

**ANALYTICAL VALIDATION OF STABILITY-INDICATING METHOD FOR
ESTIMATION OF ANTIHYPERTENSIVE DRUGS USING REVERSE PHASE ULTRA-
PERFORMANCE LIQUID CHROMATOGRAPHY: A REVIEW****Selvaraja Elumalai¹, Sanjay Saxena²**¹Research Scholar, Department of Chemistry, School of Basic and Applied Sciences, Raffles University, Neemrana-301705, 0009-0003-1203-9316.²Professor, Department of Chemistry, School of Basic and Applied Sciences, Raffles University, Neemrana-301705.***Corresponding Author: Selvaraja Elumalai**

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ABSTRACT

Antihypertensive drugs are widely used for the long-term management of elevated blood pressure and associated cardiovascular complications. Reliable analytical procedures are essential for confirming the identity, strength, purity, quality, and stability of these pharmaceutical substances and formulations. Reverse phase ultra-performance liquid chromatography (RP-UPLC) has emerged as an efficient chromatographic technique for pharmaceutical analysis because of its high separation efficiency, rapid analysis, improved resolution, reduced solvent consumption, and compatibility with different detection systems. A stability-indicating analytical method is particularly important because it should distinguish the intact drug from degradation products, process-related impurities, and formulation components. The present review discusses the principles of RP-UPLC, analytical method development, forced degradation studies, and validation of stability-indicating methods for antihypertensive drugs. Important validation characteristics, including specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability, are discussed in relation to current analytical practice. The role of analytical quality by design (AQbD), risk assessment, design of experiments, and method lifecycle management is also considered. Overall, a properly developed and validated RP-UPLC method can provide a rapid, sensitive, reproducible, and stability-indicating approach for pharmaceutical quality control and stability assessment of antihypertensive drug products.

KEYWORDS: Antihypertensive drugs, RP-UPLC, UHPLC, analytical validation, stability-indicating method, forced degradation, pharmaceutical analysis, AQbD, quality control.**1. INTRODUCTION**

Hypertension is a major chronic cardiovascular condition requiring sustained pharmacological treatment. Antihypertensive therapy encompasses several pharmacological classes, and pharmaceutical products may contain single active ingredients or combinations of therapeutically complementary agents. Because these medicines are generally administered over prolonged periods, consistent quality and stability of the active pharmaceutical ingredients (APIs) are essential for maintaining their intended therapeutic performance.

Analytical chemistry has an important role throughout pharmaceutical development, manufacturing, release testing, and stability assessment. Chromatographic techniques are extensively used because they permit separation and quantitative determination of active ingredients in the presence of impurities and formulation components. High-performance liquid chromatography (HPLC) has traditionally been one of the principal techniques used for pharmaceutical quality control, particularly because of its versatility and reproducibility.^[1-3]

The increasing demand for rapid, efficient, and resource-conscious analytical procedures has contributed to the development and application of ultra-performance liquid chromatography (UPLC), also referred to more generally as ultra-high-performance liquid chromatography (UHPLC). The technique uses highly efficient stationary phases, commonly with sub-2 μm particles, together with instrumentation capable of operating at elevated pressures. These characteristics can provide higher efficiency, improved resolution, shorter analysis times, and reduced mobile-phase consumption compared with conventional HPLC.^[4-7]

For pharmaceutical stability assessment, however, chromatographic speed alone is insufficient. The analytical procedure must demonstrate that the drug can be accurately measured in the presence of its degradation products and other potential interferents. Consequently, stability-indicating method development and validation are essential components of pharmaceutical analytical development.^[8-10]

2. Stability-Indicating Analytical Methods

A stability-indicating analytical method is an analytical procedure capable of detecting changes in the relevant quality attributes of a drug substance or drug product during storage and stress exposure. In chromatographic applications, the method should provide adequate separation between the principal drug component and degradation-related substances so that the measured assay value is not falsely increased or decreased by co-eluting components.^[8,9]

The development of stability-indicating methods normally involves understanding the degradation behavior of the drug, generating suitable stressed samples, developing adequate chromatographic separation, and demonstrating method specificity. Earlier reviews emphasized that successful stability-indicating method development requires attention to degradation pathways, separation of degradation products, formulation effects, and, where appropriate, mass balance.^[8]

More recent literature continues to emphasize forced degradation as an important component of pharmaceutical development because it can provide information about intrinsic degradation pathways and help establish suitable analytical procedures.^[10,11] Mass-balance evaluation can provide additional evidence that the disappearance of the parent drug is appropriately accounted for by degradation products and other measurable components.^[12]

3. Principle of RP-UPLC

RP-UPLC is based on chromatographic separation using a non-polar stationary phase and a relatively polar mobile phase. Reversed-phase columns containing chemically modified silica, particularly C18 phases, are commonly employed for pharmaceutical compounds.

