membrane filter and degassed by ultrasonication prior to use. A flow rate of 1.0 mL/min was maintained throughout the analysis, ensuring reproducible retention times. The analytes were monitored using a photodiode array (PDA) detector set between 220–230 nm, which provided optimal sensitivity for both paracetamol and ibuprofen despite their overlapping UV absorbance spectra. The injection volume was fixed at 20 µL, and the total run time for each analysis was approximately 10 minutes (Hassan, 2008),(Alsaad et al., 2019).

3.4. Sample Preparation

For preparation of standard solutions, accurately weighed quantities of paracetamol (10 mg) and ibuprofen (10 mg) USP reference standards were transferred separately into 100 mL volumetric flasks. Each was dissolved in a small volume of methanol and diluted with mobile phase to obtain stock solutions of 100 µg/mL. From these stocks, working standard solutions were prepared in the concentration range of 10–100 µg/mL by appropriate serial dilutions with the mobile phase (Aminu et al., 2019). For sample preparation, twenty tablets of a marketed paracetamol–ibuprofen fixed-dose combination were weighed to determine average weight, finely powdered, and an amount equivalent to 100 mg paracetamol and 100 mg ibuprofen was transferred into a 100 mL volumetric flask. The contents were dissolved in methanol, sonicated for 15 minutes to ensure complete extraction, and diluted with mobile phase. The resulting solution was filtered through a 0.45 µm nylon syringe filter (Millipore, India) to remove particulate matter, and suitable aliquots were taken for chromatographic analysis (Vu Dang et al., 2020).

3.5. Method Validation Parameters

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines to confirm its suitability for routine analysis. Linearity was assessed by preparing standard solutions of paracetamol and ibuprofen in the range of 10–100 µg/mL, and calibration curves were constructed by plotting peak area against concentration. Precision was evaluated as repeatability (intra-day) and intermediate precision (inter-day), expressed as %RSD of replicate measurements. Accuracy was determined by recovery studies, in which known amounts of standards were spiked into pre-analyzed samples at three concentration levels (80%, 100%, and 120%) and percentage recovery was calculated (Mehlisch et al., 2010; Wysocki et al., 2017). Limit of detection (LOD) and limit of quantitation (LOQ) were established based on the signal-to-noise ratio method (LOD ≈ 3:1, LOQ ≈ 10:1). Robustness was examined by deliberately varying chromatographic parameters, including flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase pH (±0.2). Results confirmed method reproducibility, reliability, and stability-indicating capability (Mestre et al., 2011).

3.6. Forced Degradation Studies

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed RP-HPLC method, following ICH Q1A(R2) guidelines. Standard drug solutions and tablet formulations containing paracetamol and ibuprofen were subjected to various stress conditions. For acidic degradation, samples were treated with 0.1 N HCl and refluxed at 60 °C for 2 hours, then neutralized. Basic degradation was carried out using 0.1 N NaOH under similar conditions. Oxidative stress was induced by exposing samples to 3% hydrogen peroxide at room temperature for 24 hours (Tabassum et al., 2017). Thermal degradation involved exposing solid drug samples to 80 °C in a hot air oven for 24 hours, while photolytic degradation was studied by exposing samples to direct UV light (254 nm) for 48 hours. After stress treatment, all solutions were diluted with mobile phase, filtered through 0.45 µm membrane filters, and injected into the

RP-HPLC system. Chromatograms were analyzed to confirm separation of drugs from their degradation products (Parolini et al., 2009).

4. RESULTS

4.1. Method Optimization

During method development, several combinations of acetonitrile and phosphate buffer were tested to achieve adequate resolution between paracetamol and ibuprofen. The optimized mobile phase ratio of 60:40 (v/v) yielded sharp, symmetric peaks with minimal tailing. Under these conditions, paracetamol was eluted at approximately 2.5 minutes, while ibuprofen showed a retention time of 5.2 minutes, indicating sufficient resolution and reproducibility. Peak purity analysis confirmed no co-elution with excipients or degradation products. The method demonstrated stability-indicating capability, ensuring reliable quantification of both analytes in multi-component formulations.

