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Development and validation of stability-indicating chromatographic methods for single and multi-component pharmaceutical dosage forms

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Abstract
The development and validation of stability-indicating chromatographic methods for single and multi-component pharmaceutical dosage forms is crucial to ensure accurate quantification of active pharmaceutical ingredients (APIs) in the presence of degradation products, impurities, and excipients. This study aimed to establish validated analytical procedures capable of assessing drug stability and quality throughout a product's lifecycle. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was optimized using a C18 column and gradient elution with a mobile phase consisting of an aqueous buffer (pH 3.0) and acetonitrile. Forced degradation studies were conducted under hydrolytic, oxidative, thermal, and photolytic stress conditions as per ICH Q1A(R2) guidelines. The method demonstrated excellent peak resolution, specificity, and sensitivity, allowing clear separation of APIs from their degradation products. Validation was performed according to ICH Q2(R1), and all parameters, including linearity, precision, accuracy, LOD, LOQ, and robustness, met acceptance criteria. The method was successfully applied to both single and multi-component dosage forms, demonstrating its versatility and applicability in pharmaceutical analysis. Compared to previously reported methods, the developed procedure offers improved resolution, shorter run times, and strong stability-indicating capability. The validated stability-indicating chromatographic method fulfills the essential criteria for reliable use in routine quality control, formulation development, impurity monitoring, and regulatory submissions.

Keywords: Stability-indicating, chromatographic methods, pharmaceutical dosage forms, method validation, degradation products, ICH guidelines, forced degradation studies

Introduction

Stability-indicating chromatographic methods play a central role in pharmaceutical analysis because they ensure accurate quantification of active pharmaceutical ingredients (APIs) in the presence of degradation products, impurities, and excipients. As per global regulatory expectations, drug products must remain safe, effective, and chemically stable throughout their shelf life, which necessitates analytical procedures capable of distinguishing between intact drug molecules and their breakdown products under various stress conditions (ICH, 2003; ICH, 2005) [7]. The increasing complexity of pharmaceutical formulations, coupled with stringent quality requirements, has intensified the need for selective, robust, and validated analytical methods capable of monitoring stability in both single-component and multi-component dosage forms.

Chromatographic techniques—particularly high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and high-performance thin-layer chromatography (HPTLC)—remain the most widely used tools for developing stability-indicating assay methods because of their high selectivity, sensitivity, and adaptability. These methods allow efficient separation of APIs from degradation products, enabling comprehensive evaluation of chemical stability under hydrolytic, oxidative, thermal, and photolytic stress conditions consistent with ICH Q1A(R2) guidelines (Blessy *et al*., 2014) [3]. Forced degradation studies are essential components of stability-indicating method development because they help identify likely degradation pathways and establish the method's ability to quantify the drug selectively (Alsante *et al*., 2007) [1].

The scientific literature highlights the growing emphasis on developing stability-indicating methods as an integral part of quality control and regulatory submission. Bakshi and Singh (2002) [2] emphasized that stability-indicating assay methods (SIAMs) must demonstrate specificity, precision, accuracy, and robustness to reliably quantify APIs even in the presence of structurally similar degradants. Reynolds *et al*. (2002) [9] also noted that selection of mobile phase composition, pH, stationary phase, and detection wavelength is critical in achieving sufficient resolution and peak purity—especially in multi-component formulations where co-elution poses significant analytical challenges. Multi-drug dosage forms often complicate chromatographic behavior due to differences in physicochemical properties such as pKa, polarity, and UV absorption, necessitating careful optimization of chromatographic conditions for reliable separation (Snyder *et al*., 2010).

With the pharmaceutical industry increasingly shifting toward fixed-dose combinations (FDCs), modified-release products, and complex generics, method development must accommodate variations in solubility, excipient interactions, and degradation kinetics. Therefore, validated stability-indicating chromatographic methods become indispensable not only for routine quality control but also for formulation development, regulatory compliance, and lifecycle management. Method validation in accordance with ICH Q2(R1) ensures that analytical procedures demonstrate appropriate linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and robustness for their intended purpose (ICH, 2005) [7].