Separation depends on the relative interactions of analytes with the stationary and mobile phases.

The use of small-particle stationary phases increases chromatographic efficiency and can improve resolution. However, smaller particles also generate greater back pressure, requiring instrumentation capable of operating at elevated pressures.^[4,5] The theoretical advantages of small particles include improved efficiency, faster mass transfer, higher optimum linear velocities, and the potential to achieve rapid separations without sacrificing resolution.^[4,6]

UPLC has consequently become an important tool in pharmaceutical analysis. Reviews have highlighted its advantages in speed, resolution, sensitivity, and solvent reduction compared with conventional HPLC.^[5,7] Applications include assay determination, impurity profiling, stability testing, dissolution-related analysis, and analysis of pharmaceutical formulations.

4. Advantages of RP-UPLC in Pharmaceutical Analysis

RP-UPLC offers several characteristics that make it suitable for analytical method development and validation:

4.1 High Separation Efficiency

The use of small-particle columns provides high chromatographic efficiency and can produce narrow peaks with improved resolution. This characteristic is particularly useful when the active drug must be separated from structurally related impurities and degradation products.^[4,6]

4.2 Reduced Analysis Time

High efficiency allows acceptable chromatographic separation to be obtained in relatively short run times. Rapid methods can increase laboratory throughput and reduce the time required for routine quality-control analysis.^[5,7]

4.3 Lower Solvent Consumption

Shorter columns and rapid separations may substantially reduce mobile-phase consumption compared with conventional methods. This can reduce analytical waste and contribute to more sustainable laboratory practices.^[7,13]

4.4 Improved Resolution

The increased efficiency of UPLC columns can provide adequate separation of closely related compounds. This is particularly important in stability-indicating procedures where degradation products may have chromatographic behavior similar to that of the parent drug.^[9,14]

4.5 Compatibility with Advanced Detection

UPLC can be coupled with UV, photodiode-array, fluorescence, and mass-spectrometric detection systems.

Such combinations may provide both quantitative determination and additional information for impurity or degradation-product characterization.^[9,15]

5. Method Development for Antihypertensive Drugs

Development of a stability-indicating RP-UPLC method requires systematic selection and optimization of chromatographic variables. The principal objective is to obtain adequate separation, acceptable peak shape, reproducible retention, suitable sensitivity, and a practical analysis time.

5.1 Selection of Stationary Phase

Selection of the stationary phase depends on the physicochemical characteristics of the analyte and the required separation. C18 reversed-phase columns are frequently considered as an initial option because of their broad applicability. However, alternative stationary phases may be required when selectivity is insufficient.

Column dimensions, particle size, pore characteristics, and surface chemistry can influence retention, efficiency, and resolution. The use of sub-2 μm particles is one of the important technological features distinguishing UPLC from conventional HPLC.^[4-6]

5.2 Mobile-Phase Selection

The mobile phase is a critical method variable. Common organic modifiers include acetonitrile and methanol, while the aqueous component may contain water, buffers, or suitable pH-modifying agents. The proportion of organic solvent influences retention time and selectivity, whereas buffer conditions may substantially affect the behavior of ionizable compounds.

For antihypertensive drugs with different ionization properties, optimization of mobile-phase pH can be particularly important. An appropriate pH may improve peak shape, retention reproducibility, and separation from degradation products.

5.3 Flow Rate

Flow rate influences retention, efficiency, resolution, pressure, and analysis time. In UPLC, the optimum flow rate must be selected with consideration of column dimensions, particle size, mobile-phase viscosity, and instrument pressure limitations.^[4,6]

5.4 Column Temperature

Temperature may influence mobile-phase viscosity, analyte retention, selectivity, and mass-transfer kinetics. Controlled temperature conditions can improve chromatographic reproducibility and may be investigated as part of method optimization and robustness evaluation.

