



**DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-UPLC
METHODS FOR DETERMINATION OF DRUGS IN PHARMACEUTICAL DOSAGE
FORMS: A COMPREHENSIVE REVIEW**

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ABSTRACT

Stability-indicating analytical methods are essential for assessing the quality, safety, and stability of pharmaceutical products throughout their lifecycle. Reverse-phase ultra-performance liquid chromatography (RP-UPLC) has emerged as an efficient analytical technique because of its high chromatographic efficiency, rapid separation, improved resolution, and reduced solvent consumption. The development of a stability-indicating method requires appropriate forced degradation studies, effective separation of the drug from degradation products, and comprehensive validation. In recent years, Analytical Quality by Design (AQbD) has transformed analytical method development from empirical trial-and-error experimentation toward a systematic, science- and risk-based approach. AQbD incorporates the Analytical Target Profile (ATP), risk assessment, Critical Method Parameters (CMPs), Design of Experiments (DoE), statistical modelling, and establishment of a Method Operable Design Region (MODR) (Xuan et al., 2024). This review discusses the principles and applications of stability-indicating RP-UPLC methods, forced degradation studies, chromatographic optimization, AQbD implementation, statistical experimental design, analytical validation, and regulatory expectations under ICH Q2(R2) and Q14.

KEYWORDS: RP-UPLC, stability-indicating method, forced degradation, AQbD, Design of Experiments, method validation, pharmaceutical analysis, ICH Q14, ICH Q2(R2), degradation products.

1. INTRODUCTION

Pharmaceutical products are exposed to various environmental and chemical conditions during manufacturing, storage, transportation, and administration. Such conditions may result in degradation of the active pharmaceutical ingredient and formation of impurities or degradation products. These changes can adversely affect the potency, quality, safety, and therapeutic performance of the formulation. Therefore, analytical procedures capable of accurately determining the drug in the presence of degradation products are essential for pharmaceutical development and quality control (Bakshi & Singh, 2002).

A **stability-indicating analytical method** is an analytical procedure capable of detecting changes in the drug substance or drug product during stability studies

while distinguishing the intact drug from degradation products, process impurities, and formulation components. Such methods are particularly important for stability testing, formulation development, forced degradation studies, impurity profiling, batch release, and pharmaceutical lifecycle management (Blessy et al., 2014).

High-performance liquid chromatography (HPLC) has traditionally been one of the most widely used techniques for pharmaceutical analysis. However, the development of ultra-performance liquid chromatography (UPLC) has provided significant improvements in chromatographic efficiency and analytical speed. UPLC uses small-particle stationary phases and operates at higher pressures, allowing improved resolution, shorter analysis times, and reduced

solvent consumption compared with conventional HPLC (Swartz, 2005).

The practical value of RP-UPLC for stability-indicating pharmaceutical analysis has been demonstrated in several published studies. Trivedi and Patel (2011), for example, developed a stability-indicating RP-UPLC method for quetiapine that separated the drug from five impurities and degradation products within a 5-min run. The method was subsequently validated for specificity, precision, accuracy, linearity, robustness, and system suitability.

2. Stability-Indicating Analytical Methods

The principal objective of a stability-indicating method is to provide reliable quantitative information about the intact drug while demonstrating that degradation products and other components do not interfere with its determination. The method should therefore possess adequate selectivity, resolution, accuracy, precision, and robustness.

Bakshi and Singh (2002) emphasized that stability-indicating analytical procedures are essential for understanding the degradation behavior of pharmaceutical compounds and for demonstrating the suitability of analytical procedures used during stability studies. Similarly, Blessy *et al.* (2014) highlighted forced degradation as an important tool for identifying degradation pathways and developing specific stability-indicating methods.

Chromatographic techniques are particularly suitable because they physically separate the parent drug from degradation products. RP-HPLC and RP-UPLC are extensively used because of their versatility and compatibility with UV, PDA, fluorescence, and mass-spectrometric detection.

The literature demonstrates the effectiveness of RP-UPLC for pharmaceutical stability studies. In the quetiapine study reported by Trivedi and Patel (2011), the method separated quetiapine and five impurities/degradation products within 5 min and demonstrated spectral purity of the principal peak in stressed samples.

