WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 14, 302-330. Review Article ISSN 2277–7105

ANALYTICAL QUALITY BY DESIGN (AQBD)-DRIVEN RP-HPLC METHOD DEVELOPMENT FOR TARGETED ANTICANCER AGENTS: REGULATORY ADVANCES, CHROMATOGRAPHIC STRATEGIES, AND FUTURE PERSPECTIVES)-DRIVEN RP-HPLC METHOD DEVELOPMENT FOR TARGETED ANTICANCER AGENTS: REGULATORY ADVANCES, CHROMATOGRAPHIC STRATEGIES, AND FUTURE PERSPECTIVES

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Article Received on 15 June 2026,
Article Revised on 05 July 2026,
Article Published on 16 July 2026,

https://doi.org/10.5281/zenodo.21357537

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How to cite this Article: *Dipali Diwate (2026). Analytical Quality By Design (Aqbd)-Driven Rp-Hplc Method Development For Targeted Anticancer Agents: Regulatory Advances, Chromatographic Strategies, And Future Perspectives)-Driven Rp-Hplc Method Development For Targeted Anticancer Agents: Regulatory Advances, Chromatographic Strategies, And Future Perspectives. World Journal of Pharmaceutical Research, 15(14), 302–330.

ABSTRACT

The increasing complexity of modern pharmaceutical products has intensified the need for robust, reliable, and scientifically justified analytical methodologies. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the most widely utilized analytical technique in pharmaceutical research and quality control because of its versatility, sensitivity, and reproducibility. Traditionally, chromatographic method development relied heavily on empirical experimentation and sequential optimization approaches, often resulting in methods with limited robustness and inadequate understanding of critical variables. The introduction of Analytical Quality by Design (AQbD) has transformed analytical method development by incorporating systematic risk assessment, statistical experimental design, lifecycle management, and regulatory science principles. AQbD facilitates a deeper understanding of analytical procedures through the identification of Analytical Target Profiles (ATPs), Critical Quality Attributes (CQAs), and Critical Method Parameters (CMPs), ultimately leading to the establishment of Method Operable Design Regions (MODRs). In parallel, recent regulatory developments, including ICH Q14 and ICH

Q2(R2), have reinforced the importance of science-based analytical development and continuous performance verification. Targeted anticancer therapies such as osimertinib present unique analytical challenges due to their complex physicochemical characteristics, stringent impurity requirements, and increasing regulatory scrutiny. This review critically examines the evolution of AQbD, modern RP-HPLC method development strategies, regulatory expectations, and recent advances in chromatographic science. Particular emphasis is placed on targeted oncology products and osimertinib as a representative case study. Emerging technologies including artificial intelligence, digital chromatography, and green analytical chemistry are also discussed as future directions for pharmaceutical analytical science.

KEYWORDS: Analytical Quality by Design; AQbD; RP-HPLC; Osimertinib; Targeted Anticancer Therapy; ICH Q14; Method Development; Pharmaceutical Analysis.

1. INTRODUCTION

Analytical methods play a critical role in pharmaceutical development by ensuring the identity, strength, purity, and quality of drug substances and drug products throughout their lifecycle. Among the available analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) remains one of the most widely used methods because of its high sensitivity, selectivity, reproducibility, and suitability for routine quality control applications.[1,2] RP-HPLC is extensively employed for assay determination, impurity profiling, dissolution testing, stability studies, and regulatory submissions.

Traditionally, analytical methods were developed using a trial-and-error approach in which individual variables were optimized sequentially. Although this strategy has been widely used, it often provides limited understanding of method variability and interactions among experimental factors. As pharmaceutical products have become more complex and regulatory expectations have evolved, there has been increasing emphasis on systematic and science-based analytical development approaches.[3,4]

Analytical Quality by Design (AQbD) has emerged as an extension of the Quality by Design (QbD) concept introduced in pharmaceutical development. AQbD applies risk assessment, statistical experimental design, and lifecycle management principles to analytical procedure development, enabling a better understanding of method performance and robustness. Through the establishment of an Analytical Target Profile (ATP), identification of Critical Method

Parameters (CMPs), and evaluation of Critical Quality Attributes (CQAs), AQbD facilitates the development of reliable and fit-for-purpose analytical methods.[5,6]

The importance of AQbD has increased following the publication of ICH Q14 (Analytical Procedure Development) and the revised ICH Q2(R2) guideline on analytical validation. These guidelines encourage a lifecycle-based approach to analytical procedure development and emphasize scientific understanding, risk management, and continuous performance verification throughout the method lifecycle.[7,8]

The application of advanced analytical strategies is particularly relevant in oncology drug development, where targeted therapies often possess complex molecular structures and require highly sensitive, stability-indicating analytical methods. The growing use of targeted anticancer agents has increased the demand for robust chromatographic methods capable of supporting pharmaceutical development, quality control, and regulatory compliance.[9,10] Osimertinib, a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, is widely used in the treatment of EGFR-mutated non-small-cell lung cancer (NSCLC). Owing to its clinical significance and complex analytical profile, osimertinib serves as an appropriate model compound for discussing modern AQbD-based analytical method development strategies.[11,12]

This review summarizes the evolution of AQbD, recent regulatory developments, and contemporary RP-HPLC method development approaches, with particular emphasis on analytical challenges associated with targeted anticancer therapies. The article further discusses current limitations, emerging technologies, and future opportunities that may influence the next generation of pharmaceutical analytical development.