5.5 Detection Wavelength

For UV or PDA detection, the wavelength should be selected according to the absorption characteristics of the analyte while minimizing interference from mobile-

phase components and formulation excipients. PDA detection can additionally assist in evaluating peak homogeneity and spectral characteristics.

6. Stability Studies and Forced Degradation

Stability testing evaluates how the quality of a drug substance or drug product changes with time under environmental factors such as temperature, humidity, and light. The ICH stability framework provides principles for generating stability data used to support shelf-life and storage conditions.^[16]

Forced degradation, or stress testing, is performed under conditions more severe than normal accelerated or long-term storage conditions. Its purpose is to understand degradation behavior and support the development of stability-indicating analytical procedures.^[8,10,11]

6.1 Acidic Stress

Acidic conditions may promote hydrolysis or other chemical transformations depending on the molecular structure. Samples exposed to acidic conditions can be analyzed using the proposed chromatographic method to determine whether degradation products are adequately separated from the parent drug.

6.2 Alkaline Stress

Basic conditions may induce hydrolytic degradation in susceptible molecules. Appropriate stress conditions should be selected scientifically rather than applying unnecessarily severe treatment.

6.3 Oxidative Stress

Oxidative degradation can occur through reactions involving susceptible functional groups. Oxidative stress studies are therefore useful for determining whether the analytical procedure can distinguish oxidation products from the intact drug.^[10,11]

6.4 Thermal Stress

Exposure to elevated temperatures can accelerate chemical degradation and may provide information about thermal sensitivity. Thermal stress conditions should be controlled and documented carefully.

6.5 Photolytic Stress

Light exposure may cause degradation in photosensitive compounds. Photostability assessment can therefore contribute to understanding the overall stability profile of a pharmaceutical product.

6.6 Humidity Stress

Moisture can affect compounds that are susceptible to hydrolysis or physical changes. Where scientifically justified, humidity-related studies may be incorporated into the overall degradation assessment.

Importantly, forced degradation should not simply aim to maximize degradation. Recent literature emphasizes the importance of scientifically justified stress conditions

and appropriate endpoints so that degradation remains relevant to pharmaceutical development.^[11,12]

7. Analytical Validation of RP-UPLC Method

Validation establishes documented evidence that an analytical procedure is appropriate for its intended purpose. The current ICH Q2(R2) guideline provides an updated framework for validation of analytical procedures, while ICH Q14 provides guidance for analytical procedure development.^[17,18]

7.1 Specificity

Specificity is the ability to measure the analyte accurately in the presence of other components that may be expected to be present. For a stability-indicating method, this includes degradation products, impurities, excipients, and other formulation-related components.

Specificity is particularly important because inadequate chromatographic separation can result in inaccurate assay values. Evaluation may include comparison of blank, placebo, standard, sample, and stressed samples. PDA spectral analysis or complementary spectroscopic techniques may provide additional evidence where appropriate.^[9,14]

7.2 Linearity and Range

Linearity evaluates whether analytical response is appropriately related to analyte concentration over the specified range. Multiple concentration levels are analyzed, and the response is evaluated using an appropriate statistical model.

The selected range should be appropriate for the intended use of the method. For an assay procedure, the range should adequately cover the expected concentration around the target level. ICH Q2(R2) emphasizes evaluation of the analytical procedure according to its intended purpose rather than applying validation characteristics without considering the application.^[17]

7.3 Accuracy

Accuracy describes the closeness of the measured value to an accepted reference value. Recovery studies using samples spiked with known amounts of analyte are commonly used for pharmaceutical assay procedures.

Accuracy should be evaluated across an appropriate range and under conditions that reflect the intended analytical procedure. Statistical evaluation should be appropriate to the experimental design and purpose of the method.^[17,19]

7.4 Precision

Precision expresses the closeness of agreement among repeated measurements. It may include repeatability and intermediate precision, with the latter considering variables such as analyst, day, instrument, or other relevant laboratory conditions.

