Ind. J. Pharm. Edu. Res., 2027; XX(XX):1-9.
https://www.ijper.org

Review Article

Stability-Indicating Chromatographic Method Development for Pharmaceutical Dosage Forms via Enhanced a QbD and DoE Strategies: A Review

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ABSTRACT

Many global regulatory organizations emphasize the need to establish a robust pharmaceutical stability profile during drug development. Stability testing plays a crucial role in determining shelf life, retesting intervals, and appropriate storage conditions for both Active Pharmaceutical Ingredients (APIs) and finished dosage forms. The International Council for Harmonization stability guidelines serve as the primary framework for such evaluations and recommend the use of forced degradation studies to simulate potential degradation pathways and impurity formation, thereby ensuring the development of stability-indicating analytical methods. In this context, the application of Quality by Design (QbD) principles, supported by Design of Experiments (DoE), enables a systematic, science- and risk-based approach for method development through identification of critical method parameters, optimization of analytical conditions, and establishment of method robustness. However, while ICH guidelines provide overarching regulatory direction, they do not specify detailed experimental procedures or standardized approaches for executing such studies. This limitation often leads to inconsistencies in the design and implementation of stability testing protocols across academic and industrial settings. Consequently, variability persists in the selection and application of stability-indicating parameters reported in the literature. This review critically examines current regulatory frameworks and analytical practices associated with pharmaceutical stability testing, and highlights QbD- and DoE-driven strategies for developing scientifically sound, robust, and regulatory-compliant stability-indicating methods for both drug substances and their corresponding formulations.

Keywords: DoE, Forced degradation condition, HPLC, HPTLC. QbD, Quality risk management, Stability indicating, Validation.

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INTRODUCTION

Stability assessment of bioactive agents across diverse dosage forms is a fundamental component of drug development, conducted in accordance with guidelines established by the International Council for Harmonization and other regulatory authorities. These studies evaluate the influence of environmental factors such as temperature, humidity, and light on pharmaceutical compounds to establish shelf life, storage conditions, and retesting intervals. Across development stages, stability testing evolves from assessing intrinsic drug stability and drug-excipient compatibility under stress conditions (e.g., pH extremes, oxidative, thermal, and photolytic environments) to confirming the integrity of clinical and commercial batches, thereby ensuring product quality, safety, and efficacy throughout its lifecycle.[1,2]

The reliability of stability assessment critically depends on the development of robust Stability-Indicating Analytical Methods (SIAMs), particularly chromatographic techniques capable of resolving and quantifying degradation products in complex matrices. Conventional method development approaches, primarily based on One-Factor-At-A-Time (OFAT) experimentation, often lack robustness, fail to capture interaction effects, and may not ensure reproducibility. Moreover, several reported methods do not fulfill the criteria of true SIAMs, as they lack comprehensive forced degradation studies, fail to demonstrate peak purity, or do not establish adequate mass balance. The absence of confirmatory tools such as Photodiode Array (PDA) spectral purity analysis and Liquid Chromatography-Mass

DOI: 10.5530/ijper.20273407

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Table 1: Comparative Evaluation of Conventional and AQbD-Based Stability-Indicating Analytical Method (SIAM) Development.

Parameter	Conventional SIAM Approach	AQbD-Based SIAM Approach
Development Strategy	Empirical, trial-and-error (OFAT approach)	Systematic, science- and risk-based approach
Regulatory Alignment	Limited alignment with modern guidelines	Fully aligned with International Council for Harmonisation and International Council for Harmonisation
Understanding of Method Variables	Limited understanding of variable interactions	Comprehensive understanding using multivariate analysis (DoE)
Optimization Approach	One-factor-At-A-Time (OFAT), ignores interaction effects	Design of Experiments (DoE) evaluates interaction and quadratic effects
Critical Method Parameters (CMPs)	Not systematically identified	Scientifically identified and controlled
Critical Quality Attributes (CQAs)	Implicit or poorly defined	Clearly defined and linked to method performance
Method Robustness	Evaluated post-development	Built into method during development (robust by design)
Design Space / MODR	Not established	Defined Method Operable Design Region (MODR)
Forced Degradation Strategy	Often inconsistent or incomplete	Systematically optimized using risk-based approaches
Peak Purity Assessment	Limited use of PDA or orthogonal tools	Mandatory use of PDA spectral purity and confirmatory tools (e.g., LC-MS)
Mass Balance Evaluation	Often neglected or inadequately demonstrated	Explicitly assessed as per regulatory expectations
Data Reliability	Moderate; may lack reproducibility	High reliability with enhanced reproducibility and traceability
Method Lifecycle Management	Static (development → validation)	Lifecycle-based (development, validation, continuous monitoring)
Risk Assessment	Rarely performed	Integrated risk assessment (e.g., FMEA, risk ranking)
Regulatory Flexibility	Limited; changes require revalidation	Greater flexibility within approved design space
Documentation and Knowledge Management	Limited scientific justification	Comprehensive knowledge management and regulatory documentation
Applicability to Complex Matrices	May face limitations	Highly suitable for complex formulations and impurity profiling
Overall Outcome	Acceptable but variable performance	Robust, reliable, and regulatory-compliant SIAM