2. Evolution of Analytical Quality by Design (AQbD) and Analytical Lifecycle Management

2.1 Emergence of AQbD in Pharmaceutical Analysis

For many years, pharmaceutical analytical methods were developed primarily through empirical experimentation, where method variables were adjusted individually until acceptable performance was achieved. Although this approach successfully supported routine analytical activities, it often provided limited understanding of the factors influencing method variability and long-term performance. As pharmaceutical products became more complex and regulatory expectations evolved, the need for a more systematic and science-based approach to analytical

development became evident.[13,14]

The concept of Quality by Design (QbD), initially introduced for pharmaceutical product and process development, provided the foundation for this transition. QbD emphasizes building quality into a process through scientific understanding, risk assessment, and effective control strategies rather than relying solely on end-product testing.[15] The successful implementation of QbD in pharmaceutical development subsequently led to the emergence of Analytical Quality by Design (AQbD), which applies the same principles to analytical procedure development.[16]

AQbD promotes a structured framework in which analytical methods are developed based on predefined objectives, systematic risk evaluation, and statistical experimentation. This approach enhances method understanding, improves robustness, and supports regulatory flexibility throughout the analytical lifecycle.[17]

Table 1: Evolution of AQbD and Major Regulatory Guidelines Influencing.

Guideline/Concept	Year	Major Focus	pact on Analytical Development
H Q10 Pharmaceutical Quality System	2008	ifecycle quality management	Promoted continuous improvement and performance monitoring
cycle Management Concepts	2010–2020	Continuous performance verification	Enhanced long-term analytical reliability
4 Analytical Procedure Development	2023	uctured analytical development	Formalized ATP-driven analytical procedures
CH Q2(R2) Validation of Analytical Procedures	2023	Lifecycle-based validation strategy	Modernized validation and analytical performance evaluation
AQbD in ICHQ14 Guidelines for Analytical Procedure Development	2024	Analytical Quality by Design integration into ICHQ14	Strengthened ATP-driven development and regulatory alignment
Advances in AQbD-Based RP-HPLC Method Development for Oncology Drugs	2025	Application of AQbD in targeted anticancer therapies	Improved robustness and sensitivity in oncology drug analysis
Integration of AQbD with Green Analytical Chemistry	2025	Sustainable pharmaceutical quality systems	Promoted eco-friendly method development with lifecycle focus
Strategic Implementation of AQbD in HPLC Method Development	2026	Lifecycle-oriented AQbD strategies	Enhanced robustness, transferability, and regulatory flexibility

Pharmaceutical Analytical Development

2.2 Fundamental Elements of AQbD

The foundation of AQbD is the establishment of an Analytical Target Profile (ATP), which defines the intended purpose of an analytical procedure and specifies the required performance characteristics necessary to achieve reliable analytical results.[18] The ATP serves as a guiding element throughout method development and helps ensure that the final procedure is fit for its intended application. Once the ATP is defined, attention is directed toward identifying Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs). In chromatographic methods, CQAs commonly include retention time, resolution, peak symmetry, theoretical plate count, and assay precision. These attributes provide measurable indicators of analytical performance.[19] CMPs represent method variables that may significantly influence one or more CQAs. In RP-HPLC methods, typical CMPs include mobile phase composition, pH, buffer concentration, flow rate, column temperature, and detection wavelength. Understanding the relationship between CMPs and CQAs is essential for achieving robust and reliable analytical performance.[20]

2.3 Risk Assessment in AQbD

Risk assessment is a critical component of AQbD because it enables the identification and prioritization of variables that may affect analytical performance. Rather than investigating all experimental factors equally, risk-based approaches focus development efforts on parameters most likely to influence method quality.[21] Several risk assessment tools have been successfully applied in analytical development. Ishikawa (fishbone) diagrams are commonly used to identify potential sources of variability associated with instrumentation, materials, analytical conditions, environmental factors, and operator-related variables. Failure Mode and Effects Analysis (FMEA) further supports risk evaluation by assessing potential failures according to severity, occurrence, and detectability, thereby enabling prioritization of development activities.[22]

The application of structured risk assessment improves development efficiency and provides a scientific basis for subsequent experimental studies.

2.4 Design of Experiments as the Core of AQbD

Design of Experiments (DoE) represents one of the most important tools within the AQbD framework. Unlike traditional one-factor-at-a-time approaches, DoE allows simultaneous investigation of multiple variables and their interactions, providing a more comprehensive

understanding of analytical behavior.[23]

Experimental designs such as factorial designs, Box–Behnken designs, and Central Composite Designs are widely employed during chromatographic method development. These approaches facilitate the identification of critical variables, evaluation of interaction effects, and optimization of analytical conditions with a reduced number of experiments.[24,25]

The integration of DoE into analytical development not only improves efficiency but also generates predictive models that support robust method operation and regulatory justification.

2.5 Method Operable Design Region (MODR)

A key outcome of AQbD-based development is the establishment of the Method Operable Design Region (MODR), which defines the multidimensional range of method conditions capable of consistently delivering acceptable analytical performance.[26] Unlike traditional methods that operate at a single optimized condition, methods developed within an MODR can tolerate minor variations without significant impact on analytical quality. This flexibility enhances robustness, facilitates method transfer between laboratories, and supports lifecycle management activities. Regulatory agencies increasingly recognize MODR-based development because it provides a stronger scientific understanding of method performance.[27]

2.6 Analytical Lifecycle Management

Modern regulatory guidance recognizes that analytical procedures continue to evolve after validation and therefore require ongoing monitoring throughout their lifecycle. This concept, referred to as Analytical Lifecycle Management, extends beyond traditional validation practices and emphasizes continuous performance verification.[28]

The analytical lifecycle can be broadly divided into three stages: analytical procedure design, performance qualification, and continued performance verification. The first stage includes ATP definition, risk assessment, experimental design, and method optimization. The second stage involves validation activities such as evaluation of accuracy, precision, specificity, robustness, and detection capability. The final stage focuses on routine performance monitoring through system suitability testing, trend analysis, and periodic review of method performance.[29]

Lifecycle management ensures that analytical procedures remain reliable and fit for purpose

throughout commercial use and aligns closely with the principles of continuous improvement promoted by modern pharmaceutical quality systems.

