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Review Paper

Analytical Quality by Design (AQbD) in HPLC Method Development: A Comprehensive Review of Principles, Tools, Regulatory Framework, and Emerging Applications

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ABSTRACT

Over the past two decades, pharmaceutical analytical science has undergone a quiet but consequential transformation in how methods are conceived, developed, and sustained across their operational lifetimes. The emergence of Analytical Quality by Design (AQbD) as a governing philosophy — rather than a procedural formality or a regulatory concession — marks a meaningful turning point in analytical thinking. Borrowed from manufacturing and formulation sciences where Quality by Design first took root, AQbD insists that measurement reliability and method robustness are not outcomes to be confirmed after the fact, but properties to be deliberately engineered from the very beginning of method development.The foundation of AQbD rests on a deceptively straightforward question: what must this method actually accomplish, and under what real-world conditions must it do so dependably? Answering that question rigorously gives rise to the Analytical Target Profile (ATP) — a performance specification that precedes any experimental work. From there, structured tools including Failure Mode and Effects Analysis (FMEA) and Ishikawa cause-and-effect diagrams guide the analyst toward the variables that genuinely drive method behavior. These Critical Method Parameters (CMPs) are then studied through Design of Experiments (DoE), producing a statistically grounded understanding of method performance across a defined space rather than at a single, potentially fragile operating point.High Performance Liquid Chromatography (HPLC) is a natural home for AQbD. It is both the dominant workhorse of pharmaceutical analysis and a technique characterized by a rich, interacting parameter space where mobile phase composition, pH, flow rate, column temperature, and stationary phase chemistry all influence outcomes in ways no one-factor-at-a-time study can fully capture. By establishing a Method Operable Design Region (MODR) — the multidimensional envelope within which the method

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consistently meets its performance requirements — AQbD transforms robustness from a post-development checkbox into a core design output.Regulatory backing for this approach has strengthened markedly. The ICH guidelines Q8(R2), Q9, Q10, Q14, and the revised Q2(R2) collectively signal an expectation that analytical development should be science-driven, risk-informed, and lifecycle-oriented. ICH Q14 in particular formalizes AQbD thinking, encouraging the definition of an Analytical Procedure Design Space and the management of methods as living assets rather than frozen procedures. This review traces the full arc of AQbD in HPLC method development — from its conceptual origins and philosophical contrast with traditional approaches, through its regulatory underpinnings, methodological components, documented applications, and into the emerging technologies reshaping its future. It is offered both as a scholarly synthesis for researchers already working in this space and as an accessible orientation for pharmaceutical analysts encountering AQbD for the first time.

INTRODUCTION

Few ideas in pharmaceutical science have generated as much sustained debate — and occasional bewilderment — as Quality by Design. Formalized in ICH Q8, Q9, and Q10, the QbD philosophy argues that product quality cannot simply be tested into existence at the end of a process. Instead, it must be systematically engineered in from the outset, built on a thorough and documented understanding of how raw materials, process variables, and environmental conditions interact to shape outcomes [1,2,3,4]. That argument, once controversial, is now broadly accepted in manufacturing and formulation sciences.

Analytical methods occupy an unusual position within this quality picture. They are simultaneously instruments for characterizing and controlling pharmaceutical products, and themselves objects of quality concern. A method that is inaccurate, imprecise, or prone to failure under routine laboratory conditions is not simply a technical nuisance — it is a direct threat to sound quality decisions and, by extension, to patient safety. Yet for much of pharmaceutical history, analytical methods were developed largely through accumulated experience, intuitive judgment, and sequential trial-and-error. Methods that passed validation were considered good methods, regardless of how little their developers understood about why they passed or how close they sat to a boundary of failure [5,10].

The one-factor-at-a-time (OFAT) paradigm, which defined analytical development for decades, illustrates the problem well. The approach is appealing in its simplicity: vary one parameter, observe the effect, move to the next. But real analytical systems are not independent aggregates of isolated variables. They are interconnected systems in which parameters interact — sometimes subtly, sometimes dramatically. An OFAT study cannot detect these interactions, and a method optimized through OFAT may rest on conditions that are optimal only because their developer never discovered that the apparently ideal pH depends critically on the organic solvent fraction, or that temperature and flow rate combine in ways that affect peak symmetry in ways neither factor reveals alone [25,26].

Analytical Quality by Design emerged as a direct response to these limitations. By bringing the systematic, risk-based, multivariate logic of QbD into the analytical laboratory, AQbD replaces reactive empiricism with prospective planning grounded in scientific understanding. Pioneered in the early 2000s by academic and industrial scientists and increasingly embraced by regulatory agencies, AQbD has matured into a well-supported framework with a coherent methodology and a growing body of demonstrated applications [5,8,10]. The publication of ICH Q14 in 2022 — explicitly formalizing AQbD elements including ATP definition, risk assessment, DoE-based optimization, and design space establishment — marks perhaps its clearest regulatory endorsement to date [5].

HPLC is the natural domain for AQbD. Used across the full spectrum of pharmaceutical analytical tasks — assay determination, impurity profiling, stability-indicating studies, dissolution testing, and bioanalytical quantification — HPLC combines analytical power with methodological complexity. Mobile phase composition, pH, buffer concentration, flow rate, column temperature, column chemistry, gradient profile, and detector settings all interact in ways that reward systematic investigation. This complexity makes HPLC methods vulnerable to variability when poorly understood, but it also means that a well-executed AQbD study produces unusually rich knowledge about method behavior [99,100].

This review is organized to follow the natural sequence of an AQbD-based development project. Section 2 contrasts traditional OFAT development with AQbD philosophically and practically. Section 3 surveys the regulatory framework supporting AQbD. Section 4 provides a detailed treatment of AQbD's key components. Section 5 reviews documented applications across pharmaceutical analytical contexts. Section 6 weighs the genuine advantages of AQbD against its real limitations. Section 7 considers emerging technological integrations. Section 8 concludes with a synthesis.

2. TRADITIONAL METHOD DEVELOPMENT VERSUS ANALYTICAL QUALITY BY DESIGN

The difference between traditional OFAT-based analytical development and AQbD is not simply a matter of technique selection or experimental efficiency. It reflects a deeper conceptual divergence in what method development is for and what it should produce. Table 1 captures the most significant contrasts across several dimensions.