Table 2: Selected reports of stability indicating methods where no stress testing has been done.

Separations	Drugs	Methodology
Separations from process impurities	Benazepril HCl	HPLC
Separations from known / potential degradation products	Canrenone Phenylbutazone Homatropine methylbromide Felodipine	HPLC HPLC UV- spectrophotometry SFC
Separations from known / potential degradation products and process impurities	Benzodiazepine Piroxicam Fenclorac Azathioprine	HPLC HPTLC GLC CE

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Table 3: Selected reports of 'Stability-Indicating' methods where only one stress condition has been employed.

Stress Conditions	Drugs	Methodology
Acid	Dyclonine HLC Lisinopril	HPLC UV- spec-trophotometry
Alkali	Allantoin Benzapril Carbachol	HPLC UV IR
Neutral	Physostigmine salicylate	HPLC
Oxidation	Nortriptyline HCl	UV
Light	Atenolol Nifedipine Ranitidine HCl	HPLC HPTLC Spectrodensitometric TLC

Table 4: Selected reports of 'stability indicating' methods where five (and additional) stress conditions have been employed.

Stress Conditions	Drugs	Methodology
Acid, alkali, oxidation, dry heat, light	Elanapril maleate	HPLC
Acid, alkali, oxidation, dry heat, light (separation from synthetic impurities also seen)	Sildenafil citrate	HPLC
Acid, neutral, alkali, oxidation, light	Nicardipine HCl	HPLC
Acid, alkali, oxidation, dry heat, wet heat, light dry, light wet	Paroxetine	HPLC
Acid, alkali, oxidation, dry heat, light, reduction	Cyproterone acetate	HPLC
Acid, alkali, oxidation, dry heat, light, moisture, sonication	Buspirone HCl	HPLC

Spectrometry (LC-MS) for degradation product identification further limits confidence in method specificity and data reliability.

To address these limitations, the paradigm has shifted toward Analytical Quality by Design (AQbD), an extension of Quality by Design (QbD), supported by Design of Experiments (DoE). This systematic, risk-based framework enables the identification of critical method parameters (CMPs), Critical Quality Attributes (CQAs), and the establishment of a Method Operable Design Region (MODR), ensuring method robustness and lifecycle adaptability. The AQbD approach aligns with emerging regulatory expectations, particularly those outlined in ICH Q14 (Analytical Procedure Development) and ICH Q2(R^2) (Analytical Procedure Validation), which emphasize a science-based, lifecycle-oriented approach to analytical method development, validation, and continuous performance verification.[3–6]

Stability studies are inherently multifactorial, governed by intrinsic factors such as physicochemical properties of the Active Pharmaceutical Ingredient (API), excipient compatibility, and formulation design, as well as extrinsic factors including temperature, humidity, light exposure, and mechanical stress. These factors influence degradation pathways such as hydrolysis, oxidation, and photolysis, potentially leading to reduced potency and formation of harmful degradation products.[7] Regulatory guidelines recommend forced degradation studies to evaluate such pathways; however, the lack of detailed standardization in stress conditions often results in variability across reported methods.[8]

In this context, the integration of AQbD and DoE facilitates systematic optimization of stress conditions and analytical parameters, enabling improved understanding of degradation behavior and enhancing method robustness. Additionally, regulatory expectations such as demonstration of mass balance, confirmation of peak purity, and characterization of degradation products using orthogonal techniques have become critical for establishing the reliability of SIAMs.