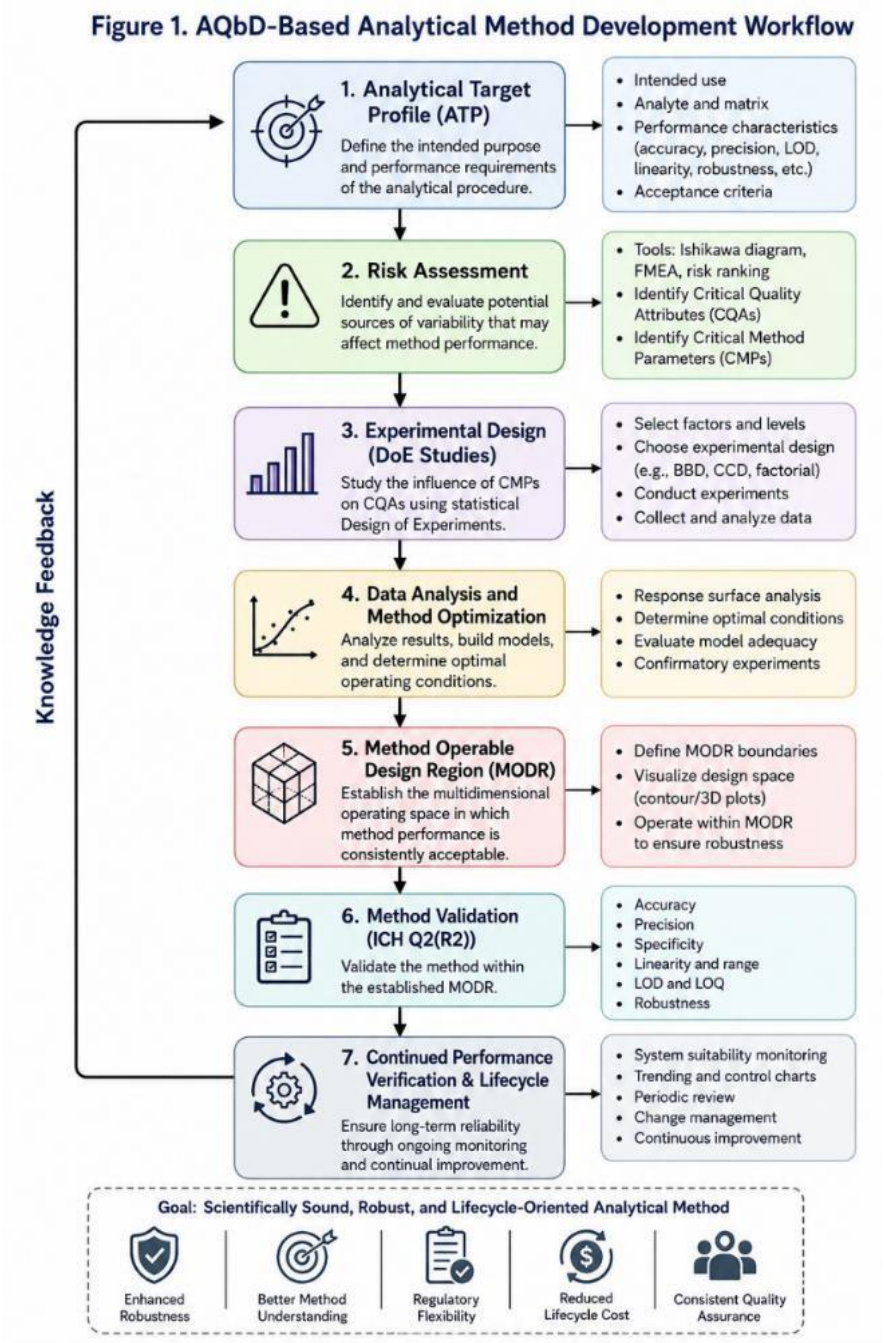

Figure 1: AQbD workflow.

illustrating the systematic progression from Analytical Target Profile establishment through risk assessment, Design of Experiments, method optimization, validation, and lifecycle management.

2.7 Advantages of AQbD

Compared with conventional analytical development approaches, AQbD provides several important advantages, including improved method understanding, enhanced robustness, greater regulatory flexibility, and reduced risk of method failure. The use of risk-based experimentation and statistical optimization facilitates the development of methods that are more resilient to routine operational variability and easier to transfer across laboratories.[30,31] As pharmaceutical products continue to increase in complexity, AQbD is expected to play an increasingly important role in ensuring analytical reliability, supporting regulatory compliance, and enabling effective lifecycle management.

3. Regulatory Framework Supporting AQbD-Based Analytical Method Development

3.1 Evolution of Regulatory Expectations

Over the past two decades, pharmaceutical regulatory expectations have evolved from a compliance-driven approach toward science- and risk-based quality systems. Historically, analytical methods were primarily evaluated through validation studies and predefined acceptance criteria. While this approach ensured basic method suitability, it often provided limited understanding of factors affecting method performance throughout its lifecycle.[32]

The introduction of Quality by Design (QbD) principles and lifecycle-based quality management significantly changed this perspective. Regulatory agencies increasingly encourage the development of analytical procedures based on scientific understanding, risk assessment, and continuous performance monitoring. This shift has laid the foundation for the widespread adoption of Analytical Quality by Design (AQbD) principles in pharmaceutical analysis.[33] Several International Council for Harmonisation (ICH) guidelines collectively provide the regulatory framework supporting modern analytical development, including ICH Q8(R2), Q9(R1), Q10, Q14, and Q2(R2).