Precision is particularly important for stability-indicating procedures because small analytical variations should not be confused with actual changes in drug concentration. The importance of precision in establishing appropriate acceptance criteria has been emphasized in pharmaceutical analytical validation literature.^[19]

7.5 Limit of Detection

The limit of detection (LOD) represents the lowest level of an analyte that can be detected but not necessarily quantified with acceptable accuracy and precision. It is particularly relevant when the analytical procedure is intended to detect low-level impurities or degradation products.

7.6 Limit of Quantification

The limit of quantification (LOQ) represents the lowest concentration that can be quantified with appropriate accuracy and precision. The LOQ is particularly relevant to impurity and degradation-product assessment.

7.7 Robustness

Robustness evaluates the capacity of an analytical procedure to remain reliable when small, deliberate variations are introduced into method parameters. Typical variables may include mobile-phase composition, flow rate, temperature, pH, and detection conditions.

Robustness is increasingly considered during method development rather than only after the method has been finalized. AQbD approaches encourage systematic identification of critical method parameters and evaluation of their effects on critical analytical responses.^[20–23]

7.8 System Suitability

System suitability testing ensures that the chromatographic system performs adequately before sample analysis. Depending on the method, parameters may include retention time, theoretical plate count, peak asymmetry, resolution, and replicate injection precision.

Appropriate system-suitability requirements provide an additional control mechanism for detecting chromatographic problems before analytical results are reported.^[24]

8. Analytical Quality by Design in RP-UPLC Development

Traditional analytical method development may rely heavily on sequential experimentation and analyst experience. Analytical Quality by Design provides a more systematic alternative based on predefined objectives, risk assessment, experimental design, and method understanding.^[20–23]

An Analytical Target Profile (ATP) can first be established to define what the method must accomplish. Critical Method Parameters (CMPs), such as mobile-

phase composition, pH, flow rate, temperature, and gradient conditions, can then be identified. Critical Analytical Attributes or responses may include retention, resolution, peak symmetry, efficiency, and assay performance.

Design of Experiments (DoE) can be used to evaluate the influence of multiple factors simultaneously. This approach may provide information about interactions between method variables that could be missed when only one variable is changed at a time.^[21,22]

ICH Q14 formally recognizes enhanced approaches to analytical procedure development and supports greater scientific understanding of method performance.^[18] Recent reviews have also emphasized the importance of design-space concepts and lifecycle management for robust chromatographic methods.^[23,25]

9. Risk Assessment in Method Development

Risk assessment helps identify analytical variables that may have the greatest potential effect on method performance. Factors such as column chemistry, mobile-phase pH, organic solvent proportion, flow rate, temperature, and injection volume may be evaluated according to their potential impact.

A risk-based approach can help prioritize experimental studies and reduce unnecessary experimentation. Literature on analytical QbD has described the use of risk assessment and DoE as tools for developing more robust analytical separation methods.^[20,21,26]

10. Application of RP-UPLC to Pharmaceutical Formulations

After successful development and validation, the RP-UPLC method can be applied to pharmaceutical dosage forms. Sample preparation should provide adequate extraction of the active ingredient without introducing significant analytical interference.

For formulation analysis, placebo studies are important because excipients may produce chromatographic signals or influence extraction efficiency. The validated method should demonstrate that the formulation matrix does not compromise assay accuracy or precision.

The literature contains examples in which stability-indicating UHPLC methods have successfully separated active ingredients from degradation products in pharmaceutical products.^[27-29] Such studies illustrate the practical value of high-efficiency chromatography for pharmaceutical quality control.

11. Role of Chromatography in Impurity and Degradation-Product Assessment

Impurity profiling is an important component of pharmaceutical quality assessment. Impurities may originate from synthesis, manufacturing processes, degradation, storage, or interactions with excipients and packaging materials.

HPLC and UHPLC are widely used for impurity analysis because they provide separation before detection. When UV or PDA detection does not provide sufficient structural information, coupling with mass spectrometry may support identification and characterization of unknown degradation products.^[9,15,29]

The development of a stability-indicating method should therefore consider not only the quantitative assay of the parent drug but also the possibility of detecting relevant degradation products at appropriate levels.

12. Method Performance and Statistical Evaluation

Statistical evaluation is an important part of analytical validation. Regression analysis can be used for evaluating calibration behavior, while standard deviation and percentage relative standard deviation can be used to describe precision. Appropriate statistical procedures should be selected according to the study design and analytical objective.