The development of a robust stability testing strategy therefore requires not only a comprehensive understanding of degradation mechanisms and regulatory frameworks but also the implementation of lifecycle-based analytical approaches. This review critically evaluates current practices in stability-indicating method development and highlights the role of AQbD- and DoE-driven strategies, aligned with modern regulatory guidelines, for developing scientifically sound, reproducible, and regulatory-compliant chromatographic methods for pharmaceutical analysis.

Stability-Indicating Analytical Methods (SIAMs) for the Quantification of Pharmaceutical Samples

The development of Stability-Indicating Analytical Methods (SIAMs) is a critical component of pharmaceutical analysis, aimed at accurately quantifying active pharmaceutical ingredients (APIs) in the presence of their degradation products, impurities, excipients, and other constituents.[9,10] Effective SIAMs must address several essential criteria, including the complete separation of degradation products, the establishment of mass balance, the application of stress testing protocols to drug formulations, and the design of analytical methods suitable for combination drug products.

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Table 5: Reports of 'stability indicating' methods on drug formulation.

Stress conditions	Drugs	Dosage form	Methodology
Acid	Fluconazole Flucytosine	Admixtures Extemporaneous solutions	GC HPLC
Acid, alkali	Gancyclovir	Capsules	HPLC
Light, thermal	Sodium levothyroxine	Tablets	HPLC
Acid, alkali, oxidation	Pentoxifylline	Suspension	HPLC
Acid, alkali,oxidation, thermal	Chlorbutanol	Ointment	HPLC
Acid, alkali,oxidation, light	Fotemustine Efavirenz	5% dextrose Capsules	HPLC HPLC
Acid, light,oxidation, thermal	Fentanyl	Injection	HPLC
Acid, alkali, light,oxidation, thermal	Cyclosporine	Oral solution	HPLC
Acid, alkali, light,thermal, 450c /75% RH for two weeks	Aspirin and warfarin sodium	Tablets	HPLC
Aged samples (3 years at 400c and 75% RH	Losartan	Tablets	HPLC and LC-MS

Although stability-indicating assays are widely employed in the pharmaceutical industry to assess the integrity of drug substances and products under stress conditions, a precise and universally accepted definition of a stability-indicating method is not provided in current International Council for Harmonization (ICH) guidelines. Nevertheless, regulatory expectations are outlined in documents such as the 1987 United States Food and Drug Administration (US-FDA) stability guidelines and the FDA draft guidance issued in 1998. These guidelines emphasize two major advancements: (i) the requirement for analytical method validation and (ii) the comprehensive assessment of degradation products and other constituents alongside the active drug.[11–13]

Forced degradation studies are mandated under various stress conditions-such as hydrolytic (acidic, basic, and neutral), photolytic, oxidative, and thermal environments-to simulate possible degradation pathways. These studies aim to ensure the selectivity and specificity of the developed method in detecting the active moiety independently from its degradation products. Notably, several reports have described SIAMs developed without subjecting the drug to forced degradation, focusing instead on the resolution of known synthetic impurities and potential degradants (as summarized in Table 2).

Additionally, various degradation pathways-including acid/base hydrolysis, oxidation, photolysis, and thermal decomposition-have been reported across different studies (refer to Tables 3 and 4).[14] In some instances, stress conditions have been applied directly to the formulated product rather than the pure drug substance to evaluate the stability-indicating capacity of the analytical method (Table 5).[15]

The literature highlights the application of several analytical techniques-such as titrimetric, spectrophotometric, and chromatographic methods-for the evaluation of stability samples. These methods are utilized for analyzing the drug within complex matrices consisting of excipients, degradation products, impurities, and co-formulated drugs. While titrimetric and spectrophotometric methods offer advantages in terms of cost-effectiveness and operational simplicity, they may be limited in terms of sensitivity and specificity. In contrast, chromatographic techniques-particularly High-Performance Liquid Chromatography (HPLC)-are now preferred due to their enhanced resolution, sensitivity, and ability to detect minor degradation products.