3.2 ICH Q8(R2): Foundation of Quality by Design

ICH Q8(R2) introduced the concept of systematic pharmaceutical development based on scientific understanding and quality risk management. One of its most influential contributions was the concept of design space, defined as the multidimensional combination of material

attributes and process parameters capable of consistently ensuring quality.[34] Although originally intended for pharmaceutical product development, the principles described in ICH Q8 have been widely adopted in analytical sciences. The concept of the Method Operable Design Region (MODR) in AQbD is directly derived from the design-space philosophy described in Q8. The guideline also established the importance of knowledge-driven development and continuous improvement, which remain central to modern analytical method development.[35]

3.3 ICH Q9(R1): Quality Risk Management

ICH Q9(R1) provides a structured framework for identifying, evaluating, and controlling risks that may affect pharmaceutical quality. Within AQbD, risk assessment serves as a critical step for identifying analytical variables that may significantly influence method performance.[36] Tools such as Ishikawa diagrams, Failure Mode and Effects Analysis (FMEA), and risk ranking methodologies are frequently employed to identify Critical Method Parameters (CMPs) and prioritize experimental efforts. The application of risk management principles improves development efficiency and supports scientifically justified decision-making throughout the analytical lifecycle.[37]

3.4 ICH Q10: Pharmaceutical Quality System

ICH Q10 extends the concepts introduced in Q8 and Q9 by establishing a lifecycle-oriented pharmaceutical quality system. The guideline emphasizes continual improvement, change management, corrective and preventive actions (CAPA), and ongoing performance monitoring.[38]

These principles are equally relevant to analytical procedures. Modern analytical methods require continuous evaluation to ensure that performance remains consistent throughout routine use. Trend analysis, system suitability monitoring, and periodic performance reviews are increasingly recognized as essential components of analytical lifecycle management.[39]

3.5 ICH Q14 and ICH Q2(R2): Modern Analytical Development and Validation

The publication of ICH Q14 and the revised ICH Q2(R2) in 2023 marked a significant advancement in regulatory expectations for analytical procedures.[40,41] ICH Q14 formally introduces a structured approach to analytical procedure development based on predefined objectives, scientific understanding, risk assessment, and experimental evidence. Central to the guideline is the Analytical Target Profile (ATP), which defines the intended purpose and

required performance characteristics of an analytical procedure. The guideline also encourages the use of Design of Experiments (DoE), response surface methodologies, and knowledge-based development strategies to improve method understanding and robustness.[40] Complementing Q14, ICH Q2(R2) modernizes analytical validation by integrating validation activities within the broader analytical lifecycle. Rather than viewing validation as a standalone event, the guideline promotes continuous assurance of analytical performance through lifecycle management principles. Traditional validation characteristics, including accuracy, precision, specificity, detection capability, linearity, and robustness, remain essential but are now considered within a more comprehensive quality framework.[41] Together, ICH Q14 and Q2(R2) establish a regulatory foundation that aligns closely with AQbD principles and supports the development of robust, reliable, and fit-for-purpose analytical procedures.

3.6 Impact of Regulatory Guidance on RP-HPLC Method Development

The combined influence of ICH Q8, Q9, Q10, Q14, and Q2(R2) has transformed RP-HPLC method development from an empirical process into a structured scientific activity. Modern chromatographic development increasingly relies on risk assessment, statistical experimentation, and lifecycle management rather than solely on trial-and-error optimization.[42] For complex pharmaceutical products, particularly targeted anticancer therapies, these regulatory expectations are especially important. Analytical methods must reliably support assay determination, impurity profiling, stability assessment, and product quality evaluation while maintaining robustness under routine operating conditions.[43] Consequently, AQbD-based RP-HPLC development has become an important strategy for achieving regulatory compliance, improving method reliability, and facilitating lifecycle management.

3.7 Future Regulatory Perspectives

Regulatory expectations continue to evolve alongside advances in analytical science. Emerging technologies such as artificial intelligence, machine learning, automated experimentation, and digital analytical platforms are expected to influence future method development strategies. Regulatory agencies are increasingly recognizing the value of data-driven decision-making and predictive analytics in supporting pharmaceutical quality systems.[44] As analytical technologies become more sophisticated, future regulatory frameworks will likely place greater emphasis on knowledge management, continuous verification, and integration of digital tools within AQbD-based development programs.

4. AQbD-Based RP-HPLC Method Development Strategies for Targeted Anticancer Drugs

4.1 Role of RP-HPLC in Oncology Drug Analysis

Reverse-phase high-performance liquid chromatography (RP-HPLC) remains one of the most widely applied analytical techniques in pharmaceutical development because of its versatility, sensitivity, reproducibility, and regulatory acceptance. In oncology drug development, RP-HPLC is routinely employed for assay determination, impurity profiling, stability studies, dissolution testing, and quality control of both drug substances and finished dosage forms.[45,46]

The increasing use of targeted anticancer therapies has introduced additional analytical challenges due to the structural complexity of these molecules, the need for low-level impurity detection, and stringent regulatory requirements. Consequently, analytical methods must provide adequate selectivity, sensitivity, and robustness while maintaining suitability for routine laboratory applications.[47]

4.2 Critical Chromatographic Variables Influencing Method Performance

Successful RP-HPLC method development depends on understanding the influence of chromatographic variables on analytical performance. Among these variables, stationary phase selection, mobile phase composition, pH, flow rate, and detection conditions are particularly important because they directly affect retention, selectivity, resolution, and peak symmetry.[48] C18 stationary phases remain the most frequently employed columns for pharmaceutical applications due to their broad applicability and ability to provide adequate retention for moderately to highly lipophilic compounds. However, column selection should always be guided by analyte characteristics and separation objectives rather than routine preference alone.[49]

Mobile phase composition is another critical factor affecting chromatographic behavior. Acetonitrile and methanol continue to be the most commonly used organic modifiers, while phosphate, acetate, formate, and ammonium-based buffers are frequently employed to control analyte ionization and improve reproducibility. Appropriate selection of buffer systems is particularly important for compounds containing ionizable functional groups, as small changes in pH may significantly influence retention and peak shape.[50] Flow rate and column temperature also contribute to chromatographic performance. Although increasing flow rate may reduce analysis time, excessive flow rates can compromise resolution and sensitivity.