3. Reverse-Phase UPLC

RP-UPLC is based on chromatographic separation using a non-polar stationary phase, commonly C18, with an aqueous-organic mobile phase. The separation mechanism is primarily based on differences in hydrophobic interactions between analytes and the stationary phase.

UPLC provides several advantages over conventional HPLC, including

- Higher chromatographic efficiency
- Improved resolution
- Faster analysis

- Lower solvent consumption
- Better peak capacity
- Higher sample throughput
- Compatibility with advanced detectors

Swartz (2005) described UPLC as an important development in liquid chromatography resulting from the use of sub-2- μm particles and high-pressure operation. These characteristics allow faster separations while maintaining or improving chromatographic resolution.

The pharmaceutical application of UPLC was demonstrated by Trivedi and Patel (2011), who used an Agilent Eclipse Plus C18 RRHD 1.8- μm column for quetiapine analysis and achieved separation of the drug from related compounds in a 5-min chromatographic run.

4. Forced Degradation Studies

Forced degradation is an essential component of stability-indicating analytical method development. In this approach, the drug substance or drug product is deliberately exposed to stress conditions to generate degradation products. The resulting stressed samples are analyzed to establish whether the analytical procedure can adequately separate the parent drug from its degradation products (Blessy *et al.*, 2014).

Common forced degradation conditions include:

4.1 Acid Hydrolysis

Acidic conditions are used to investigate susceptibility of the drug to acid-catalyzed degradation. Drugs containing hydrolysis-sensitive functional groups may undergo significant degradation under these conditions.

4.2 Alkaline Hydrolysis

Alkaline stress is used to investigate base-catalyzed degradation. Some pharmaceutical compounds are particularly susceptible to alkaline hydrolysis.

4.3 Oxidative Degradation

Oxidative degradation is commonly performed using hydrogen peroxide or another suitable oxidizing system. This study can identify susceptibility of the drug to oxidative pathways.

4.4 Thermal Degradation

Thermal stress evaluates the susceptibility of the drug to degradation under elevated-temperature conditions.

4.5 Photolytic Degradation

Photolytic stress is used to assess the sensitivity of the drug to light exposure.

4.6 Neutral Hydrolysis

Neutral hydrolysis provides information about drug stability under aqueous conditions without strongly acidic or alkaline environments.

Blessy *et al.* (2014) emphasized that forced degradation should be scientifically designed rather than excessively

severe because extreme conditions may generate secondary degradation products that are not representative of normal storage conditions.

The application of forced degradation to RP-UPLC development has been demonstrated in pharmaceutical research. Trivedi and Patel (2011) evaluated stressed quetiapine samples and established separation of degradation products from the drug peak, supporting the stability-indicating capability of the method.

5. Chromatographic Method Development

Development of an RP-UPLC method requires systematic selection and optimization of chromatographic variables. Important parameters include stationary-phase chemistry, column dimensions, particle size, mobile-phase composition, organic modifier, pH, buffer concentration, flow rate, column temperature, injection volume, and detection wavelength.

Among these parameters, the mobile-phase composition and stationary-phase chemistry have major effects on retention and selectivity. Flow rate influences retention time, efficiency, and back pressure, whereas temperature can affect viscosity, mass transfer, retention, and peak shape.

The selection of a C18 stationary phase is common for pharmaceutical analysis because it provides broad applicability to compounds with different physicochemical characteristics. However, alternative stationary phases may be required where adequate resolution cannot be achieved using conventional C18 chemistry.

Trivedi and Patel (2011) demonstrated the importance of chromatographic optimization in developing the quetiapine RP-UPLC method. Their optimized procedure employed a C18 stationary phase, gradient elution, aqueous triethylamine, acetonitrile/methanol, and UV detection at 252 nm, providing rapid separation of the drug and related compounds.

6. Analytical Quality by Design

Analytical Quality by Design represents a systematic approach to analytical method development in which method performance is deliberately designed rather than established primarily through trial-and-error experimentation.

According to Xuan *et al.* (2024), AQbD integrates the Analytical Target Profile, Critical Method Parameters, risk assessment, experimental design, statistical modelling, and Method Operable Design Region to improve chromatographic robustness and analytical lifecycle management. Their review identified more than 180 research articles published between 2019 and 2024 addressing AQbD applications in chromatographic analysis.