Table 1. Comparative Summary of Traditional OFAT and AQbD Approaches in HPLC Method Development

Dimension	Traditional / OFAT Approach	AQbD Approach
Starting Point	Prior experience and literature	Analytical Target Profile (ATP)
Experimental Strategy	Sequential, one variable at a time	Multivariate Design of Experiments
Risk Management	Informal, experience-based	Formal: FMEA, Ishikawa, risk ranking
Interaction Effects	Not studied	Systematically captured and modeled
Robustness Testing	Post-development, during validation	Built in during development via MODR
Design Space	Not established	Defined statistically; regulatory flexibility
Documentation	Validation report focused	ATP, risk reports, DoE models, MODR, lifecycle plan
Regulatory Flexibility	Low; any change requires submission	High within approved design space
Lifecycle Approach	Limited post-approval monitoring	Continuous monitoring and verification

Dimension	Traditional / OFAT Approach	AQbD Approach
Knowledge Management	Implicit, expert-dependent	Explicit, documented, transferable

2.1 Philosophical Orientation

Traditional analytical development is organized around a single practical objective: producing a method that passes its validation criteria. Once that objective is achieved, the method is considered complete, regardless of whether its developer possesses any deep understanding of how it works or how close it sits to a performance boundary. AQbD, by contrast, is built around understanding as a primary outcome. The goal is not merely a method that performs well at one set of conditions, but a method whose behavior across a defined range of conditions can be predicted with statistical confidence and defended with data.

That philosophical difference carries practical weight. A method optimized by OFAT may sit very close to a region of poor performance without its developer knowing it. Small disturbances — a slightly different column lot from a new supplier, a minor shift in ambient temperature, a batch of buffer with marginally different purity — may push the method across an invisible boundary into failure. The AQbD developer, having mapped the performance landscape through DoE and established a MODR, knows not just the optimal conditions but the extent of the safe operating region around them. That knowledge supports confident method transfer, reliable troubleshooting, and rational lifecycle management [11,12,13].

2.2 Experimental Strategy: OFAT versus Multivariate Designs

In conventional OFAT experimentation, the analyst begins with a reference condition drawn from literature or accumulated experience, varies one parameter at a time across a feasible range, and observes the effect on one or more chromatographic responses. The process may consume dozens or hundreds of experiments, yet the information it produces is structurally limited: main effects are estimated, but interaction effects — what happens when two or more variables change simultaneously — remain invisible.

Design of Experiments replaces this sequential approach with a structured multivariate design in which all selected factors vary simultaneously according to a statistically chosen pattern. A well-constructed design with fifteen experimental runs may generate more useful information — including interaction terms and curvature effects — than fifty OFAT experiments. Statistical analysis of the DoE results produces mathematical models that describe the relationship between operating conditions and analytical responses, enabling optimization through navigation of the response surface rather than point-by-point searching [25,26,27,28].

For HPLC method development, the practical implications are considerable. A Plackett-Burman screening design can efficiently identify, from among ten or more candidate variables, the three or four that genuinely govern chromatographic performance. A Central Composite or Box-Behnken Design can then characterize those critical parameters in detail, yielding a response surface model that predicts resolution, tailing factor, and retention time as continuous functions of experimental conditions. That model becomes the scientific basis for design space definition and MODR establishment [29,67].

2.3 Risk Management Formality

Traditional development incorporates risk management informally — experienced chromatographers know which variables tend to cause problems and manage those risks through intuition and accumulated judgment. AQbD makes

the same process explicit and structured. Tools such as FMEA and Ishikawa diagrams impose a discipline on risk identification that captures potential failure modes more comprehensively than individual recollection allows. The Risk Priority Number (RPN) framework of FMEA provides a quantitative basis for directing experimental effort toward the variables most likely to cause significant problems [31,32,30].

This formality also produces documentation — a recorded account of what was considered, what was found, and what decisions were made — that proves valuable during regulatory submissions, method transfer activities, and future troubleshooting. In a traditional project, much of this knowledge lives only in laboratory notebooks and in the memories of scientists who may move on. In an AQbD project, it is preserved in structured, retrievable form.

2.4 Robustness: Tested versus Built In

The contrast between traditional and AQbD approaches to robustness is perhaps the sharpest practical distinction between the two paradigms. In traditional development, robustness is assessed during the validation phase by deliberately introducing small perturbations to method parameters and confirming that performance remains acceptable. When a method fails this test, re-optimization must occur at a late and expensive stage of the development cycle.

In AQbD, robustness is not confirmed after the fact — it is established during development as a direct consequence of design space characterization. By identifying the MODR, the analyst demonstrates statistically that the method performs acceptably across a scientifically characterized region of parameter space, not merely at one validated point. That is a meaningfully stronger and more honest assurance of robustness [10,11,38].

2.5 Regulatory Implications

The regulatory implications of design space establishment represent one of the most compelling arguments for AQbD adoption in industrial pharmaceutical development. Under traditional development, the submitted method is defined by a fixed set of operating conditions. Any subsequent adjustment — even a minor modification to accommodate a change in column supply or instrument configuration — constitutes a post-approval change requiring regulatory notification or formal variation submission. This burden creates an incentive to avoid technically rational improvements.

Under AQbD, changes that remain within an approved design space generally do not require regulatory submission. The reviewing authority has already evaluated the evidence demonstrating that any point within the design space delivers acceptable method performance. This flexibility is both a practical efficiency gain and a genuine incentive to invest in the upfront science of design space establishment [2,3,4,5].

3. REGULATORY FRAMEWORK SUPPORTING AQBD IN ANALYTICAL METHOD DEVELOPMENT

AQbD does not exist in a regulatory vacuum. Its adoption has been propelled, at least in part, by a coherent and mutually reinforcing body of ICH guidance that collectively defines an expectation for systematic, science-based, and lifecycle-oriented analytical development. Understanding this regulatory context is essential for implementing AQbD in a way that satisfies external requirements while also delivering genuine scientific value.