Among chromatographic techniques, HPLC has emerged as the most widely employed approach for developing SIAMs. Its robustness, specificity, and applicability to thermally unstable, polar, ionic, or non-volatile compounds make it particularly well-suited for comprehensive stability studies. Other chromatographic techniques, such as Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), Gas Chromatography (GC), and capillary electrophoresis, have also been explored.[16]

Although TLC has historically been used in SIAM development due to its simplicity, modern techniques such as HPTLC offer improved reliability, faster analysis times, and reduced solvent consumption, making them more practical for high-throughput analysis.

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Although TLC has historically been used in SIAM development due to its simplicity, modern techniques such as HPTLC offer improved reliability, faster analysis times, and reduced solvent consumption, making them more practical for high-throughput analysis. However, the regulatory guidelines and pharmacopoeias do not contain information on the fundamental procedures for developing and validating stability indicating methods. As a result, the practical procedures for developing SIAMs using HPLC are carried out, given that 85-90% of the methods are documented in literature.

Analytical Method Development and Its Applications in Pharmaceutical Sciences

The generation of accurate, precise, and reproducible analytical data is fundamental to the successful development of pharmaceutical compounds across various stages of drug discovery, formulation, and manufacturing. A well-structured analytical method development process ensures the integrity of data used to support regulatory submissions and product lifecycle management.[17]

Key elements in analytical method development include

a) The strategic selection of representative sample sets, which is critical for ensuring method applicability across a diverse range of formulations and matrices.
b) The systematic screening of chromatographic conditions-such as stationary phases and mobile phase compositions-guided by predictive models like the Linear Solvent Strength (LSS) theory of gradient elution, which facilitates the efficient separation of analytes.
c) Method optimization, which involves fine-tuning parameters to enhance method ruggedness and robustness, ensuring consistent performance under variable laboratory conditions.

Applications in Pharmaceutical Development

Analytical methods serve as indispensable tools in multiple domains of pharmaceutical research and development, particularly for assessing the identity, purity, potency, safety, and stability of Active Pharmaceutical Ingredients (APIs) and finished dosage forms. These methods are employed across a wide spectrum of therapeutic categories and compound classes, including but not limited to: Analgesics, Antibacterial agents, Anticonvulsants, Antidepressants, Tranquilizers, Anticancer drugs, Local anesthetics, Steroidal compounds, Biopharmaceuticals and macromolecules derived from natural, synthetic, or recombinant sources. Through continuous advancements in analytical science, the precision and reliability of these methods support regulatory compliance and contribute significantly to ensuring the quality and safety of pharmaceutical products throughout their development and commercialization.[18]

Bioanalytical Method Development and Validation: Principles and Applications

The accurate quantification of drugs and their metabolites in biological matrices is a cornerstone of preclinical, biopharmaceutical, and clinical pharmacology research. To achieve this, highly selective and sensitive bioanalytical techniques are required. The validation of bioanalytical methods involves a comprehensive framework of procedures designed to confirm that the analytical method employed provides reliable, reproducible, and accurate measurements of target analytes within complex biological matrices such as plasma, serum, blood, and urine. Pre-study method development, often referred to as analytical method development, focuses on establishing an appropriate bioanalytical strategy through the systematic optimization of assay parameters, considering the physicochemical properties of the analyte and the matrix complexity. Once developed, method application pertains to the practical use of the validated analytical procedure for the quantitative evaluation of samples collected in Bioavailability (BA), Bioequivalence (BE), and Pharmacokinetic (PK) studies. The use of rigorously validated and well-characterized bioanalytical methods is indispensable for generating data that are scientifically robust and suitable for regulatory submission. Since analytical performance can vary depending on the specific analyte and matrix under investigation, method validation must often be tailored to the unique attributes of each compound. In studies conducted across multiple analytical laboratories or sites, independent method validation at each location is critical to ensure consistency, harmonization, and reproducibility of results across different settings.[19]

Current regulatory expectations emphasize clearly defined validation criteria for both chromatographic techniques (e.g., LC-MS/MS) and ligand-binding assays, particularly in the context of quantitative bioanalysis. It is essential that both qualitative and quantitative components of method validation are thoroughly addressed to maintain the integrity and interpretability of the resulting data and to comply with international regulatory guidelines such as those set forth by the FDA, EMA, and ICH.[20,21]

Key Stages in Bioanalytical Method Development and Validation

The development and validation of bioanalytical methods for quantitative drug analysis involve a structured, phase-wise approach, typically comprising the following key stages:

Preparation and Qualification of Reference Standards: This step involves the selection, verification, and qualification of a reference standard representative of the target analyte, ensuring traceability and analytical reliability.