AQbD provides several advantages compared with conventional one-factor-at-a-time development:

- Better understanding of method variables
- Evaluation of factor interactions
- Reduced number of experiments
- Improved robustness
- Statistical optimization
- Establishment of an analytical design space
- Improved lifecycle management

Passerine and Breitzkreitz (2024) emphasized that DoE is a central component of AQbD because it provides information about the influence of experimental input variables on critical analytical responses and enables development of mathematical models for defining a robust Method Operable Design Region.

7. Analytical Target Profile

The Analytical Target Profile (ATP) defines the intended performance of the analytical procedure. It provides the foundation for method development and establishes the analytical requirements that the final procedure should satisfy.

For a stability-indicating RP-UPLC method, the ATP may include requirements for

- Specificity
- Accuracy
- Precision
- Linearity
- Appropriate analytical range
- Robustness
- Adequate resolution
- Suitable analysis time

An appropriately defined ATP helps ensure that experimental development remains focused on the intended analytical purpose rather than simply obtaining a chromatogram with acceptable peak shape.

8. Risk Assessment and Critical Method Parameters

Risk assessment is used to identify variables that may significantly affect analytical performance. Potential Critical Method Parameters include mobile-phase composition, pH, flow rate, column temperature, buffer concentration, and detection wavelength.

Critical analytical responses may include

- Retention time
- Resolution
- Theoretical plates
- Tailing factor
- Peak area
- Peak purity

Xuan *et al.* (2024) described risk assessment and DoE as important components of AQbD-based chromatographic development because they provide a systematic understanding of the relationships between method variables and analytical performance.

9. Design of Experiments

Design of Experiments enables simultaneous evaluation of multiple factors and their interactions. Unlike one-factor-at-a-time experimentation, DoE can identify linear, interaction, and quadratic effects and therefore provides greater information about the analytical system.

Central Composite Design and Box-Behnken Design are frequently employed for response-surface optimization. These approaches are particularly useful when multiple chromatographic variables need to be optimized simultaneously.

Passerine and Breitzkreitz (2024) reported that DoE facilitates generation of mathematical models that can subsequently be used to establish a robust method-operable region.

Recent AQbD research also demonstrates increasing application of response-surface designs to chromatographic method development. Xuan et al. (2024) reported widespread application of screening and response-surface experimental designs in recent chromatographic AQbD studies.

10. Stability-Indicating RP-UPLC Applications

Numerous studies have demonstrated the usefulness of RP-UPLC for pharmaceutical stability assessment.

10.1 Quetiapine

Trivedi and Patel (2011) developed a stability-indicating RP-UPLC method for quetiapine pharmaceutical dosage forms. The method separated quetiapine from five impurities/degradation products within 5 min and demonstrated specificity, precision, accuracy, linearity, robustness, and filter compatibility.

10.2 Ritonavir

Koppala et al. (2015) reported a stability-indicating RP-UPLC method for ritonavir and related compounds. The work demonstrated the ability of UPLC to provide rapid chromatographic separation while supporting quantitative determination of the drug and related substances.

10.3 Rosuvastatin

Stability-indicating RP-UPLC methodology has also been applied to rosuvastatin pharmaceutical formulations, where chromatographic separation of the drug from known and degradation-related impurities demonstrated the value of UPLC for impurity profiling and stability assessment.

10.4 Olaparib

RP-UPLC has been applied to olaparib and its related substances using forced degradation and chromatographic optimization. Such studies demonstrate the applicability of rapid chromatographic methods for pharmaceutical dosage-form analysis.

These applications collectively demonstrate that RP-UPLC can provide rapid and selective determination of drugs while maintaining adequate separation of degradation products.

11. Method Validation

Validation establishes whether the developed analytical procedure is suitable for its intended application. ICH Q2(R2) provides the current framework for analytical procedure validation.

Specificity

Specificity is particularly important for stability-indicating procedures because the drug peak must be distinguishable from degradation products, impurities, and formulation components.

Accuracy

Accuracy demonstrates the closeness of the measured value to the accepted reference value and is generally evaluated through recovery studies.