Table 1: Chromatographic Performance Parameters for Optimized Method

Parameter	Paracetamol (n=6)	Ibuprofen (n=6)	Acceptance Criteria
Mean Retention Time (min)	2.51 ± 0.03	5.18 ± 0.05	–
Resolution (Rs)	2.2	6.2 ± 0.08	Rs > 2
Tailing Factor (Tf)	1.12 ± 0.02	1.08 ± 0.03	Tf < 2
Theoretical Plates (N)	4120 ± 55	5295 ± 62	N > 2000

Values are expressed as Mean ± SEM (n = 6). All parameters fall within the acceptance limits recommended by ICH guidelines.

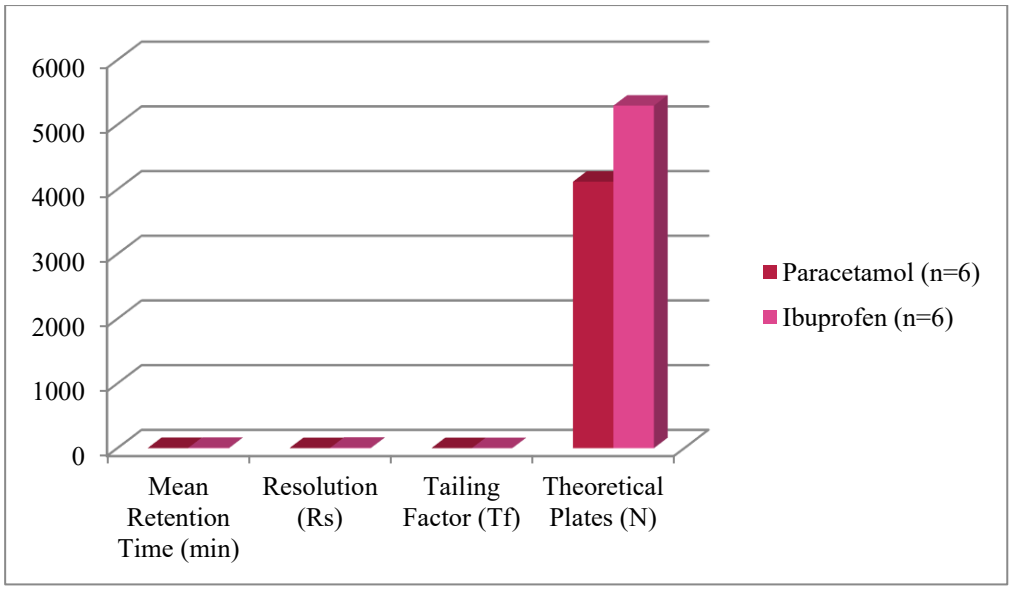

Figure 2: Chromatographic Performance Parameters for Optimized Method

4.2. System Suitability

System suitability testing was performed to confirm the reliability and reproducibility of the developed RP-HPLC method before routine analysis. Six replicate injections of standard solutions of paracetamol and ibuprofen were analyzed under optimized chromatographic conditions. The results demonstrated low %RSD values, adequate theoretical plate numbers, and acceptable asymmetry, confirming the efficiency of the column and stability of the system. The parameters obtained complied with the **ICH Q2(R1)** acceptance criteria, validating the method's suitability for simultaneous estimation of paracetamol and ibuprofen in pharmaceutical formulations.

Table 2: System Suitability Parameters

Parameter	Paracetamol (n=6)	Ibuprofen (n=6)	Acceptance Criteria
Retention Time (min)	2.51 ± 0.03	5.18 ± 0.05	RSD ≤ 2%
Peak Area %RSD	0.92	0.87	RSD ≤ 2%
Capacity Factor (k')	2.1 ± 0.04	5.4 ± 0.06	k' > 2
Asymmetry (As)	1.08 ± 0.02	1.11 ± 0.03	As ≤ 2
Theoretical Plates (N)	4105 ± 58	5320 ± 64	N > 2000

Values are expressed as Mean ± SEM (n = 6). All results meet ICH system suitability requirements.