Method Development and Optimization: A robust bioanalytical method is designed and optimized, taking into account the chemical nature of the analyte, matrix interferences, and assay

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sensitivity requirements. Optimization ensures selectivity, accuracy, precision, and minimal matrix effects.

Routine Application and Full Method Validation: Once validated, the method is routinely employed for sample analysis in pivotal BA/BE and PK studies. Performance characteristics such as accuracy, precision, linearity, selectivity, stability, recovery, and carry-over are evaluated using predefined acceptance criteria.

This systematic methodology guarantees that the bioanalytical method is not only scientifically sound but also regulatory-compliant, reproducible across laboratories, and fit for its intended purpose throughout the drug development lifecycle.

Reference Standards and Bioanalytical Method Development

Foundation for Robust Quantification in Drug Analysis; The accurate quantification of pharmaceutical compounds and their metabolites in biological matrices is fundamentally dependent on the use of well-characterized reference standards. Calibration curves and Quality Control (QC) samples are prepared by fortifying biological matrices-such as plasma, serum, urine, or whole blood-with known concentrations of the analyte. The reliability and reproducibility of analytical results hinge on the integrity of these standards, which serve as critical benchmarks for assessing assay performance.

Reference standards commonly fall into three categories

Certified Reference Materials (CRMs): Highly characterized compounds accompanied by certificates of analysis from recognized regulatory or standard-setting bodies, confirming identity, potency, and purity.

Commercial Standards: Materials sourced from reputable suppliers, accompanied by detailed documentation verifying their suitability for analytical applications.

Custom-Synthesized Compounds: Substances synthesized by specialized laboratories, provided with comprehensive supporting data ensuring structural confirmation and purity specifications. The appropriate selection of a reference standard is vital for ensuring the analytical method's compliance with regulatory expectations and its overall accuracy and precision.[22]

Bioanalytical Method Development

Bioanalytical method development involves a structured evaluation of key performance attributes-including selectivity, sensitivity, accuracy, precision, and stability-within the selected biological matrix. These parameters are rigorously optimized to ensure that the developed method is fit for its intended use in Pharmacokinetic (PK), Bioavailability (BA), and Bioequivalence (BE) studies.[23]

Table 6: Validation criteria to be studied in pre-validation and validation steps.

Criteria	Pre-Validation Step	Validation Step
Absolute recovery	+	+
Selectivity	(+)	(+)
Response function	(+)	+
Linearity	+	+
Accuracy	(+)	+
Precision	(+)	+
Limit of detection	+	+

(+): estimation of the criteria; **+:** validation of the criteria.

Analyte Stability in Biological Matrices

Stability assessments are essential to ensure the integrity of the analyte throughout all stages of sample handling and analysis. Importantly, the stability of a compound can be highly specific to the matrix-container combination used and cannot be generalized across other systems. Therefore, stability testing must be customized to replicate conditions experienced during collection, short- and long-term storage, freeze-thaw cycles, and post-preparative holding.

Stability evaluations must be conducted using freshly prepared solutions of the analyte spiked into an analyte-free matrix devoid of interferences. Stock solutions should be accurately prepared in appropriate solvents at known concentrations and stored under controlled conditions to maintain consistency throughout the analytical workflow.[24]

Freeze-Thaw Stability: To assess freeze-thaw stability, at least three aliquots at low and high concentration levels must undergo a minimum of three complete freeze-thaw cycles. Samples are frozen at the designated storage temperature (typically ≤ -20°C or -70°C for labile compounds) for a minimum of 24 hr, followed by passive thawing at ambient temperature. After thawing, samples are refrozen for an additional 12-24 hr. This process is repeated twice, with the final analysis conducted after the third thaw to evaluate any degradation resulting from repeated temperature cycling.[25]

Short-Term (Room Temperature) Stability: Short-term stability is determined by allowing spiked samples at both low and high concentrations to remain at room temperature for durations ranging from 4 to 24 hr, reflective of expected sample processing times. Post-exposure, these samples are analyzed to assess any significant loss in analyte concentration.