Similarly, column temperature influences analyte mass transfer and selectivity and should be optimized as part of a systematic development strategy.[51]

Detection conditions must provide adequate sensitivity and specificity. UV and photodiode array (PDA) detectors remain the most commonly used detectors in pharmaceutical quality control laboratories because of their simplicity and regulatory acceptance. PDA detection additionally enables peak purity assessment and supports stability-indicating method development.[52]

4.3 Application of AQbD Principles in RP-HPLC Development

The adoption of AQbD has significantly improved chromatographic method development by replacing empirical optimization strategies with structured scientific approaches. Development begins with defining an Analytical Target Profile (ATP), followed by identification of Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs) that may influence analytical performance.[53]

Risk assessment tools such as Ishikawa diagrams and Failure Mode and Effects Analysis (FMEA) are commonly employed to identify variables requiring further investigation. Parameters considered high risk are subsequently evaluated through statistically designed experiments, enabling efficient exploration of factor effects and interactions.[54]

Among the various experimental designs used in chromatographic development, Box–Behnken Design (BBD) and Central Composite Design (CCD) are particularly popular because they efficiently evaluate nonlinear relationships while minimizing experimental burden. These designs facilitate optimization of critical variables such as mobile phase composition, pH, flow rate, and temperature.[55,56]

The resulting mathematical models provide a deeper understanding of chromatographic behavior and support the establishment of robust operating conditions.

4.4 Method Operable Design Region and Robustness Optimization

One of the most important outcomes of AQbD-based development is the establishment of a Method Operable Design Region (MODR). Rather than identifying a single optimum condition, MODR defines a multidimensional region within which acceptable analytical performance can consistently be achieved.[57] Operation within an established MODR improves method robustness by reducing sensitivity to routine variability associated with

instrument settings, reagent preparation, and environmental conditions. This approach also facilitates method transfer between laboratories and supports regulatory flexibility during post-approval lifecycle management.[58]

Robustness evaluation is particularly important for oncology products because analytical procedures frequently require accurate quantification of low-level impurities and degradation products. Methods capable of maintaining performance despite small operational variations are less likely to generate atypical or out-of-specification results during routine use.[59]

4.5 AQbD Applications in Targeted Anticancer Drug Analysis

The analytical requirements associated with targeted anticancer therapies make them well suited for AQbD-based development approaches. Many kinase inhibitors and related oncology products contain complex molecular structures with multiple ionizable groups and structurally similar impurities that can complicate chromatographic separation.[60]

AQbD facilitates systematic optimization of chromatographic conditions and enables a more comprehensive understanding of variables affecting assay performance, impurity resolution, and stability-indicating capability. Published studies have demonstrated that AQbD-developed methods generally exhibit improved robustness, reproducibility, and transferability compared with conventionally developed procedures.[61]

These advantages are particularly valuable in regulatory environments where analytical methods are expected to support product development, commercialization, and long-term lifecycle management. RP-HPLC continues to serve as a cornerstone of pharmaceutical analysis because of its reliability, versatility, and regulatory acceptance. The integration of AQbD principles into chromatographic method development has transformed traditional optimization practices by emphasizing scientific understanding, risk assessment, and statistical experimentation. Through the application of Design of Experiments, risk-based decision-making, and Method Operable Design Regions, AQbD enables the development of robust analytical methods capable of supporting the increasingly complex requirements of targeted anticancer therapies.

5. Osimertinib and Targeted Anticancer Therapy: Analytical Challenges and Opportunities

5.1 Clinical Importance of NSCLC and EGFR-Targeted Therapy

Non-small cell lung cancer (NSCLC) remains the most common form of lung cancer and is responsible for a large proportion of cancer-related mortality worldwide. Despite advances in chemotherapy and radiotherapy, long-term survival outcomes were historically limited due to non-specific cytotoxic effects and the development of drug resistance.

The identification of activating mutations in the epidermal growth factor receptor (EGFR) gene significantly changed the treatment landscape of NSCLC. These mutations are frequently observed in adenocarcinoma patients, particularly among non-smokers and Asian populations, and lead to continuous activation of downstream signaling pathways that promote uncontrolled cell proliferation and survival. This discovery enabled the development of EGFR tyrosine kinase inhibitors (TKIs), which selectively block aberrant signaling and represent a major step toward precision oncology (Herbst et al., 2018; Passaro et al., 2021).

5.2 Evolution of EGFR Tyrosine Kinase Inhibitors

EGFR-targeted therapies have evolved across three generations. First-generation inhibitors such as gefitinib and erlotinib demonstrated significant clinical benefit but were limited by the emergence of resistance mutations. Second-generation agents such as afatinib provided broader inhibition but were associated with increased toxicity.

The development of third-generation inhibitors marked a major advancement, with osimertinib emerging as the most clinically successful agent in this class. It was specifically designed to overcome the T790M resistance mutation while maintaining activity against sensitizing EGFR mutations, with improved central nervous system penetration and a more favorable safety profile (Cross et al., 2014; Soria et al., 2018).