3.1 ICH Q8(R2) — Pharmaceutical Development

ICH Q8 introduced Quality by Design into pharmaceutical regulatory guidance and defined

the design space as the multidimensional combination and interaction of input variables and process parameters that provide assurance of quality. Although primarily focused on formulation and manufacturing, its conceptual architecture — understanding factor interactions, establishing a design space, demonstrating that quality is built in rather than tested in — translates directly into analytical development [2]. The MODR concept in AQbD is the analytical counterpart of the design space as originally framed in ICH Q8.

3.2 ICH Q9 — Quality Risk Management

ICH Q9 provides the methodological foundation for the risk assessment component of AQbD. It articulates a framework for risk identification, analysis, evaluation, reduction, and review, and it endorses specific tools — FMEA, fault tree analysis, hazard analysis — as appropriate instruments for managing quality risks in pharmaceutical contexts [3]. In AQbD practice, FMEA and Ishikawa diagrams are the most widely applied of these, converting qualitative understanding of failure modes into a prioritized list of critical parameters for experimental attention.

3.3 ICH Q10 — Pharmaceutical Quality System

ICH Q10 describes the broader quality management system within which AQbD-developed methods must operate. Its emphasis on lifecycle management, change control, continual improvement, and knowledge management supports the AQbD philosophy of treating methods as assets requiring ongoing attention [4]. The principle that knowledge generated during development should remain accessible and actionable throughout the product lifecycle applies as directly to analytical methods as it does to manufacturing processes.

3.4 ICH Q14 — Analytical Procedure Development

ICH Q14, finalized in 2022, is the most directly relevant guideline for AQbD practitioners. It explicitly applies systematic development principles to analytical procedures, encouraging ATP definition, risk assessment, DoE-based optimization, and the establishment of an Analytical Procedure Design Space [5]. ICH Q14 distinguishes between enhanced approaches — encompassing these AQbD elements — and minimal approaches that simply follow existing practice, providing incentives for enhanced approaches through increased regulatory flexibility.

Importantly, Q14 also introduces the Analytical Procedure Control Strategy: a planned set of controls derived from development knowledge that assures ongoing method performance. This strategy may incorporate system suitability tests, statistical process control monitoring, and periodic lifecycle reviews — all elements of the continuous management philosophy that AQbD embodies.

3.5 ICH Q2(R2) — Validation of Analytical Procedures

The revised ICH Q2(R2), issued concurrently with ICH Q14, updates the longstanding validation framework to accommodate the AQbD lifecycle approach. Where the original Q2(R1) treated validation as a discrete endpoint activity, Q2(R2) explicitly recognizes that validation data can accumulate continuously throughout development and that the boundary between development, optimization, and validation is not sharp in an AQbD context [41]. Robustness receives particular prominence in Q2(R2) as a characteristic most meaningfully established through DoE and design space activities rather than through limited perturbation experiments at a single operating point.

3.6 USP <1220> — Analytical Procedure Lifecycle

The USP chapter <1220> on Analytical Procedure Lifecycle provides a complementary framework aligned with ICH Q14 and Q2(R2). It describes three lifecycle stages: procedure design and development (Stage 1), procedure performance qualification (Stage 2), and continued procedure performance verification (Stage 3). This maps naturally onto AQbD: Stage 1 encompasses ATP definition, risk assessment, DoE, and design space establishment; Stage 2 covers formal validation; Stage 3 describes ongoing monitoring and lifecycle management [6].

3.7 US FDA Perspectives

The FDA has consistently advocated for QbD approaches in pharmaceutical development since its Pharmaceutical cGMPs for the 21st Century initiative in 2004 [7]. The Process Analytical Technology (PAT) framework, issued the same year, extended this philosophy to real-time process monitoring [53]. More recently, the FDA has aligned with ICH Q14 in encouraging AQbD-based analytical development. Submissions incorporating design space justifications and statistical modeling generally receive favorable regulatory scrutiny because they demonstrate a depth of scientific understanding that traditional submissions rarely achieve.

4. KEY ELEMENTS OF ANALYTICAL QUALITY BY DESIGN

4.1 Analytical Target Profile (ATP)

The Analytical Target Profile is, in essence, the founding document of any AQbD project. It is written before any experiment is run, and it defines the method's purpose in performance terms rather than procedural ones. The ATP does not specify a C18 column with a particular mobile phase; it specifies what the method must accomplish — the analyte to be measured, the matrix, the required accuracy and precision, the working concentration range, and the minimum acceptable limit of quantitation [10,11,5].

This performance-first framing has real methodological consequences. By articulating requirements before selecting methodology, the analyst preserves the freedom to explore multiple chromatographic approaches and evaluate them against consistent criteria. More importantly, the ATP defines the very basis for design space establishment: the design space is precisely the region of parameter space within which the method meets the ATP requirements. Without an ATP, there is no coherent foundation for defining or defending a design space.

A well-constructed ATP typically specifies analyte identity, sample matrix characteristics, required measurement range, acceptable bias limits, repeatability and intermediate precision requirements, specificity requirements including known interferences, and any special requirements tied to the method's intended application [5,6,44]. It should be developed collaboratively by analytical chemists, formulation scientists, regulatory affairs specialists, and — where relevant — clinical and pharmacokinetic teams, since the intended use of the data shapes the performance requirements.

4.2 Critical Analytical Attributes (CAAs) and Critical Method Parameters (CMPs)

With the ATP established, the next step is to identify which measurable method properties are most directly tied to meeting those requirements (Critical Analytical Attributes, or CAAs) and which experimental variables most powerfully influence those properties (Critical Method Parameters, or CMPs).

In HPLC method development, CAAs typically include chromatographic resolution between critical peak pairs, peak tailing factor, theoretical plate count, retention time reproducibility, signal-

to-noise ratio at the LOQ level, and peak purity. Each can be measured experimentally and assessed against acceptance criteria derived from the ATP.

CMPs are the variables the analyst controls that have been identified through risk assessment as significantly affecting one or more CAAs. Typical HPLC candidates include mobile phase pH, organic solvent percentage (and gradient profile in gradient methods), buffer type and concentration, column temperature, flow rate, column dimensions, particle size, and stationary phase chemistry. The goal of AQbD development is to characterize how each CMP — individually and in combination — affects the CAAs, and to use that characterization to establish a MODR where all CAAs simultaneously satisfy their acceptance criteria [10,11,5].