Long-Term Stability: For long-term stability, analyte integrity must be maintained over periods exceeding the time span between the first and last sample analysis within a study. A minimum of three replicates per concentration level should be stored under

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the same conditions used for study samples. Sufficient sample volume must be available to perform at least three independent analytical runs. Stability is confirmed by comparing the analyte concentrations in stored samples to the initially calculated concentrations from calibration standards analyzed on the first day of the study.

Stock Solution Stability: Stock solution stability assessments include storage at ambient temperatures for at least 6 hr and under refrigerated or frozen conditions for extended periods. Stability is confirmed by comparing instrument responses of the stored stock solutions to those from freshly prepared standards, ensuring there is no significant degradation.

Post-Preparative Stability: Post-preparative stability evaluates how long processed samples (e.g., autosampler-resident extracts) retain their integrity during the expected batch analysis duration. This involves comparing analyte and internal standard concentrations using validation samples against original calibration data. Traditional direct comparisons are acceptable; however, alternative statistical approaches-such as confidence interval-based models-may also be employed, provided they are detailed in Standard Operating Procedures (SOPs) and include clear acceptance criteria. In certain cases, stability testing of incurred samples from dosed subjects may also be warranted to ensure method applicability under real-world conditions.[26]

Method Validation Strategy: A comprehensive validation strategy is essential to confirm that a bioanalytical method is suitable for regulatory submission and routine use. This includes pre-validation, full validation, partial validation, and cross-validation, each tailored to specific stages of method lifecycle management.

Pre-Validation: Pre-validation involves preliminary assessment of method performance using a single analytical run of spiked samples. This step is crucial for identifying potential issues in assay design and ensuring that the method is sufficiently robust to proceed to formal validation. Parameters such as linearity, precision, and assay viability are typically examined during this phase (outlined in Table 6).[27–30]

Full Validation

Full validation is mandatory when

A new bioanalytical method is developed.

The method is applied to a novel molecular entity.

Major modifications are introduced to an existing validated method, such as the inclusion of additional analytes.

This comprehensive process confirms the method's reliability in terms of accuracy, precision, sensitivity, selectivity, reproducibility, and analyte stability under defined laboratory conditions.[31]

Partial Validation

Partial validation is required when minor modifications are made to an already validated method. Depending on the nature of the change, the scope may range from a single performance parameter to an extensive re-evaluation. Common triggers for partial validation include:

- Laboratory transfer or analyst change.
- Modifications in anticoagulant.
- Alteration in detection systems or instrument platforms.
- Matrix change within the same species (e.g., plasma to urine).
- Transition to a different species for the same matrix.
- Changes in sample preparation or calibration range.
- Situations involving small-volume samples (e.g., pediatric studies).
- Assessment of selectivity in the presence of metabolites.[32]

Cross-Validation

Cross-validation is employed when multiple analytical methods or laboratories are involved in a single study or across related studies. Typically, a newly developed method is compared against a pre-validated reference method, ensuring analytical consistency between the two. Cross-validation must also be performed when studies are conducted at different sites or use different detection platforms (e.g., LC-MS/MS versus ELISA). Bidirectional analysis, using both spiked QC samples and subject-derived specimens, ensures data equivalency and inter-laboratory harmonization. This multi-phase validation framework ensures that bioanalytical methods are scientifically rigorous, reproducible, and fully aligned with international regulatory expectations.

Documentation Requirements

Validation activities must be comprehensively documented and traceable. All experimental details-including method development records, validation data, SOPs, and QC performance results-must be included in the method validation report.

Regulatory submissions should be accompanied by

- A concise summary of key findings.
- Detailed descriptions of method development and validation procedures.
- Bioanalytical study reports demonstrating routine method application.
- Any supplementary information relevant to the assay's performance and implementation.
- Proper documentation ensures transparency, facilitates audits and inspections, and supports the integrity of bioanalytical results submitted for regulatory review.