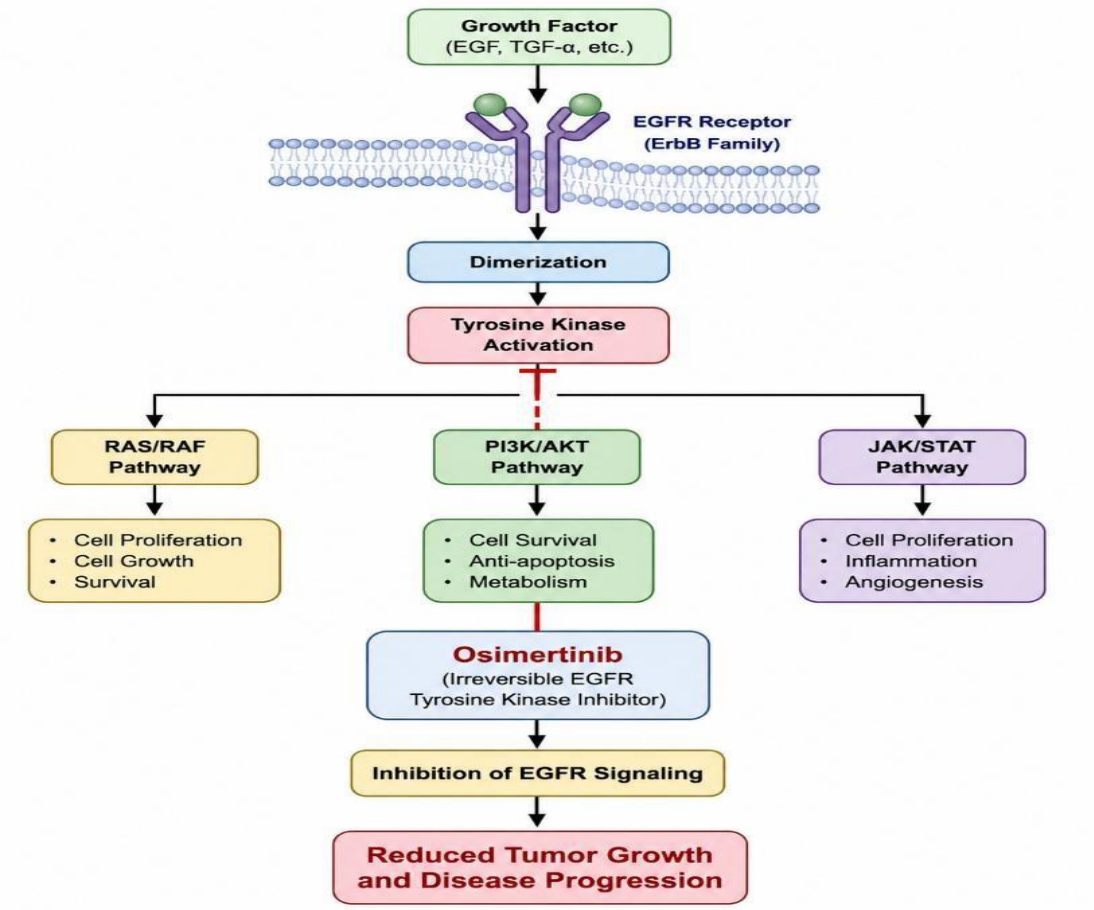

Figure 2: Simplified representation of EGFR-mediated intracellular signaling pathways and the inhibitory mechanism of osimertinib in EGFR-mutated non-small-cell lung cancer.

5.3 Osimertinib: Pharmacological Relevance

Osimertinib is an orally active, irreversible EGFR tyrosine kinase inhibitor that has demonstrated strong clinical efficacy in both first-line and resistant settings of EGFR-mutated NSCLC. The landmark FLAURA trial established osimertinib as a first-line treatment option, showing improved progression-free and overall survival compared with earlier generation EGFR inhibitors (Soria et al., 2018; Ramalingam et al., 2020).

From an analytical perspective, osimertinib presents a complex profile due to its potency, structural characteristics, and presence of multiple related impurities and degradation products that must be controlled during development and manufacturing.

5.4 Physicochemical and Stability Challenges

The analytical behavior of osimertinib is strongly influenced by its physicochemical properties.

The molecule contains multiple aromatic and heterocyclic systems along with ionizable functional groups, which contribute to variable retention behavior in reversed-phase chromatography.

Its lipophilic nature leads to strong interactions with non-polar stationary phases such as C18 columns, making RP-HPLC the method of choice for routine analysis. However, the ionizable groups make chromatographic performance highly sensitive to pH variations, which can affect retention time, peak symmetry, and resolution.

In addition, osimertinib is known to undergo degradation under acidic, basic, oxidative, and photolytic conditions. These degradation pathways necessitate the development of stability-indicating analytical methods capable of separating the parent compound from its degradation products with adequate resolution (Ballard et al., 2016; Leonetti et al., 2019).

5.5 Analytical Challenges in Osimertinib Quantification

The analysis of osimertinib in pharmaceutical dosage forms involves several challenges.

One of the primary challenges is the detection and control of low-level impurities. Many structurally related impurities exhibit similar chromatographic behavior, making their separation difficult without systematic optimization of chromatographic conditions.

Another important requirement is the development of stability-indicating methods. Regulatory expectations require that analytical procedures must reliably distinguish the active pharmaceutical ingredient from degradation products formed during manufacturing, storage, and handling.

In addition, sensitivity requirements are high because osimertinib is administered at low doses, requiring analytical methods capable of accurate quantification at trace levels without compromising precision or robustness. These challenges highlight the need for structured analytical development approaches that go beyond empirical optimization strategies.

5.6 RP-HPLC and AQbD Approaches for Osimertinib

RP-HPLC remains the most widely used technique for osimertinib analysis due to its robustness, accessibility, and regulatory acceptance. Most reported methods utilize C18 columns with aqueous buffer–organic solvent systems and UV or PDA detection.

However, a significant proportion of published methods rely on conventional one-factor-at-a-

time optimization approaches, which may not fully capture interactions between critical chromatographic variables.

The application of Analytical Quality by Design (AQbD) offers a more systematic alternative. AQbD-based development allows identification of Critical Method Parameters (CMPs) such as mobile phase composition, pH, flow rate, and column temperature, followed by their systematic evaluation using Design of Experiments (DoE).