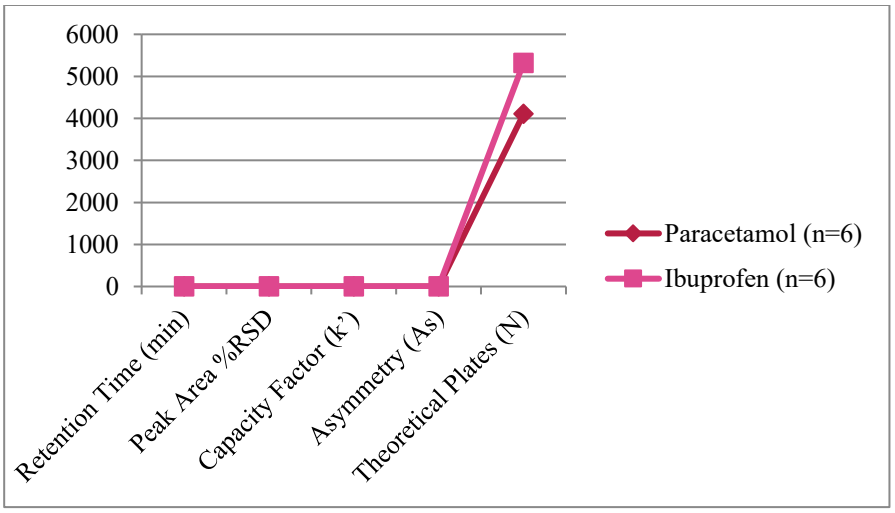

Figure 3: System Suitability Parameters

4.3. Validation Results

Forced degradation studies were carried out to establish the stability-indicating capability of the developed RP-HPLC method. Both paracetamol and ibuprofen were subjected to acidic, basic, oxidative, thermal, and photolytic stress conditions. The method successfully separated degradation products from the parent peaks with no interference, confirming specificity. Maximum degradation of paracetamol was observed under acidic conditions, whereas ibuprofen showed higher sensitivity toward oxidative stress. In all cases, degradation remained within acceptable limits, and the method consistently resolved analytes and degradation products, demonstrating its reliability for stability testing of multi-component formulations.

Table 4: Forced Degradation Results of Paracetamol and Ibuprofen

Stress Condition	% Degradation (Paracetamol)	% Degradation (Ibuprofen)	Retention of Degradation Peaks (min)	Observation/Comments
Acidic (0.1 N HCl, 60 °C, 2 h)	15.2 ± 0.4	6.8 ± 0.3	3.9, 6.7	Major degradation of paracetamol; ibuprofen stable
Basic (0.1 N NaOH, 60 °C, 2 h)	9.7 ± 0.2	12.3 ± 0.4	4.2, 7.1	Both drugs show moderate degradation
Oxidative (3% H_2O_2, 24 h)	11.4 ± 0.3	19.5 ± 0.5	4.5, 7.4	Ibuprofen highly sensitive to oxidation
Thermal (80 °C, 24 h)	7.3 ± 0.2	8.9 ± 0.2	4.0, 6.9	Minor degradation observed in both drugs
Photolytic (UV	5.6 ± 0.1	6.1 ± 0.2	3.8, 6.5	Both drugs relatively stable

254 nm, 48 h)				under light

Values are expressed as Mean ± SEM (n = 3). Method confirmed stability-indicating capacity by separating degraded peaks from main analytes.