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Documentation for Method Development and Validation

Records related to method development and validation should include the following:

- A detailed operational procedure of the analytical technique.
- Verification of the identity and purity of the drug, metabolites, and internal standards utilized during validation.
- Descriptions and supporting data from stability studies.
- Comprehensive accounts of experiments conducted to assess parameters such as accuracy, precision, recovery, selectivity, lower limit of quantification, and calibration curve data (including equations and any weighting functions applied).
- Evidence of both intra- and inter-assay accuracy and precision.

Application of a Validated Method in Routine Drug Analysis

Analysis of all biological matrix samples containing the analyte should be completed within the established stability period supported by validated stability data. Typically, if the assay demonstrates acceptable variability as confirmed during validation, each biological sample may be analyzed once, without requiring duplicates or replicates.[33] The following considerations are important when implementing a bioanalytical method for routine drug analysis:

Response Function: The standard calibration curve used during routine analysis should employ the same fitting model, weighting method, and fit criteria as those established during pre-study validation. The response function should be verified using appropriate statistical analysis based on the actual calibration standards in each analytical run. Any discrepancies in the response relationship between the pre-validation phase and the routine application may signal underlying issues.

Quality Control (QC) Samples: QC samples, which consist of biological matrices spiked with the analyte, should be utilized to determine whether a run is acceptable or must be discarded.

System Suitability

Depending on the analyte and analytical technique employed, a dedicated Standard Operating Procedure (SOP) or sample protocol should be used to confirm that the analytical system is functioning at optimal performance levels.[34–39]

CONCLUSION

Analytical methodologies play a critical role in ensuring the reliable generation of data required to evaluate pharmaceutical stability and quality. The effectiveness of stability-indicating methods is defined by their ability to demonstrate specificity, accuracy, precision, and robustness, particularly in the presence of degradation products and complex sample matrices. However, insights from the literature indicate that many reported analytical methods lack comprehensive compliance with current regulatory expectations, particularly in terms of systematic impurity profiling and resolution of degradation products. The increasing emphasis of pharmacopoeial and regulatory frameworks, including those of the International Council for Harmonisation, on impurity identification and quantification underscores the necessity for well-characterized and validated analytical procedures. The isolation and characterization of degradation products remain essential for establishing safety profiles and understanding degradation pathways, thereby reinforcing the importance of stability-indicating capability in analytical method development.

A key observation from the reviewed studies is the growing need to transition from conventional trial-and-error approaches toward more systematic, science- and risk-based methodologies. In this regard, the integration of Quality by Design (QbD) principles with Design of Experiments (DoE) offers a robust framework for method development by enabling the identification of critical method parameters, optimization of analytical conditions, and establishment of method robustness through a defined design space. Such approaches significantly enhance method reproducibility, reliability, and regulatory acceptability. Furthermore, the variability observed in stability testing strategies and analytical practices highlights the lack of standardized experimental approaches, particularly in forced degradation studies. Addressing these inconsistencies through structured, QbD-driven methodologies can improve the scientific rigor and comparability of stability data across studies and regulatory submissions.

Overall, the adoption of systematic, QbD- and DoE-based analytical strategies, coupled with rigorous validation practices, is essential for developing robust, stability-indicating methods that meet evolving regulatory expectations. Such approaches not only strengthen the quality and reliability of pharmaceutical analysis but also support the development of safe, effective, and globally compliant drug products.

ABBREVIATIONS

QbD: Quality by Design; **DoE:** Design of Experiment; **HPLC:** high-performance liquid chromatography**; BA:** Bioavailability; **BE:** Bioequivalence; **QC:** Quality Control.

CONFLICT OF INTEREST

The authors declared they do not have any conflict of interest.

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AUTHOR CONTRIBUTION STATEMENTS

SK and **MH** were responsible for the conceptualization and design of the study. **SK** and **MH** actively participated in data acquisition, analysis, and interpretation. The initial draft of the manuscript was prepared by **SK** and **MH**. All authors contributed to the critical evaluation of the results, as well as the preparation and comprehensive review of the final manuscript.

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Cite this article: Khade S, Harde M. Stability-Indicating Chromatographic Method Development for Pharmaceutical Dosage Forms via Enhanced a QbD and DoE Strategies: A Review. Indian J of Pharmaceutical Education and Research. 2027;XX(XX):1-9.

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