USP General Chapter <1210> addresses statistical tools associated with analytical procedure validation, while USP <1225> provides general principles for validation of compendial procedures.^[24,30]

The use of appropriate statistical tools improves confidence in analytical conclusions and helps distinguish genuine method performance characteristics from experimental variability.

13. Comparison of Conventional HPLC and RP-UPLC

Parameter	Conventional RP-HPLC	RP-UPLC/UHPLC
Particle size	Generally larger	Often sub-2 μm
Pressure	Lower	Higher
Analysis time	Usually longer	Generally shorter
Efficiency	Good	Very high
Resolution	Good	Often improved
Solvent consumption	Relatively higher	Generally lower
Throughput	Moderate	High
Instrument requirement	Conventional HPLC	High-pressure UHPLC/UPLC system
Method optimization	Established	Requires careful optimization
Suitability for rapid stability analysis	Good	Particularly advantageous

The advantages of small-particle chromatography and high-pressure operation have been demonstrated extensively in the development of fast liquid chromatographic methods.^[4–7] However, method transfer between HPLC and UPLC should be performed carefully because changes in column dimensions, particle size, flow rate, dwell volume, and other system characteristics can influence chromatographic behavior.^[6]

14. Current Trends and Future Perspectives

Current analytical development increasingly emphasizes method robustness, sustainability, lifecycle management, and scientific understanding. AQbD approaches are gaining attention because they provide a structured framework for identifying critical method variables, establishing a method operable design region, and implementing an analytical control strategy.^[18,20–23]

Green analytical considerations are also becoming increasingly important. Reduction in organic-solvent consumption and shortening of analytical run time are among the advantages associated with UHPLC approaches.^[7,13]

Another important development is the combination of chromatographic separation with advanced detectors. UPLC coupled with mass spectrometry can facilitate characterization of unknown impurities and degradation products, while PDA detection can provide useful information concerning peak homogeneity.^[9,15,29]

Future stability-indicating methods for antihypertensive drugs are therefore likely to emphasize rapid separation, improved sensitivity, simultaneous analysis of multiple components, environmentally conscious solvent selection, and lifecycle-based method management.

15. Research Gap

Although numerous chromatographic methods have been reported for pharmaceutical analysis, differences in drug structure, formulation composition, degradation behavior, and analytical requirements mean that a previously reported method may not necessarily be suitable for every application. Stability-indicating procedures require demonstrated separation of the active component from relevant degradation products rather than simply acceptable assay performance.

The literature also indicates that forced degradation studies can vary considerably in stress conditions, endpoints, and interpretation.^[10–12] Consequently, systematic development, scientifically justified stress testing, and comprehensive validation remain important when establishing an RP-UPLC procedure for antihypertensive drugs.

Integration of ICH Q14 development principles with ICH Q2(R2) validation requirements provides an opportunity to improve method understanding, robustness, and lifecycle management.^[17,18] This approach is particularly

relevant for the development of rapid and reliable RP-UPLC procedures intended for routine pharmaceutical quality control.

16. CONCLUSION

RP-UPLC represents an advanced and efficient approach for the analytical evaluation of antihypertensive drugs. Its high chromatographic efficiency, rapid separation, improved resolution, and reduced solvent consumption provide important advantages for pharmaceutical quality-control laboratories. However, the performance of the technique depends on appropriate method development, optimization, and validation.

A stability-indicating RP-UPLC method should demonstrate adequate specificity and should effectively distinguish the active pharmaceutical ingredient from degradation products, impurities, and formulation components. Forced degradation studies provide valuable information about degradation behavior and help establish the specificity of the analytical procedure. Validation of linearity, accuracy, precision, sensitivity, robustness, and system suitability provides evidence that the method is fit for its intended purpose.

The incorporation of analytical quality-by-design concepts, risk assessment, design of experiments, and lifecycle management can further improve the reliability and robustness of RP-UPLC methods. In accordance with current analytical-development principles, a well-designed and validated RP-UPLC procedure can provide a dependable tool for routine assay determination, stability testing, impurity assessment, and quality control of antihypertensive pharmaceutical products.^[12,17,18,23]

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