This approach enables the development of predictive models and facilitates the establishment of a Method Operable Design Region (MODR), within which consistent performance can be maintained despite minor variations in experimental conditions (Reid et al., 2013; Swain et al., 2020).

5.7 Current Gaps and Future Opportunities

Despite the availability of several analytical methods for osimertinib, most studies still rely on traditional optimization strategies. The adoption of AQbD principles in osimertinib analysis remains limited, particularly in the context of impurity profiling and long-term lifecycle management.

Another important gap is the limited integration of green analytical chemistry principles. Many reported RP-HPLC methods continue to use high volumes of organic solvents such as acetonitrile and methanol, with limited focus on sustainability or solvent reduction strategies.

Emerging opportunities include the application of artificial intelligence and machine learning for chromatographic optimization, predictive impurity profiling, and automated method development. These approaches have the potential to significantly reduce experimental workload while improving method robustness and efficiency.

Osimertinib represents a clinically important targeted anticancer agent with significant analytical challenges due to its structural complexity, stability concerns, and strict regulatory requirements. While RP-HPLC remains the primary analytical tool, conventional development approaches are increasingly being replaced by AQbD-based strategies.

The integration of risk assessment, experimental design, and lifecycle management provides a more systematic framework for developing robust, stability-indicating, and regulatory-compliant analytical methods. Future advancements are expected to focus on AQbD

implementation, sustainable analytical practices, and the integration of digital technologies in chromatographic method development.

6. DISCUSSION AND CRITICAL ANALYSIS

6.1 Transition from Conventional Methods to AQbD

Analytical method development in pharmaceutical sciences has traditionally relied on empirical optimization, where chromatographic conditions are adjusted one factor at a time. Although this approach is simple and widely practiced, it often provides limited understanding of variable interactions and method behavior under changing conditions.

In contrast, Analytical Quality by Design (AQbD) introduces a structured, science-based framework that emphasizes method understanding, risk assessment, and statistical optimization. Instead of focusing only on meeting validation criteria, AQbD aims to build robustness into the method from the early stages of development.

This transition reflects a broader shift in regulatory science toward lifecycle-oriented quality systems supported by ICH Q8(R2), Q9, Q10, Q14, and Q2(R2), which collectively encourage proactive quality design rather than retrospective quality testing (ICH Q14, 2023; ICH Q2(R2), 2023).

6.2 Strengths and Limitations of AQbD Implementation

The primary strength of AQbD lies in its ability to improve method robustness and reduce variability during routine analysis. By identifying Critical Method Parameters (CMPs) and Critical Quality Attributes (CQAs), AQbD enables systematic optimization through Design of Experiments (DoE), resulting in more predictable chromatographic performance.

Another key advantage is the establishment of a Method Operable Design Region (MODR), which allows controlled flexibility in method parameters without compromising analytical performance. However, AQbD implementation is not without challenges. Many laboratories still face limitations due to lack of statistical expertise, limited access to DoE software, and increased initial development time. These constraints often restrict AQbD adoption to well-equipped industrial or academic research settings.

Despite these challenges, AQbD has demonstrated clear long-term benefits in terms of reduced method failures, improved transferability, and better regulatory compliance (Swain et al., 2020; Reid et al., 2013).

6.3 Analytical Challenges in Targeted Oncology Drugs

Targeted anticancer drugs such as osimertinib present unique analytical challenges due to their structural complexity, low-dose administration, and strict impurity requirements. These compounds often exhibit multiple degradation pathways, making stability-indicating method development essential.

Another major challenge is impurity separation, where structurally similar degradants require highly selective chromatographic conditions. Small variations in pH, mobile phase composition, or column chemistry can significantly impact resolution and peak symmetry. Therefore, conventional trial-and-error approaches are often insufficient for such molecules, and systematic AQbD-based strategies provide a more reliable alternative for ensuring consistent analytical performance (Leonetti et al., 2019; Ballard et al., 2016).

6.4 Osimertinib as a Model Compound for Modern Analytical Development

Osimertinib serves as a representative example of modern targeted therapy requiring advanced analytical control strategies. Its widespread clinical use in EGFR-mutated NSCLC has led to increased demand for robust analytical methods supporting assay, impurity profiling, and stability studies.

Although several RP-HPLC and LC-MS methods have been reported, most rely on conventional optimization approaches. Only a limited number of studies have implemented AQbD principles, highlighting a clear gap in systematic method development for this drug.

The application of AQbD in osimertinib analysis has shown improved robustness and better understanding of critical variables, suggesting that future analytical development should increasingly adopt structured design-based approaches rather than empirical optimization (Soria et al., 2018; Ramalingam et al., 2020).

6.5 Green Analytical Chemistry Perspective

An emerging concern in pharmaceutical analysis is environmental sustainability. Conventional RP-HPLC methods often rely heavily on organic solvents such as acetonitrile and methanol, leading to high solvent consumption and environmental burden.

Green analytical chemistry aims to minimize this impact through reduced solvent usage, shorter run times, and the use of environmentally friendly alternatives. Although this concept is gaining attention, its integration with AQbD frameworks remains limited.

Future analytical development should therefore focus on combining robustness (AQbD) with sustainability (green chemistry) to achieve both regulatory compliance and environmental responsibility.

6.6 Digital Transformation in Analytical Science

The future of pharmaceutical analysis is increasingly influenced by digital technologies such as artificial intelligence (AI), machine learning (ML), and predictive modeling. These tools have the potential to transform chromatographic method development by predicting optimal conditions, reducing experimental workload, and improving method robustness.

Machine learning models can analyze historical chromatographic data to identify relationships between molecular properties and retention behavior, while AI-based systems may eventually enable automated method development and troubleshooting.