Distinguishing critical from non-critical parameters matters practically because it focuses experimental resources where they matter most. With ten adjustable parameters, a full factorial DoE would require 1,024 experiments. By first identifying three or four truly critical parameters through risk-based screening, the subsequent optimization study can be conducted in fifteen to twenty runs — capturing the essential parameter space without exhausting available resources.

4.3 Risk Assessment Methodology

Risk assessment in AQbD bridges the gap between qualitative scientific knowledge and the structured, quantitative logic of experimental design. It converts the question of what might go wrong into a prioritized list of variables worth studying systematically [30,31,32].

4.3.1 Ishikawa (Fishbone) Diagrams

The Ishikawa diagram, named for quality management pioneer Kaoru Ishikawa, organizes potential causes of method variability into a structured visual form. In a typical HPLC application, the effect of interest — poor chromatographic resolution, for example — is placed at the head of the diagram, and branches represent major cause categories: the method itself, the instrument, materials (reagents, columns, standards), the environment, and the analyst. Sub-branches identify specific variables within each category.

The value of the Ishikawa diagram lies partly in the process of constructing it. A team working through it together will identify factors that might not occur to any individual working alone. The completed diagram provides a starting inventory of potential sources of variability for the more quantitative analysis that follows through FMEA [32].

4.3.2 Failure Mode and Effects Analysis (FMEA)

FMEA takes the potential failure modes identified through tools like the Ishikawa diagram and subjects them to systematic quantitative evaluation. For each failure mode, three scores are assigned [31]:

Severity (S): A score from 1 to 10 reflecting the seriousness of the consequence if this failure occurs. A failure that could produce a false negative impurity result — potentially releasing a harmful product — warrants a high severity score. A failure affecting only the aesthetic quality of a chromatogram warrants a low one.

Occurrence (O): A score from 1 to 10 reflecting the likelihood of the failure under realistic operating conditions. A tightly instrument-controlled parameter such as UV wavelength receives a low occurrence score; a parameter subject to inter-laboratory variability such as mobile phase pH may receive a higher one.

Detectability (D): A score from 1 to 10 reflecting the ease of detection before the failure affects the final result. A failure immediately flagged by system suitability receives a low detectability

score; one that affects assay accuracy without triggering any alert receives a high score.

The Risk Priority Number (RPN) is the product of these three scores: RPN = S × O × D. Variables with high RPNs are designated as CMPs and targeted for DoE characterization; low-RPN variables may be held constant, conserving experimental resources for the factors that truly matter [30].

It is worth acknowledging that FMEA scores are inherently subjective and dependent on the knowledge of the team conducting the assessment. Different teams may reach different CMP designations for the same method. This limitation is mitigated by ensuring diverse team composition, anchoring scores to explicit criteria, and documenting the rationale for each assignment.

4.4 Design of Experiments in HPLC Method Development

Design of Experiments is the analytical engine of AQbD. It provides the structured, statistically interpretable framework for characterizing the relationship between CMPs and CAAs, and the resulting models form the scientific basis for design space establishment, optimization, and robustness prediction.

4.4.1 Screening Designs

When the initial candidate CMP list is long — as is typical early in HPLC development — screening designs efficiently separate the variables that matter from those that do not. Plackett-Burman designs are the most widely used tools for this purpose. A 12-run Plackett-Burman design can accommodate up to eleven factors, enabling a comprehensive initial survey of HPLC parameters at modest experimental cost [28]. The limitation is that Plackett-Burman designs do not estimate interaction effects, but this is generally acceptable at the screening stage where the goal is simply to reduce the candidate list.

Fractional factorial designs offer a useful middle ground — more informative than Plackett-Burman but more efficient than full factorials — providing estimates of main effects and many two-factor interactions for four to five factors in sixteen runs [25].

4.4.2 Response Surface Designs

Once the critical few CMPs have been identified, response surface designs model the full relationship between these variables and the key responses across the experimental region of interest. Unlike factorial designs that use only two or three factor levels and fit only linear models, response surface designs incorporate axial and center points that allow quadratic effects to be captured.

The Central Composite Design (CCD) is the most widely applied response surface design in HPLC AQbD. Built on a factorial core supplemented with axial and center point replicates, it enables fitting of a full quadratic model with main effects, two-factor interactions, and curvature terms. The CCD is particularly valuable when responses such as chromatographic resolution are expected to show a maximum within the feasible parameter range [27,51].

The Box-Behnken Design offers an alternative that avoids extreme combinations of factor levels — useful when the axial points of a CCD would fall outside practical operating ranges, for example when very high column temperatures combined with very low flow rates might damage the column [27,51].

Mixture designs represent a specialized category relevant to mobile phase optimization, where components must sum to a fixed total and classical factorial approaches are inapplicable. Simplex-centroid and simplex-lattice designs accommodate this constraint and allow systematic exploration of the mobile phase composition space [29].

4.4.3 Statistical Analysis and Response Surface Modeling

DoE data are analyzed by regression to fit mathematical models relating factor settings to each response. For a two-factor quadratic model: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2$, where Y is the response, X_1 and X_2 are coded factor values, and the β coefficients represent the intercept, main effects, interaction, and quadratic terms. Coefficient significance is assessed by ANOVA and t-tests; non-significant terms are removed through stepwise elimination to yield a parsimonious model [50,52].

Model quality is evaluated through several diagnostics: the coefficient of determination (R^2) measures explained variance; the adjusted R^2 penalizes unnecessary terms; the lack-of-fit test evaluates structural adequacy; and residual plots assess whether model assumptions are satisfied. A validated response surface model can predict responses at untested conditions within the experimental region with quantified uncertainty [50,51,52].

Contour plots are the primary visualization tool: overlaying acceptance boundaries for each response on a two-factor plot, the MODR is identified as the region where all criteria are simultaneously satisfied. In multi-response systems, the MODR emerges from the intersection of all individually acceptable regions — most efficiently identified using desirability function optimization or Monte Carlo simulation [27,51,56].