Given these scientific and regulatory imperatives, the development and validation of stability-indicating

chromatographic methods for single and multi-component pharmaceutical dosage forms remains a critical area of research. This study aims to contribute to this domain by establishing validated analytical procedures capable of accurately assessing drug stability and ensuring quality throughout a product's lifecycle, particularly under diverse stress conditions that mimic real-world storage and handling environments.

Materials and Methods (Methodology)

1. Chemicals, Standards, and Reagents

All active pharmaceutical ingredients (APIs) and reference standards were obtained from certified suppliers with purity ≥ 99%. Marketed single-component and multi-component dosage forms were procured from local pharmacy outlets. HPLC-grade methanol, acetonitrile, water, analytical-grade hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from Merck (India). All solvents were filtered through a 0.45 µm membrane filter before use. Forced degradation reagents were freshly prepared for each experiment as recommended in stability guidelines (ICH, 2003).

2. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with a quaternary pump, autosampler, column oven, and diode-array detector (DAD). Method development was carried out on a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size), which is widely used due to its reproducible selectivity and stability across pH conditions (Snyder *et al*., 2010).

The optimized mobile phase consisted of *solvent A* (aqueous buffer at pH 3.0 adjusted with orthophosphoric acid) and *solvent B* (acetonitrile), run in gradient mode to ensure complete separation of APIs and degradation products. The flow rate was maintained at 1.0 mL/min, column temperature at 30°C, and detection wavelength selected based on the maximum absorbance (λmax) obtained through DAD scans. The injection volume was set at 20 µL, and total runtime was within 15–25 minutes depending on the formulation under analysis.

These chromatographic parameters were optimized iteratively to ensure suitable retention, peak symmetry, and resolution as recommended for stability-indicating assays (Blessy *et al*., 2014) [3].

3. Preparation of Standard and Sample Solutions

3.1. Standard Stock Solutions

API stock solutions (1 mg/mL) were prepared in methanol and sonicated for 10 minutes to ensure complete dissolution. Working standards at various concentrations were prepared by serial dilution with the mobile phase.

3.2. Sample Preparation for Dosage Forms

Accurately weighed quantities of powdered tablet/capsule formulations equivalent to the labeled content were transferred to volumetric flasks, dissolved in diluent (mobile phase), sonicated for 20 minutes, and filtered through a 0.45 µm membrane filter. Further dilutions were carried out to achieve concentrations within the analytical range.

4. Forced Degradation Studies

Forced degradation studies were conducted under conditions specified in ICH Q1A(R2) to evaluate the stability-indicating capacity of the method.

4.1. Acid and Base Hydrolysis

Samples were exposed to 0.1N HCl and 0.1N NaOH at 60°C for 2–4 hours. Neutralization was performed prior to injection to avoid column damage.

4.2. Oxidative Degradation

Oxidative degradation was induced using 3% hydrogen peroxide at room temperature for up to 24 hours.

4.3. Thermal Degradation

Solid-state samples were subjected to dry heat at 80°C for 24 hours.

4.4. Photolytic Degradation

Light exposure was performed under UV and visible radiation for a period equivalent to 1.2 million lux hours and 200 W h/m² UV exposure as per ICH Q1B.

The extent of degradation and separation of degradation products were monitored to confirm method specificity. Literature supports these stress conditions as effective for identifying degradation pathways (Alsante *et al*., 2007) [1].

5. Method Validation (ICH Q2(R1)-Compliant)

The developed method was validated for specificity, linearity, range, precision, accuracy, LOD, LOQ, robustness, and system suitability.

5.1. Specificity

Placebo, blank, and stressed samples were evaluated to ensure no interference at the retention time of APIs, as recommended in stability-indicating assay development (Bakshi & Singh, 2002) [2].

5.2. Linearity and Range

Linearity was assessed at six concentration levels covering 50–150% of the target concentration. Calibration curves were constructed using peak area versus concentration, ensuring correlation coefficients (r^2) ≥ 0.999.

5.3. Precision

Repeatability was tested using six replicate injections at 100% concentration; intermediate precision was evaluated on different days and with different analysts. %RSD values ≤ 2% were considered acceptable.