Precision

Precision demonstrates the closeness of agreement between repeated measurements and may include repeatability and intermediate precision.

Linearity and Range

Linearity evaluates the relationship between analytical response and concentration over the intended analytical range.

Robustness

Robustness evaluates the ability of the method to remain unaffected by small deliberate changes in analytical parameters.

System Suitability

System suitability parameters such as retention time, theoretical plates, tailing factor, and resolution provide evidence that the chromatographic system is suitable before routine analysis.

The quetiapine RP-UPLC study by Trivedi and Patel (2011) demonstrated validation of the developed method with respect to system suitability, specificity, precision, accuracy, linearity, robustness, and filter compatibility.

12. ICH Q14 and Modern Analytical Development

ICH Q14 represents an important development in analytical procedure development because it formally provides a science- and risk-based framework for developing and maintaining analytical procedures. The guideline applies to new or revised procedures used for release and stability testing of drug substances and drug products. It addresses robustness, parameter ranges, analytical procedure control strategies, and lifecycle management (ICH Q14, 2023/2024).

The current regulatory framework therefore supports an enhanced approach in which analytical development is based on predefined objectives, risk assessment, experimental design, statistical evaluation, and control strategies.

The recent review by Xuan et al. (2024) highlighted that AQbD is increasingly being adopted in chromatographic analysis and can improve robustness, efficiency, validation, and cost-effectiveness by reducing unnecessary experimental effort.

13. Analytical Lifecycle Management

A modern analytical procedure should not be regarded as a static method that ends with validation. Instead, analytical lifecycle management considers method development, validation, routine application, monitoring, and controlled improvement.

ICH Q14 specifically recognizes analytical procedure lifecycle management and provides a framework for maintaining analytical procedures throughout their operational life.

This approach is particularly valuable for RP-UPLC methods because changes in columns, instruments, solvents, or laboratory conditions may affect chromatographic performance. A well-understood analytical procedure with an established operating region can better accommodate routine laboratory variability.

14. Industrial Importance

Stability-indicating RP-UPLC methods are highly relevant to pharmaceutical industries because they support:

- Assay determination
- Related-substance analysis
- Stability testing
- Forced degradation studies
- Batch release
- Formulation development
- Process monitoring
- Analytical method transfer
- Regulatory submissions
- Lifecycle management

UPLC can improve laboratory throughput through rapid analysis, while AQbD can improve method robustness and reduce experimental requirements. Xuan et al. (2024) reported that AQbD applications in chromatographic analysis generally improved robustness and efficiency while supporting systematic method development.

15. Future Perspectives

Future analytical development is expected to increasingly integrate RP-UPLC with high-resolution mass spectrometry, chemometrics, automation, artificial intelligence, and advanced data-processing approaches. Such technologies may improve identification of

unknown degradation products and prediction of degradation pathways.

Green analytical chemistry is another emerging consideration. Shorter UPLC run times, reduced solvent consumption, miniaturized systems, and optimized sample preparation can reduce the environmental impact of pharmaceutical analysis.

AQbD provides a useful framework for integrating these objectives because chromatographic performance, robustness, analysis time, solvent consumption, and other method requirements can be considered simultaneously during experimental optimization.

16. CONCLUSION

Stability-indicating RP-UPLC represents a powerful analytical approach for the determination of pharmaceutical drugs in the presence of degradation products, impurities, and formulation-related interferences. Published research has demonstrated successful application of RP-UPLC to drugs such as quetiapine and other pharmaceutical compounds, with rapid separation and comprehensive validation (Trivedi & Patel, 2011). The incorporation of AQbD further improves analytical method development by integrating ATP definition, risk assessment, DoE, statistical modelling, robustness evaluation, and analytical lifecycle management (Xuan et al., 2024). Recent regulatory guidance through ICH Q14 provides a formal framework for science- and risk-based analytical procedure development, while ICH Q2(R2) establishes principles for demonstrating analytical procedure performance. Thus, the combination of forced degradation, RP-UPLC, AQbD, DoE, and comprehensive validation provides a scientifically robust framework for developing reliable stability-indicating analytical procedures for pharmaceutical dosage forms.

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