Table 2. Commonly Used DoE Designs in AQbD-Based HPLC Method Development

Design Type	Primary Purpose	Factors	Key Feature	Typical Application
Plackett-Burman	Screening	Up to 11 in 12 runs	Highly efficient; no interactions	Initial CMP identification
Fractional Factorial	Screening/Prelim. Opt.	4–7	Main effects + some interactions	Narrowing variable set
Full Factorial	Comprehensive screening	2–4	All main effects and interactions	Small factor sets
Central Composite Design	Optimization	2–5	Quadratic model; curvature	Response surface modeling
Box-Behnken Design	Optimization	3–5	No extreme combinations	When axial points impractical
Mixture Design	Composition optimization	2–4 components	Component sum constrained	Mobile phase optimization
D-Optimal Design	Custom constraints	Any	Flexible; handles constraints	Irregular experimental regions

4.5 Design Space and Method Operable Design Region (MODR)

The design space — and its practical expression as the MODR — is the culminating product of the DoE and modeling work. It represents both the scientific knowledge achieved through development and the regulatory basis for operational flexibility. The concept deserves careful attention because it is sometimes misunderstood in ways that undermine both its scientific value and its regulatory benefits.

Formally adapted for analytical methods under ICH Q14, the Analytical Procedure Design Space is the multidimensional combination of method parameters within which the analytical procedure, when operated, is predicted to meet ATP requirements with a defined level of statistical confidence [2,5,38]. The word "predicted" is significant: the design space is not a region tested exhaustively at every point, but a region inferred from a model validated against experimental data. Statistical confidence reflects the uncertainty in model predictions; regions closest to the experimental center, where predictions are most reliable, are often designated as the operating range.

The MODR is a practical refinement of the design space: the subset that is best suited for routine operation, considering not only modeled performance but also practical constraints such as instrument capability, reagent stability, and analyst convenience. It is typically centered on optimal conditions and defined with enough margin to accommodate normal operating variation — column-to-column differences, between-batch mobile phase preparation variability, within-day temperature fluctuations — without pushing the method toward the performance boundary [56].

Defending a design space in a regulatory submission requires demonstrating that the underlying DoE data, the fitted model, and the model-derived predictions are of sufficient quality to support the claimed boundaries. This typically means demonstrating adequate model fit by ANOVA and lack-of-fit testing, verifying predictions with confirmation experiments at selected design space points, and reporting prediction intervals rather than point estimates to convey the uncertainty around the claimed boundaries [38,56].

4.6 Method Validation in an AQbD Context

Validation in an AQbD framework is conceptually different from traditional validation, though the characteristics to be demonstrated — specificity, linearity, accuracy, precision, robustness, LOD, LOQ — are largely the same as those required by ICH Q2(R2). The difference lies in how these characteristics are established and in their relationship to the development process that precedes them.

4.6.1 Specificity

Specificity is evaluated through forced degradation studies integrated into AQbD development for stability-indicating methods. Stress conditions — acidic, alkaline, oxidative, thermal, photolytic — are applied to the drug substance or product, and the resulting samples confirm that all degradation products are resolved from the parent compound. Peak purity testing using diode array detection or mass spectrometric confirmation adds further evidence. In a well-designed AQbD project, specificity is not merely demonstrated retrospectively; it is built into the method through deliberate optimization of conditions to maximize resolution between the analyte and all known interferences [5,6].

4.6.2 Linearity and Range

Linearity is assessed across the range specified in the ATP. Statistical analysis of the calibration data — regression coefficient, slope, intercept, residuals, and formal linearity tests — confirms whether a linear relationship adequately describes the system over the specified range. The ATP-driven framing matters here: the range is defined by intended use, not maximized arbitrarily, and the linearity demonstration is confined accordingly [41,6].

4.6.3 Accuracy and Precision

Accuracy (recovery or bias) and precision (%RSD) are demonstrated by analyzing spiked samples or reference standards at multiple

concentration levels within the reportable range. The ATP acceptance criteria provide the statistical benchmarks against which results are evaluated. Operating within the MODR provides assurance that accuracy and precision demonstrated during validation will be maintained under routine conditions [41,5].

4.6.4 Robustness as an AQbD Output

Robustness receives special treatment in AQbD. In traditional validation, small deliberate perturbations are introduced one at a time and performance is confirmed to remain acceptable — a limited approach that captures neither interaction effects nor the full shape of the performance landscape. In AQbD, robustness is established during development as a consequence of design space characterization. Demonstrating that the MODR has meaningful extent — that it is a region, not a point — is equivalent to demonstrating robustness, and it is a considerably stronger form of evidence [11,39,5].

4.6.5 Lifecycle Perspective on Validation

Both ICH Q2(R2) and USP <1220> emphasize that validation data accumulate naturally throughout the development and optimization process rather than in a single discrete post-development study. Experiments conducted during DoE that yield accuracy and precision data at multiple points across the design space contribute to the validation dataset. Confirmation experiments that verify design space predictions serve simultaneously as validation replicates. This integration reduces the total experimental burden compared to treating validation as a completely separate activity [41,6,44].

5. APPLICATIONS OF AQBD IN HPLC METHOD DEVELOPMENT

AQbD has been applied across a diverse and expanding range of pharmaceutical analytical contexts. The sections below survey the major application areas and reference published examples that illustrate what AQbD delivers in each. Table 3 summarizes selected literature examples.

5.1 Stability-Indicating HPLC Methods

Stability-indicating methods are arguably where AQbD delivers its most compelling benefits. These methods must achieve adequate chromatographic separation of the API from all expected degradation products, maintain quantitative accuracy and precision across the concentration range required for stability study samples, and remain reliable throughout a product's shelf life — which may span five years or more. The consequences of a non-robust stability-indicating method are serious: missed degradation, incorrect purity assignments, or invalid stability data can affect shelf-life determination and, ultimately, patient safety.