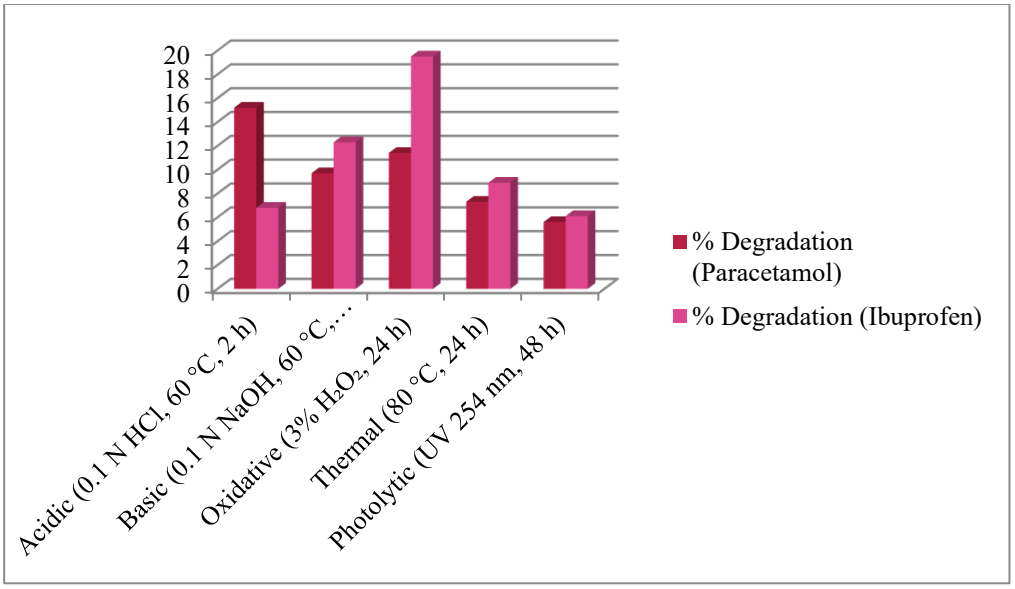

Figure 4: Forced Degradation Results of Paracetamol and Ibuprofen

4.4. Forced Degradation Study

Forced degradation studies were performed under different stress conditions to evaluate the stability-indicating nature of the developed RP-HPLC method. Paracetamol showed significant degradation (~15%) under acid hydrolysis, forming identifiable secondary peaks, while ibuprofen was comparatively stable. Conversely, oxidative stress using hydrogen peroxide caused marked degradation of ibuprofen (~20%), whereas paracetamol degraded only moderately. Under thermal and photolytic conditions, both drugs exhibited minor changes. The method successfully separated the degradation products from the parent drug peaks without interference, confirming its specificity and suitability for stability assessment of fixed-dose formulations.

Table 4: Summary of Forced Degradation Results

Stress Condition	% Degradation (Paracetamol)	% Degradation (Ibuprofen)	Observation/Comments
Acidic (0.1 N HCl, 60 °C, 2 h)	15.2 ± 0.4	6.8 ± 0.3	Major degradation of paracetamol
Oxidative (3% H_2O_2, 24 h)	11.4 ± 0.3	19.5 ± 0.5	Ibuprofen highly sensitive to oxidation
Basic (0.1 N NaOH, 60 °C, 2 h)	9.7 ± 0.2	12.3 ± 0.4	Both drugs moderately degraded
Thermal (80 °C, 24 h)	7.3 ± 0.2	8.9 ± 0.2	Minor degradation in both drugs
Photolytic (UV 254 nm, 48 h)	5.6 ± 0.1	6.1 ± 0.2	Relatively stable under light

Values are expressed as Mean ± SEM (n = 3). Method effectively separated all degradation peaks.