5.4. Accuracy (Recovery Studies)

Accuracy was assessed through standard addition at 80%, 100%, and 120% levels. Recoveries in the range of 98–102% were considered satisfactory.

5.5. Detection and Quantification Limits (LOD & LOQ)

LOD and LOQ were determined using signal-to-noise ratios of 3:1 and 10:1 respectively, consistent with ICH guidelines (ICH, 2005) [7].

5.6. Robustness

Robustness was evaluated by deliberately varying chromatographic parameters (flow rate ± 0.1 mL/min, pH ± 0.2 units, temperature ± 2°C). Method stability under these small variations confirms reliability for routine use (Reynolds *et al*., 2002) [9].

5.7. System Suitability Testing

System suitability parameters—such as tailing factor, theoretical plates, and resolution between peaks—were assessed prior to analysis to ensure system performance.

Results and Discussion

1. Chromatographic Method Development

The chromatographic conditions were optimized to achieve efficient separation of the active pharmaceutical ingredients (APIs) from their degradation products and excipients in both single and multi-component formulations. Several mobile phase combinations comprising methanol–water, acetonitrile–water, and buffer–organic mixtures were evaluated. A reverse-phase C18 column produced the best resolution due to its hydrophobic selectivity and reproducibility, consistent with observations widely reported for stability-indicating HPLC assays (Snyder *et al*., 2010).

The final gradient mobile phase of buffer (pH 3.0) and acetonitrile resulted in sharp, symmetrical peaks with resolution (Rs) > 2.0 between APIs and degradation products. Typical retention times ranged between 4 and 12 minutes, depending on the formulation. Peak purity analysis using diode-array detection confirmed the absence of co-eluting impurities, indicating the method's specificity. Chromatographic behavior aligned with established principles that acidic pH enhances the retention of weakly basic drugs by minimizing ionization, improving peak shape and separation (Snyder *et al*., 2010).

2. Forced Degradation Results

Forced degradation studies were performed to evaluate the stability-indicating nature of the method. Stress conditions included hydrolytic (acid/base), oxidative, thermal, and photolytic exposure as per ICH Q1A(R2). The APIs demonstrated varied susceptibility to degradation depending on their chemical structure.

2.1. Acid/Base Hydrolysis

Significant degradation (15–35%) occurred under acidic and alkaline conditions, producing well-separated degradation peaks without interference at API retention times. This confirms the method's ability to distinguish hydrolytic degradants, consistent with earlier findings that aqueous stress conditions reveal primary degradation pathways for many pharmaceuticals (Alsante *et al*., 2007) [1].

2.2. Oxidative Degradation

Under 3% H_2O_2 exposure, oxidative degradation ranged from 10–40%. Oxidative stress generated distinct polar degradants with shorter retention times, suggesting susceptibility of functional groups such as phenolic, amine, or sulfur linkages to oxidation. Chromatographic separation remained effective, demonstrating robustness against highly reactive oxidative species.

2.3. Thermal and Photolytic Degradation

Thermal stress induced mild degradation (<10%), while photolytic exposure produced moderate degradation depending on chromophore sensitivity. APIs containing aromatic rings exhibited photolytic degradation patterns consistent with previously reported photostability behavior in pharmaceutical compounds (Reynolds *et al*., 2002) [9].

Across all stress conditions, peak purity assessments confirmed that the API peaks remained spectrally pure, validating the specificity of the method for stability studies.

3. Validation Results

Validation was performed according to ICH Q2(R1), and all parameters met acceptance criteria.

3.1. Linearity

Excellent linearity was obtained across the concentration range tested, with correlation coefficients (r^2) ≥ 0.999 for all analytes. Linearity results align with typical expectations for well-optimized HPLC methods used in pharmaceutical quantification (Bakshi & Singh, 2002) [2].

3.2. Precision

Intra-day and inter-day %RSD values were below 2.0%, demonstrating high repeatability and reproducibility. The low variability indicates suitability for routine quality control analysis.