Gurrala and colleagues developed an AQbD-based RP-HPLC stability-indicating method for metformin hydrochloride, using FMEA to identify mobile phase pH, organic solvent percentage, and column temperature as CMPs, and a Box-Behnken Design to map the response surface [35]. The resulting MODR accommodated normal laboratory variability while assuring resolution greater than 2.0 between metformin and its stress degradation products. Rao and colleagues similarly demonstrated AQbD for a stability-indicating assay, concluding that DoE-based design space characterization delivered superior robustness evidence compared to traditional Youden testing [61].

The typical AQbD sequence for stability-indicating methods proceeds as: forced degradation to identify relevant degradation products; Ishikawa and FMEA-based risk assessment to identify CMPs; screening DoE to confirm which parameters most affect resolution; response surface DoE to optimize and characterize

those parameters; and design space establishment with confirmation experiments. This sequence efficiently produces a method with documented, statistically validated robustness — an asset of particular value for methods that must perform reliably over multi-year stability programs.

5.2 Impurity Profiling Methods

Impurity profiling presents some of the most demanding analytical challenges in pharmaceutical quality control. ICH guidelines Q3A, Q3B, and Q3C impose strict limits on impurity levels — often requiring detection and quantification at concentrations of 0.05% or below relative to the API. Methods must be highly sensitive and selective, capable of resolving multiple structurally similar impurities from one another and from the parent compound.

AQbD is well-suited to impurity profiling because the challenge is inherently multivariate. Small changes in pH, gradient slope, or column temperature can dramatically shift selectivity for specific impurity pairs, and a method optimized by OFAT at one set of conditions may be fragile under routine variation. AQbD systematically maps the selectivity landscape, identifies the conditions where all required separations are achieved simultaneously, and establishes a MODR that assures those separations are maintained [60].

Patel and colleagues described an AQbD approach to impurity profiling of an oral solid dosage form, employing a Central Composite Design to optimize gradient HPLC conditions for simultaneous resolution of six process-related impurities from the API [60]. The response surface models accurately predicted separation performance across the design space, and the established MODR accommodated inter-batch pH variability without compromising impurity resolution. The regulatory flexibility enabled by the design space was cited as a significant practical advantage for method transfer to multiple manufacturing sites.

5.3 Assay Determination

Quantitative assay methods for API content in drug substances and products are the most common analytical procedures in pharmaceutical quality control, and they represent a natural starting point for organizations building AQbD capability. While generally simpler than stability-indicating or impurity profiling methods, assay methods still benefit meaningfully from AQbD in terms of robustness assurance and regulatory flexibility.

Kokilambigai and Lakshmi developed an AQbD-based RP-HPLC assay for atorvastatin, applying FMEA to identify mobile phase pH and organic solvent percentage as CMPs and a CCD to model their effects on retention time, tailing factor, and theoretical plate count [34]. The resulting design space covered a range of conditions within which all three responses met their acceptance criteria. The design space was substantially larger than the traditional robustness range that would have been defined by OFAT, reflecting the greater method understanding achieved through multivariate experimentation.

Azhakesan and colleagues applied AQbD to develop an HPLC assay and stability-indicating method for canagliflozin, identifying column temperature and mobile phase pH as dominant CMPs through a Plackett-Burman design followed by a CCD, and establishing a MODR within which the method consistently met resolution, accuracy, and precision requirements [33].

5.4 Dissolution Testing Methods

Dissolution testing is a critical quality attribute for oral solid dosage forms, and the HPLC methods used to quantify drug release in dissolution samples must be reliable across the range of samples generated in routine testing. AQbD has

been applied to both the dissolution procedure itself and the analytical method used to assay dissolution samples.

Dissolution media can be complex — particularly biorelevant media designed to simulate gastrointestinal conditions — and may contain surfactants, fatty acids, and other components that cause retention time shifts, peak distortion, or baseline interference. AQbD addresses these challenges by incorporating dissolution medium characteristics as factors in the DoE, enabling the analyst to characterize the effect of medium composition on chromatographic performance and to establish a MODR robust to the variability inherent in medium preparation.

5.5 Bioanalytical HPLC Methods

Bioanalytical methods — those quantifying drugs, metabolites, and biomarkers in biological matrices such as plasma, urine, and tissue — represent the most technically demanding application of AQbD. Biological matrices contain thousands of endogenous components that can interfere with detection, cause matrix effects in ionization, or compete with the analyte during sample preparation.

Singh and colleagues reported an AQbD-based approach to LC-MS/MS bioanalytical method development for a pharmaceutical compound in human plasma [59]. Using Ishikawa diagrams and FMEA to identify CMPs including mobile phase pH, organic modifier percentage, column temperature, and protein precipitation conditions, and a CCD to characterize their effects on analyte response, matrix factor, and recovery, they established a MODR within which all bioanalytical acceptance criteria were met. The documented design space also supported successful method transfer to a contract research organization.

5.6 AQbD in Herbal Drug and Phytochemical Analysis

HPLC analysis of herbal drugs and phytochemical preparations presents distinctive challenges arising from the chemical complexity of plant-derived matrices. Herbal medicines may contain hundreds of active and inactive constituents, many structurally similar, and qualitative and quantitative composition can vary with geographic origin, growing conditions, harvest timing, and processing. AQbD has been applied to develop robust standardization methods for herbal preparations, with the design space assuring performance across matrix variability [62,75,78].

Mukherjee and colleagues applied AQbD to HPLC standardization of an Ayurvedic polyherbal formulation, using DoE to optimize resolution among multiple marker compounds and establishing a MODR robust to the matrix variability characteristic of herbal preparations [62]. The systematic identification of CMPs through FMEA and CCD was particularly valuable given the absence of prior literature for the specific formulation under study. Published AQbD-based methods for Picroside II quantification [75] and cold and cough formulation analysis [76] similarly illustrate the value of AQbD's systematic approach in complex, variable-matrix analytical contexts.