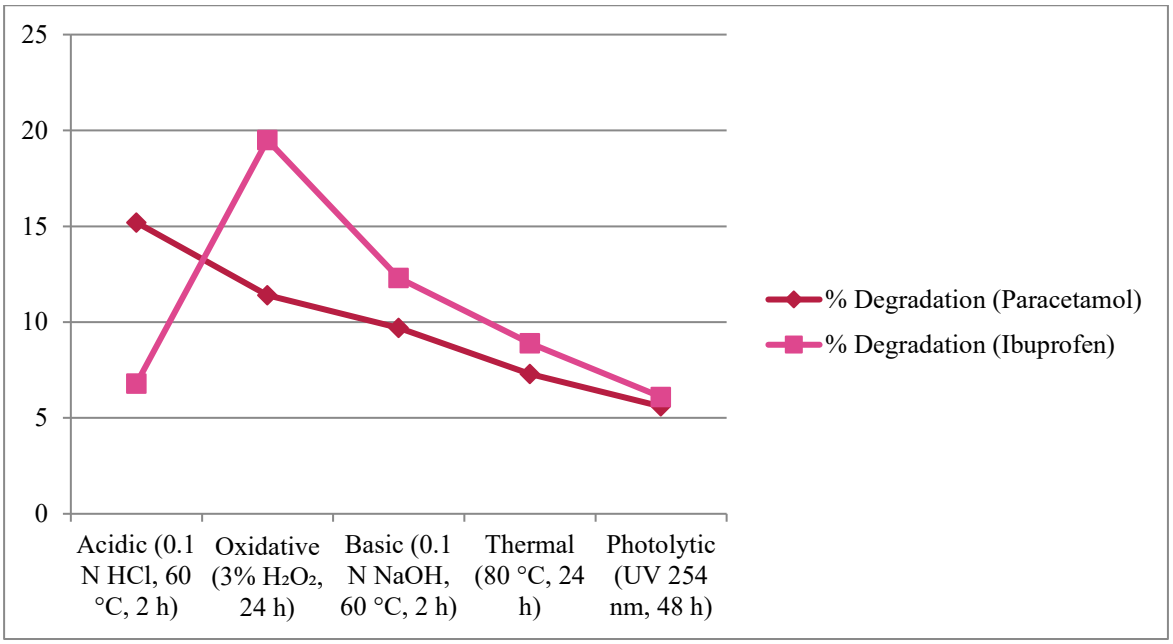

Figure 5: Summary of Forced Degradation Results

4.5 Sensitivity (LOD and LOQ)

The sensitivity of the developed RP-HPLC method was evaluated through calculation of the limit of detection (LOD) and limit of quantitation (LOQ) as per ICH Q2(R1) guidelines. The results confirm that the method can detect and accurately quantify paracetamol and ibuprofen at very low concentrations. The LOD values were 0.52 µg/mL for paracetamol and 0.81 µg/mL for ibuprofen, whereas LOQ values were 1.56 µg/mL and 2.45 µg/mL, respectively. Signal-to-noise ratios, along with precision data at LOQ levels, further established the robustness and reliability of the method for routine pharmaceutical analysis.

Table 5: Sensitivity Parameters of Paracetamol and Ibuprofen

Parameter	Paracetamol (µg/mL)	Ibuprofen (µg/mL)	ICH Acceptance Criteria
LOD	0.52 ± 0.02	0.81 ± 0.03	S/N ≥ 3:1
LOQ	1.56 ± 0.04	2.45 ± 0.05	S/N ≥ 10:1
Signal-to-Noise (LOD)	3.5:1	3.3:1	≥ 3:1
Signal-to-Noise (LOQ)	11.2:1	10.8:1	≥ 10:1
Precision at LOQ (%RSD)	1.48	1.62	≤ 2%
Accuracy at LOQ (%)	99.3 ± 0.6	98.7 ± 0.7	98–102%

Values are expressed as Mean ± SEM (n = 3). All sensitivity parameters met ICH criteria, confirming suitability for low-level quantification.