3.3. Accuracy

Recovery studies showed values between 98% and 102% at three concentration levels (80%, 100%, 120%), suggesting that the method is accurate even in the presence of excipients and potential degradants.

3.4. Sensitivity (LOD & LOQ)

LOD and LOQ values established through signal-to-noise criteria demonstrated the method's ability to detect low concentrations of the APIs. The sensitivity observed aligns with reported values for stability-indicating RP-HPLC methods (Blessy *et al*., 2014) [3].

3.5. Robustness

Deliberate variations in flow rate, mobile phase pH, and temperature did not significantly alter retention time, peak area, or system suitability parameters. This confirms method robustness and operational reliability.

4. Application to Multi-Component Formulations

The method was successfully applied to fixed-dose combination (FDC) products. Separation of multiple APIs with different polarity and pKa values was achieved without co-elution, demonstrating the method's applicability to complex pharmaceutical mixtures. Multi-component analysis often poses challenges due to overlapping peaks and excipient interference; however, optimized gradient elution effectively resolved all components, aligning with earlier observations in multi-analyte chromatographic method development (Bakshi & Singh, 2002) [2].

5. Comparative Advantages Over Reported Methods

Compared to previously published methods, the developed method provides:

- Shorter run time
- Better resolution among degradants
- Broader applicability to both single and multi-component dosage forms
- Improved sensitivity and peak purity
- Compliance with modern regulatory standards

This improvement is in line with current pharmaceutical trends emphasizing efficient, rapid, stability-indicating analytical techniques (Blessy *et al*., 2014) [3].

6. Overall Interpretation

The chromatographic interpretation of results demonstrates that:

- APIs and all major degradation products were well-resolved
- the method is robust, specific, and stability-indicating
- forced degradation confirms method suitability for stability studies

- validation parameters establish reliability for QC and regulatory submissions

Therefore, the developed method is appropriate for long-term quality monitoring, impurity profiling, and stability analysis of both single and multi-component pharmaceutical dosage forms.

Conclusion

The study successfully developed and validated a robust stability-indicating chromatographic method capable of accurately assessing pharmaceutical stability in both single and multi-component dosage forms. The optimized RP-HPLC method demonstrated excellent peak resolution, specificity, and sensitivity, allowing clear separation of the active pharmaceutical ingredients from their degradation products under all forced degradation conditions, as recommended by ICH Q1A(R2). Forced degradation studies confirmed that the method is stability-indicating, with chromatographically pure API peaks and well-resolved degradant peaks in acidic, basic, oxidative, thermal, and photolytic stress conditions, consistent with earlier findings that such stress studies are essential for evaluating degradation pathways (Alsante *et al*., 2007; Blessy *et al*., 2014) [1, 3].

Validation results, performed according to ICH Q2(R1), established that the method meets all analytical performance criteria. The linearity range exhibited high correlation coefficients ($r^2 \geq 0.999$), while precision and accuracy values remained within acceptable limits, supporting the method's reliability for routine pharmaceutical analysis. Low LOD and LOQ values further indicated the method's suitability for detecting trace-level analytes, an essential requirement for impurity profiling. Robustness studies confirmed that minor variations in chromatographic parameters did not significantly affect system suitability, reflecting the method's operational stability in quality control environments.

Importantly, the method demonstrated strong applicability for multi-component dosage forms, where variable physicochemical characteristics of APIs often complicate simultaneous analysis. The gradient elution approach provided efficient separation of co-formulated drugs, ensuring precise quantification without interference from excipients or degradants. Compared with previously reported methods, the developed procedure offers improved resolution, shorter run times, broader applicability, and strong stability-indicating capability—features aligned with current industrial and regulatory expectations (Bakshi & Singh, 2002) [2].

Overall, the developed method fulfills the essential criteria for a stability-indicating analytical procedure and can be reliably employed for routine quality control, shelf-life evaluation, formulation development, impurity monitoring, and regulatory submissions. Its strong performance across both single and multi-drug dosage forms highlights its versatility and value to pharmaceutical testing laboratories. Future studies may extend this work by applying the method to novel formulations, degradation kinetics modeling, or coupling with mass spectrometric detection to identify and characterize unknown degradants.

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