Table 3. Selected Literature Examples of AQbD-Based HPLC Method Development

Reference	Drug / Analyte	Method Type	DoE Design	Key CMPs	Outcome
Azhakesan et al. [33]	Canagliflozin	Stability-indicating assay	PB + CCD	pH, column temp.	MODR established; ICH criteria met

Reference	Drug / Analyte	Method Type	DoE Design	Key CMPs	Outcome
Kokilambigai & Lakshmi [34]	Atorvastatin	RP-HPLC assay	CCD	pH, organic solvent %	Design space with regulatory flexibility
Gurrala et al. [35]	Metformin HCl	Stability-indicating	Box-Behnken	pH, ACN%, column temp.	Resolution >2.0 assured across MODR
Patel et al. [60]	Oral solid form	Impurity profiling	CCD	Gradient, pH, temp.	Six impurities resolved; MODR defined
Singh et al. [59]	Pharma compound	LC-MS/MS bioanalytical	CCD	pH, organic modifier, temp.	Bioanalytical criteria met; transfer supported
Marie et al. [77]	Metformin/Linagliptin/Empagliflozin	Simultaneous estimation	DoE-based	pH, flow, solvent ratio	Design space in RP-HPLC established
Kotadiya [73]	Multiple compounds	UHPLC review	Various	Multiple	Systematic review of innovations 2014–2025
Chawla & Ambekar [71]	General AQbD/ICH Q14	Review and DoE synthesis	N/A	N/A	Synergistic paradigm for quality and efficiency

6. ADVANTAGES, LIMITATIONS, AND CHALLENGES OF AQBD IMPLEMENTATION

6.1 Advantages

6.1.1 Superior Method Understanding

The most fundamental advantage of AQbD is the depth of understanding it generates. A DoE-based development project produces mathematical models predicting method performance across a defined parameter space — a qualitatively richer description of method behavior than any OFAT study can provide. This understanding supports confident method transfer, troubleshooting, and adaptation. When a method fails system suitability in a new laboratory, an analyst with design space knowledge can immediately assess whether the observed conditions fall outside the MODR and identify which adjustments would restore performance. Without that knowledge, the same analyst must rely on empirical troubleshooting — more time-consuming and less reliable [11,12,13].

6.1.2 Regulatory Flexibility

The ability to operate anywhere within an approved design space without regulatory notification provides significant practical advantages for methods maintained over the long commercial lifecycle of a pharmaceutical product. Column supply changes, solvent lot variations, instrument upgrades, and transfers to contract laboratories all create potential needs for method adjustment. Under AQbD, adjustments within the approved design space can be implemented immediately, without filing a variation — saving time and supporting continuous improvement [2,3,4,5].

6.1.3 Reduced Method Failure

Methods developed with explicit MODR definition fail less frequently under routine operation than methods developed by OFAT. The MODR, by construction, encompasses the normal operating variability of the laboratory, providing assurance that acceptable performance is maintained despite minor fluctuations in real-world conditions. Out-of-specification results attributable to method failure rather than genuine product non-conformance impose significant investigational costs and can affect batch disposition [11,13].

6.1.4 Lifecycle Management

AQbD documentation — ATP, risk assessment records, DoE reports, design space justification — provides a structured knowledge base supporting the full lifecycle of the analytical method. As the product matures and operational experience accumulates, continuous verification data confirm that the method remains in statistical control and alert the analyst to any drift requiring corrective action [5,6,44,46].

6.2 Limitations and Challenges

6.2.1 Resource Requirements

AQbD development demands more upfront investment in time, personnel, and analytical resources than OFAT development. The risk assessment requires structured team engagement; the DoE requires careful planning, execution across more experiments, and statistical analysis requiring appropriate software and expertise. For smaller organizations or projects with constrained timelines, these upfront costs can be a real barrier even when long-term benefits are well understood [10,11].

6.2.2 Statistical Expertise

Effective AQbD requires statistical knowledge that may not be widely available in analytical laboratories. DoE design, response surface modeling, model diagnostics, and design space estimation all require expertise beyond the standard training of a pharmaceutical analyst. Organizations adopting AQbD must either develop this expertise internally or establish partnerships with statisticians — a worthwhile investment, but a real hurdle for organizations beginning their AQbD journey [67].

6.2.3 Model Limitations

Response surface models are empirical approximations. They accurately describe the factor-response relationship within the experimental region but cannot reliably predict performance at conditions outside it, and their predictions carry uncertainty that must be explicitly acknowledged in design space justification. A design space claimed without adequate confirmation experiments, or extending beyond the region of reliable model prediction, may fail to deliver the assurance it promises [50,51,52].

6.2.4 Regulatory Maturity

While regulatory support for AQbD is clear and growing, not all jurisdictions have equally mature frameworks for reviewing AQbD submissions.

Reviewers unfamiliar with design space concepts or response surface methodology may require additional information or may apply requirements developed for traditional submissions in ways poorly suited to AQbD approaches. The increasing alignment around ICH Q14 and Q2(R2) is improving this situation, but organizations submitting AQbD-based dossiers should anticipate reviewer queries about methodology [5,41].

7. FUTURE PERSPECTIVES: EMERGING TECHNOLOGIES AND THE EVOLVING AQBD LANDSCAPE

AQbD continues to evolve. Since its early articulations in the mid-2000s, several important technological and regulatory developments have reshaped its trajectory, and the next decade promises further transformation.

7.1 Artificial Intelligence and Machine Learning in AQbD

The integration of artificial intelligence and machine learning with AQbD is perhaps the most transformative near-term development. Classical DoE-based AQbD requires the experimental design to be fully specified in advance. AI and ML approaches challenge that assumption through adaptive experimentation: algorithms analyze data as they accumulate and propose the next most informative experiment, reaching an optimized design space in fewer runs when the response surface is complex or the most informative parameter region is not known in advance.

Bayesian optimization is attracting particular attention. Rather than following a fixed experimental plan, it builds a probabilistic model of the response surface and selects experiments that maximize expected improvement in a desirability objective [9]. Neural network models offer a complementary capability: capturing highly nonlinear factor-response relationships that quadratic polynomial models cannot represent — important for complex gradient HPLC methods with four or more CMPs, or for methods where resolution depends sensitively on a narrow pH range.

Practical adoption of AI/ML in AQbD is still emerging, and important questions remain about model validation, communication of AI-based design space justifications to regulators, and compliance with pharmaceutical quality system documentation standards. These questions are being actively addressed by regulatory science groups, and relevant guidance is expected over the next several years.