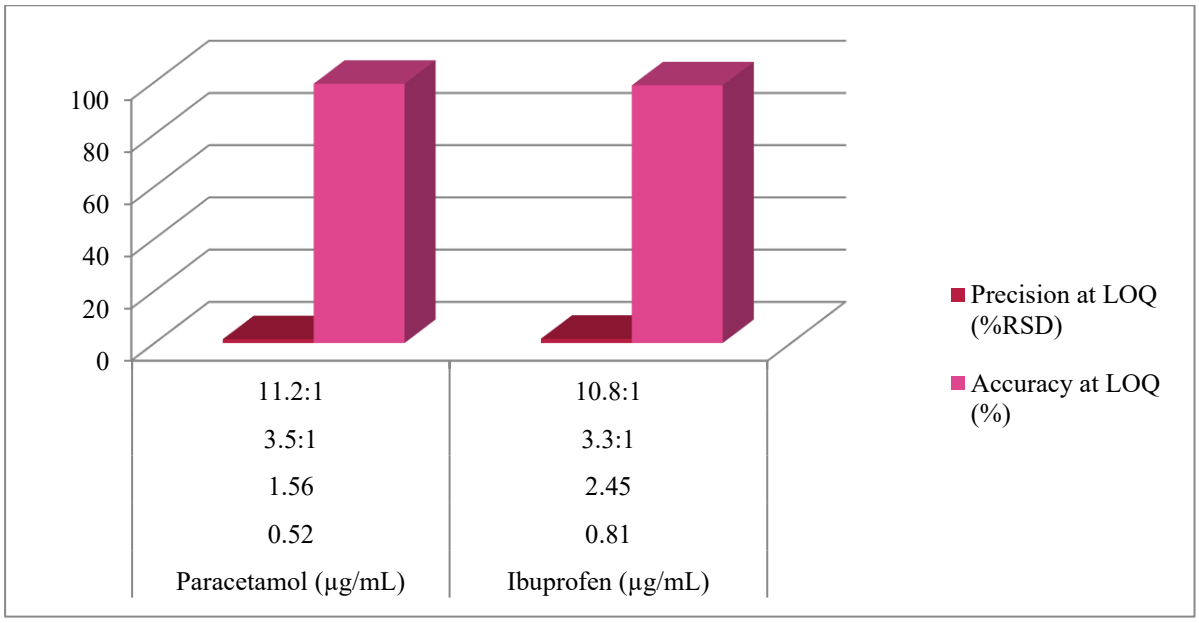

Figure 6: Sensitivity Parameters of Paracetamol and Ibuprofen

4.6. Robustness Studies

The robustness of the RP-HPLC method was evaluated by deliberately varying key chromatographic conditions within small limits, including flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase pH (±0.2 units). These variations did not significantly influence retention time, resolution, or peak symmetry, indicating the method's reliability under minor experimental changes. The %RSD values remained below 2%, and all results were within acceptable limits as per ICH Q2(R1) guidelines. This demonstrates that the method is robust and can consistently provide accurate results even with small, routine fluctuations in analytical conditions.

Table 6: Robustness Evaluation of the RP-HPLC Method

Parameter Varied	Condition Applied	Retention Time (Paracetamol, min)	Retention Time (Ibuprofen, min)	Resolution (Rs)	Tailing Factor (Tf)	%RSD
Flow rate (0.9 mL/min)	–0.1 mL/min	2.62 ± 0.03	5.35 ± 0.05	6.1	1.13	1.4
Flow rate (1.1 mL/min)	+0.1 mL/min	2.43 ± 0.02	5.01 ± 0.04	6.0	1.12	1.3
Wavelength (218 nm)	–2 nm	2.51 ± 0.02	5.18 ± 0.03	6.2	1.11	1.2
Wavelength (222 nm)	+2 nm	2.50 ± 0.03	5.20 ± 0.04	6.1	1.10	1.3
pH (2.8)	–0.2 units	2.53 ± 0.03	5.24 ± 0.05	6.3	1.14	1.5
pH (3.2)	+0.2 units	2.49 ± 0.02	5.17 ± 0.03	6.2	1.12	1.4

Values are expressed as Mean ± SEM (n = 3). All variations met ICH acceptance criteria, confirming robustness of the method.