Although these technologies are still in early stages of adoption, they represent a significant opportunity for next-generation AQbD implementation and lifecycle management strategies (Desmet & Cabooter, 2010).

6.7 Key Gaps Identified in Literature

Despite rapid progress in analytical science, several important gaps remain:

- Limited AQbD application for newly developed targeted oncology drugs
- Incomplete lifecycle-based method evaluation after validation
- Poor integration of green analytical chemistry principles
- Limited use of AI/ML tools in chromatographic optimization
- Lack of standardized AQbD reporting structure in literature

Addressing these gaps will be essential for improving analytical reliability and aligning with future regulatory expectations.

The evolution from conventional analytical development to AQbD represents a major advancement in pharmaceutical science. For complex targeted therapies such as osimertinib, AQbD offers a more systematic and reliable framework for method development by integrating risk assessment, experimental design, and lifecycle thinking. However, challenges such as statistical complexity, resource requirements, and limited digital integration still restrict widespread adoption. Future progress will likely depend on combining AQbD with green

chemistry principles and digital technologies to achieve more efficient, sustainable, and intelligent analytical systems.

7. Future Perspectives and Emerging Technologies

7.1 Artificial Intelligence-Assisted Method Development

Artificial intelligence is expected to transform analytical science over the next decade.

Current RP-HPLC method development frequently requires extensive experimentation involving multiple variables. AI-based systems may dramatically reduce development time by predicting optimal chromatographic conditions using historical datasets and predictive algorithms.[102]

Potential applications include

- **Mobile phase optimization**
- **Column selection**
- **Retention time prediction**
- **Impurity separation strategies**
- **Automated troubleshooting**

Such technologies could significantly enhance analytical efficiency while reducing development costs. Machine Learning and Predictive Chromatography Machine learning represents one of the most promising developments in pharmaceutical analytics.

By analyzing large chromatographic datasets, machine learning models can identify complex relationships among:

- Molecular descriptors
- Experimental variables
- Chromatographic responses

Future systems may be capable of automatically proposing optimized analytical conditions prior to experimental evaluation.[103]

7.2 Green Chromatography and Sustainable Analytical Practices

Environmental sustainability is expected to become a major focus of analytical development. Future chromatographic systems will likely emphasize:

- Reduced solvent consumption
- Eco-friendly mobile phases

- Shorter analysis times
- Lower energy requirements

UHPLC and microfluidic technologies are expected to play important roles in achieving these objectives.[104]

7.3 Integration of AQbD with Digital Analytics

Future analytical development strategies will likely combine:

- AQbD principles
- Artificial intelligence
- Advanced process analytics
- Real-time monitoring systems

This integration may facilitate continuous analytical optimization and support lifecycle management activities more effectively than current approaches.[105]

7.4 Future Regulatory Evolution

The publication of ICH Q14 and ICH Q2(R2) represents only the beginning of a broader regulatory transformation.

Future guidance documents are likely to address:

- Artificial intelligence validation
- Digital twin technologies
- Real-time release testing
- Advanced lifecycle management
- Predictive analytics

Regulatory agencies are expected to increasingly encourage data-driven analytical development approaches capable of enhancing product quality while reducing development timelines.[76]

7.5 Future Outlook for Osimertinib Analytics

As osimertinib continues to play a central role in NSCLC treatment, analytical methodologies will need to evolve accordingly.

Future research should focus on

- AQbD-driven stability-indicating methods
- Green analytical approaches
- Digital chromatographic optimization

- Predictive impurity profiling
- Lifecycle-based analytical control strategies

These developments will support regulatory compliance while ensuring consistent product quality throughout the commercial lifecycle.[78]

8. CONCLUSION

Analytical Quality by Design (AQbD) has significantly changed the way analytical methods are developed in the pharmaceutical industry. Rather than relying solely on trial-and-error approaches, AQbD encourages a deeper scientific understanding of analytical procedures through risk assessment, systematic experimentation, and continuous monitoring. This structured methodology helps ensure that analytical methods remain reliable, consistent, and capable of meeting evolving regulatory expectations throughout their lifecycle.

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) continues to be one of the most important analytical techniques used in pharmaceutical research and quality control. Its versatility, accuracy, and ability to analyze a wide variety of pharmaceutical compounds have made it a preferred choice for routine and advanced analytical applications. When AQbD principles are incorporated into RP-HPLC method development, analysts gain a better understanding of the factors that influence method performance, allowing the development of more robust and dependable analytical procedures.

The growing use of targeted anticancer drugs such as Osimertinib has introduced new analytical challenges. The complex nature of these molecules, along with strict requirements for impurity profiling and stability studies, demands highly efficient and well-optimized analytical methods. Although several RP-HPLC methods have been reported for the analysis of such compounds, there is still considerable potential to strengthen method development through the broader application of AQbD concepts, environmentally friendly analytical practices, and emerging digital technologies.

Recent revisions to international regulatory guidelines, including ICH Q14 and ICH Q2(R2), further emphasize the importance of science-based analytical development. These guidelines encourage a greater focus on method understanding, risk management, and continuous performance evaluation, helping to ensure that analytical procedures remain suitable for their intended purpose throughout their use.

Looking to the future, advances in artificial intelligence, machine learning, and predictive modeling are expected to play an increasingly important role in analytical science. These technologies have the potential to streamline method development, improve decision-making, and enhance overall analytical efficiency. As digital tools become more integrated into pharmaceutical research, they are likely to contribute to the development of smarter and more adaptive analytical systems.

In summary, the combination of AQbD principles with RP-HPLC methodology represents a progressive and scientifically sound approach to pharmaceutical analysis. By integrating regulatory expectations, technological advancements, and a thorough understanding of analytical processes, this strategy can support the development of high-quality methods that contribute to drug quality, patient safety, and the successful development of modern therapeutic products.

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