7.2 Process Analytical Technology Integration

Process Analytical Technology (PAT) involves online or at-line measurement for real-time process monitoring and control. Integration of AQbD with PAT represents a natural extension of both frameworks: AQbD provides systematic assurance of analytical measurement reliability; PAT provides the real-time monitoring capability to deploy that measurement in manufacturing contexts [53].

In an integrated AQbD-PAT system, online HPLC or spectroscopic methods — developed and validated under AQbD principles — provide continuous monitoring of critical quality attributes such as API content and impurity levels. The design space assures that normal process variability will not compromise measurement reliability. Statistical process control applied to the continuous data stream detects early signs of method or process drift, enabling corrective action before non-conforming product is generated. This integration supports the real-time release testing paradigm that regulators are increasingly pursuing.

7.3 Digital Chromatography Modeling and Simulation

Digital chromatography modeling uses computational simulation to predict HPLC retention and separation behavior from physicochemical properties of analytes and column characteristics, without requiring all experiments to be performed physically. Commercially available simulation software has been validated for selected applications, particularly reversed-phase HPLC on well-characterized column chemistries.

Integration of digital modeling with AQbD can substantially reduce experimental burden. Rather than conducting a full bench DoE, the analyst can first simulate the response surface in silico to identify the most promising parameter region, then confirm predictions with a focused set of experimental verification runs. This hybrid approach combines AQbD's systematic rigor with the efficiency of computational prediction, potentially reducing development timelines for well-characterized compound-column combinations [9].

7.4 Continuous Analytical Verification

Traditional validation treats method performance as something demonstrated once and thereafter assumed stable. Continuous analytical verification, promoted by both USP <1220> and ICH Q2(R2), replaces this static view with an ongoing monitoring framework in which statistical process control techniques are applied to system suitability and performance indicator data accumulated during routine use [6,41].

In a continuous verification framework, each system suitability test provides a data point for control charts tracking resolution, tailing factor, or theoretical plate count. Trends suggesting column degradation, mobile phase pH drift, or other systematic changes are detected before they cause method failure. Design space knowledge informs control limits: a trend pushing toward the MODR boundary triggers corrective action; a trend well within the MODR is informative but not immediately concerning. Periodic lifecycle reviews — structured assessments of accumulated performance data — complete the cycle [44,46].

7.5 Green Analytical Chemistry and Sustainability

Green analytical chemistry (GAC) seeks to minimize the environmental impact of analytical methods by reducing solvent consumption, eliminating hazardous reagents, and lowering energy use. AQbD provides a natural framework for integrating GAC objectives into method development, because the DoE-based approach can incorporate sustainability metrics — such as NEMI or AGREE scores — as additional optimization responses alongside traditional chromatographic performance criteria.

An AQbD project simultaneously optimizing chromatographic performance and environmental impact can identify conditions that achieve acceptable separation with reduced organic solvent consumption, lower buffer concentrations, or more sustainable solvent choices. The design space framework ensures that sustainability optimization does not compromise analytical reliability — the MODR is defined to satisfy both performance and sustainability criteria together.

7.6 Harmonized Global Regulatory Adoption

Global harmonization of AQbD-related regulatory guidance through the ICH Q14 and Q2(R2) process is expected to accelerate adoption across all major pharmaceutical markets. As regulatory authorities in the US, EU, Japan, China, and other jurisdictions align their expectations, the incentive for global companies to implement AQbD as their standard development approach strengthens considerably. A single AQbD dossier prepared under ICH Q14 principles should be acceptable in

all ICH member jurisdictions — a significant efficiency advantage over current situations where different markets may hold different expectations [5,8].

CONCLUSION

Analytical Quality by Design represents a genuinely significant advance in pharmaceutical analytical science — not a superficial procedural update, but a fundamental shift in how method development is conceived, executed, documented, and managed across the analytical lifecycle. By insisting that quality must be engineered into analytical methods from the outset, guided by predefined performance requirements in the ATP, systematic risk assessment, and multivariate DoE, AQbD produces a depth of method understanding that traditional OFAT development cannot match.

In HPLC method development specifically, where the interaction of multiple chromatographic parameters creates a rich and complex performance landscape, AQbD is particularly valuable. The ability to map that landscape through response surface modeling, to identify the MODR within which the method performs reliably, and to communicate that understanding to regulatory authorities in the form of a scientifically justified design space — these capabilities transform method development from an art into a genuine engineering discipline. Methods developed through AQbD fail less often, transfer more reliably, adapt more flexibly to post-approval changes, and generate richer knowledge for troubleshooting and continuous improvement.

The regulatory environment now supports AQbD strongly. With the publication of ICH Q14 and the revised ICH Q2(R2), regulatory expectations have been formalized in ways that reflect AQbD principles. USP <1220> provides a complementary lifecycle management structure integrating development, validation, and ongoing monitoring into a single coherent system. Together, these frameworks create both opportunity and expectation that analytical development in pharmaceutical science will increasingly be conducted under AQbD principles.

The future of AQbD is being shaped by artificial intelligence and machine learning, digital chromatography modeling, PAT integration, and continuous analytical verification — developments promising to make AQbD faster, more predictive, and more tightly coupled with manufacturing quality systems. Green analytical chemistry is also finding a natural home within the AQbD framework, enabling method development that simultaneously optimizes analytical performance and environmental sustainability.

For pharmaceutical scientists and analysts approaching AQbD for the first time, the learning curve is real but manageable. DoE, response surface modeling, and design space estimation are well-established techniques with extensive tutorial literature and commercial software support. FMEA and Ishikawa diagrams are straightforward tools that reward structured team engagement. The regulatory framework, centered on ICH Q14 and Q2(R2), provides clear and practical guidance on what is expected.

AQbD is not a niche technique or a passing regulatory fashion. It is a durable paradigm for pharmaceutical analytical method development that delivers real scientific and operational value, aligns with modern regulatory expectations, and is positioned to strengthen further as emerging technologies extend its reach. Organizations that invest in AQbD capability today are equipping themselves for the analytical challenges of tomorrow — and building a foundation of method knowledge and reliability that will serve their products, their patients, and their regulators for the full commercial lifecycle of every medicine they analyze.

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