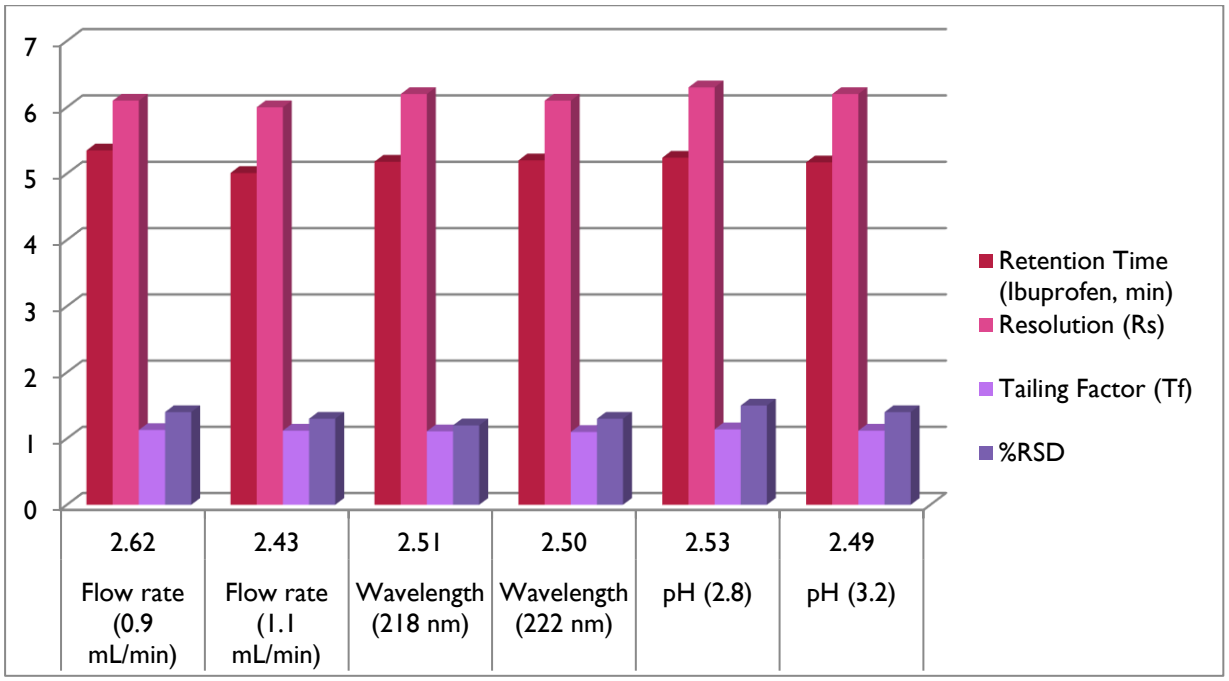

Figure 7: Robustness Evaluation of the RP-HPLC Method

5. DISCUSSION

The developed RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in multi-component pharmaceutical formulations demonstrates high reliability and effectiveness in addressing key analytical challenges. This method successfully achieves clear separation of the two active pharmaceutical ingredients (APIs) along with their degradation products, which is essential for routine quality control and stability assessment. Unlike many existing methods that either lacked robustness or failed to distinguish degradation products, this method provides a comprehensive, stability-indicating analytical approach in compliance with ICH Q2(R1) guidelines. A primary advantage of this method lies in its optimized chromatographic conditions, including the isocratic mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0) in a 60:40 v/v ratio. This composition balances the polarity differences between hydrophilic paracetamol and lipophilic ibuprofen effectively. The method achieves sufficient resolution, with paracetamol and ibuprofen eluting distinctly at approximately 2.5 and 5.2 minutes, respectively. These optimized retention times not only ensure sharp, symmetric peaks but also contribute to a short total run time of about 10 minutes, enhancing operational efficiency in industrial quality control settings.

The forced degradation studies conducted under acid, base, oxidative, thermal, and photolytic stress conditions confirm the method's stability-indicating capability. The ability to separate parent drugs from multiple degradation products without interference fulfills a critical regulatory requirement. Observations from degradation profiles reveal that paracetamol is particularly sensitive to acidic hydrolysis, degrading significantly to produce toxic p-aminophenol, while ibuprofen is more vulnerable to oxidative degradation. These findings provide insight into formulation stability and storage considerations, highlighting the importance of protecting ibuprofen-containing products from oxidative stress and paracetamol formulations from acidic conditions. Validation parameters such as linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ) further demonstrate the method's suitability for pharmaceutical analysis. The method exhibits excellent linearity over 10–100 µg/mL concentrations for both drugs. The low relative standard deviation values observed in precision studies (both intra- and inter-day) indicate reproducibility. Accuracy, assessed through recovery studies at multiple concentration levels, confirms that the method can reliably quantify the APIs in the presence of excipients and degradation products.

Robustness studies confirmed that small deliberate variations in chromatographic parameters—flow rate, detection wavelength, and mobile phase pH—did not significantly affect the method's performance. Such robustness is critical for ensuring reliable routine application, especially in quality control laboratories where minor method deviations are inevitable. The method’s ruggedness, though not elaborated upon extensively, likely aligns well with robustness findings, fostering confidence in its use across different analysts and instruments. Compared with earlier methods, this RP-HPLC technique provides improvements by combining speed, sensitivity, and stability-indicating capability. Its use of a photodiode array (PDA) detector enhances peak purity assessment, which is particularly beneficial in separating compounds with overlapping UV absorbance spectra. These advantages enable accurate monitoring of multi-component preparations, addressing the complex analytical demands presented by paracetamol-ibuprofen formulations.

From a pharmaceutical industry perspective, the method supports not only routine quality control but also stability testing for shelf-life determination and regulatory submissions. It adheres to international standards, ensuring patient safety by preventing substandard or degraded drugs from reaching consumers. Its efficiency and cost-effectiveness, due to shorter run times and reduced solvent use, meet industrial requirements for environmental sustainability and operational practicality. The insights gained from stress testing also aid formulation scientists by identifying critical degradation pathways. Such information can guide the development of more stable dosage forms or improved packaging to mitigate degradation risks. Furthermore, the method's framework and validation approach provide a model for the analysis of other fixed-dose combinations, especially those involving NSAIDs and analgesics, fostering the broader application of stability-indicating RP-HPLC methods in pharmaceutical research and development.

Table 7: Key Features and Validation Results of the RP-HPLC Method

Feature	Result/Value	ICH Acceptance Criteria
Mobile Phase	Acetonitrile: Phosphate buffer (pH 3.0), 60:40 v/v	Optimized for resolution and peak shape
Retention Time	Paracetamol: 2.5 min; Ibuprofen: 5.2 min	Accurate and reproducible
Resolution (Rs)	6.2	Rs > 2
Tailing Factor (Tf)	Paracetamol: 1.12; Ibuprofen: 1.08	Tf < 2
Theoretical Plates (N)	Paracetamol: 4120; Ibuprofen: 5295	N > 2000
Linearity Range	10–100 µg/mL	Correlation coefficient (r^2) > 0.999
Precision (%RSD)	< 2%	≤ 2%
Accuracy (Recovery %)	98–102%	Within ±2% of theoretical value
LOD	Paracetamol: 0.52 µg/mL; Ibuprofen: 0.81 µg/mL	S/N ≥ 3:1
LOQ	Paracetamol: 1.56 µg/mL; Ibuprofen: 2.45 µg/mL	S/N ≥ 10:1
Robustness	Variations in flow rate, pH, wavelength stable	%RSD < 2% across variations
Forced Degradation Sensitivity	Paracetamol: 15.2% (acidic); Ibuprofen: 19.5% (oxidative)	Confirmed stability indication and specificity
Run Time	~10 minutes	Efficient for industrial QC

This method thus represents a dependable, validated analytical tool capable of assuring the quality, efficacy, and safety of paracetamol-ibuprofen combination formulations, meeting both scientific and regulatory expectations comprehensively.

6. CONCLUSION

The present study successfully developed and validated a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of paracetamol and ibuprofen in multi-component pharmaceutical formulations. The method addressed critical analytical challenges, including overlapping UV absorbance spectra, polarity differences, and susceptibility to chemical degradation. Through careful optimization of chromatographic parameters, particularly the mobile phase composition and pH, the method achieved sharp, well-resolved peaks for both analytes with retention times of approximately 2.5 minutes for paracetamol and 5.2 minutes for ibuprofen. Validation according to ICH Q2(R1) guidelines confirmed the method's reliability and suitability for pharmaceutical analysis. Linearity, precision, and accuracy studies demonstrated reproducibility and compliance with international standards. Sensitivity testing established low limits of detection and quantitation, while robustness evaluation confirmed that minor variations in chromatographic conditions did not significantly affect system performance. Forced degradation studies further demonstrated the stability-indicating nature of the method, with paracetamol showing pronounced degradation under acidic stress and ibuprofen under oxidative conditions. Importantly, the method effectively resolved degradation products from the parent compounds, ensuring specificity and reliability in stability testing. The findings highlight the method's practical applicability for routine quality control and long-term stability studies in pharmaceutical industries. By ensuring consistent performance across multiple parameters, the method not only enhances confidence in therapeutic efficacy but also safeguards patient safety by detecting potential degradation products. Furthermore, the principles and validation framework established here can be extended to other fixed-dose combination therapies, supporting broader applications in pharmaceutical research and

development. In conclusion, the validated RP-HPLC method provides a robust, accurate, and stability-indicating analytical tool that meets both industrial and regulatory requirements, ensuring the quality and safety of paracetamol–ibuprofen formulations.

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