

ANALYTICAL QUALITY BY DESIGN (AQBD): CURRENT ADVANCES AND FUTURE PERSPECTIVES**Kowsalya Devi M., Karthiraja A. S.*, Dr. Saravanan V. S., Logesh P.**

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ABSTRACT

Analytical methods play a vital role in pharmaceutical development, quality control, stability testing, and regulatory decision-making, as the reliability of drug product identity, strength, purity, safety, and stability depends on the quality of analytical data generated. Conventional analytical method development has traditionally followed trial-and-error and one-factor-at-a-time approaches, which can require extensive experimentation and may fail to adequately evaluate interactions among method variables. Analytical Quality by Design (AQbD) has emerged as a systematic, science-based, and risk-based approach for developing analytical procedures that are robust, reliable, and fit for their intended purpose. The AQbD framework generally begins with defining an Analytical Target Profile (ATP), followed by identification of critical analytical attributes and performance characteristics, risk assessment, identification of critical method parameters, application of Design of Experiments (DoE), establishment of a Method Operable Design Region (MODR), and development of an appropriate analytical control strategy. The recently finalized ICH Q14 guideline provides a harmonized framework for analytical procedure development and lifecycle management, while ICH Q2(R2) establishes an updated framework for analytical procedure validation. Together, these guidelines emphasize a science- and risk-based approach extending beyond validation as a standalone activity. Recent advances in AQbD include integration with multivariate analysis, chemometrics, automation, artificial intelligence, machine learning, green analytical chemistry, and real-time analytical technologies. This review discusses the fundamental concepts, workflow, statistical tools, applications, regulatory significance, advantages, challenges, and future perspectives of AQbD in pharmaceutical analysis. The integration of AQbD with digital and sustainable technologies is expected to enhance method robustness, reduce experimental burden, and support continuous analytical procedure improvement throughout the analytical lifecycle.

KEYWORDS: Analytical Quality by Design; AQbD; Analytical Target Profile; Design of Experiments; Risk Assessment; Critical Method Parameters; Method Operable Design Region; ICH Q14; ICH Q2(R2); Analytical Method Development.

1. INTRODUCTION

Analytical procedures are essential components of pharmaceutical development and manufacturing because they generate the information used to establish the identity, strength, quality, purity, safety and stability of pharmaceutical products.^[1,2,3] Every decision made across the product lifecycle from early formulation screening through stability studies, batch release and

post-approval change management is only as trustworthy as the analytical procedure that produced the underlying data. The reliability of analytical data therefore directly influences product release, stability assessment, process understanding and regulatory decision-making, and any weakness in the analytical method itself propagates forward into every decision that depends on it.^[3,4]

Historically, analytical method development has frequently followed a one-factor-at-a-time (OFAT) or trial-and-error strategy, in which a chromatographer or analyst varies a single parameter for example mobile-phase pH while holding all others constant, observes the effect on a chosen response, fixes that parameter at its apparently optimal value, and then moves on to the next variable in sequence. Although such approaches can and do produce acceptable analytical methods, particularly for relatively simple matrices, they may require numerous experiments to converge on an acceptable condition and generally provide limited information about interactions between analytical parameters. Because two or more variables frequently influence a response jointly rather than independently, an OFAT-optimized condition can appear satisfactory during development yet still sit close to an edge of failure that only becomes apparent once the method is transferred or subjected to the ordinary day-to-day variability of a working laboratory.^[5,6] Consequently, a method optimized under a particular, narrowly explored set of conditions may demonstrate inadequate robustness when small, unavoidable changes occur during routine laboratory operation, such as a slightly different column lot, a marginal shift in mobile-phase pH during preparation, or a modest change in ambient column-oven temperature.^[7]

Analytical Quality by Design (AQbD) applies the broader Quality by Design (QbD) philosophy first articulated for pharmaceutical manufacturing processes to analytical procedure development. It emphasizes predefined analytical objectives, scientific understanding of the relationship between method variables and method performance, structured risk management, and systematic, statistically designed experimentation in place of ad hoc trial and error. Rather than treating an analytical procedure as a fixed recipe that is validated once and then frozen, AQbD aims to develop analytical procedures that are not merely validated but are designed to be inherently robust and suitable for their intended purpose across the range of conditions the method is likely to encounter over its working life.^[8,9]

The AQbD concept has gained considerable importance following the finalization of ICH Q14, "Analytical Procedure Development," together with ICH Q2(R2), "Validation of Analytical Procedures." These two guidelines were developed and issued together, reflecting the regulatory recognition that development and validation of an analytical procedure are not independent activities but two connected stages of a single analytical lifecycle. Together, they provide harmonized principles for science- and risk-based analytical procedure development and validation, and they give pharmaceutical organizations a common regulatory vocabulary.^[1,2,3] Analytical Target Profile, critical method parameters, method operable design region, established conditions, analytical control strategy for

describing and justifying how an analytical procedure was developed and how it is expected to behave.

This review is intended to consolidate, in a single narrative, the conceptual foundations of AQbD, the statistical and risk-management tools it draws upon, its application across the major analytical platforms used in pharmaceutical laboratories, its regulatory basis under ICH Q14 and Q2(R2), and the directions in which the framework appears to be evolving as artificial intelligence, automation and sustainability considerations become more prominent in analytical laboratories.^[10,11,12]

2. Evolution from Conventional Method Development to AQbD

Conventional analytical method development generally involves selecting an analytical technique on the basis of the analyte's physicochemical properties, conducting preliminary range-finding experiments, optimizing individual parameters sequentially, and subsequently validating the developed procedure against a fixed set of validation characteristics defined by guidance documents. This sequence has been used successfully for decades and remains adequate for many straightforward analytical problems, but it carries a number of structural limitations that become more consequential as analytes, matrices and regulatory expectations become more complex.^[6,13]

The major limitations of the conventional approach include a dependence on trial-and-error experimentation that can be time and resource-intensive; a limited understanding of interactions between method variables, since OFAT experimentation by construction cannot detect an interaction between two factors; an increased number of experimental runs relative to a properly designed experiment covering the same variable space; difficulty in defining a scientifically justified acceptable operating range around the chosen conditions, as opposed to a single point that happened to work; a limited ability to predict method performance after even minor changes, because the underlying variable relationships were never mapped; a tendency toward reactive troubleshooting during routine analysis, in which failures are investigated and patched after the fact rather than anticipated; and potential difficulty in managing post-approval analytical changes, since without a mapped design space every change to a validated method risks being treated as a new method requiring full revalidation.^[5,7]

AQbD provides a more systematic alternative by integrating prior product and process knowledge, structured risk assessment, statistical experimental design, and lifecycle management into a single continuous activity that begins before the first experiment is run and continues through routine use of the method.^[3,14] A simplified comparison between the two approaches is presented in Table 1.^[15]

Table 1: Comparison of conventional analytical method development with the AQbD approach.

Conventional Approach	AQbD Approach
Trial-and-error	Science- and risk-based
One-factor-at-a-time experiments	Design of Experiments (DoE)
Limited parameter interaction assessment	Interaction effects evaluated
Method optimized at a single condition	Method operable region established
Reactive troubleshooting	Proactive risk reduction
Validation mainly after development	Development and validation are interconnected
Limited lifecycle perspective	Lifecycle management approach

3. Fundamental Principles of AQbD

AQbD is best understood not as a single technique but as a sequence of interlinked activities, each of which builds on the output of the one before it.^[16] The major principles of AQbD include, in approximate order of execution: definition of the Analytical Target Profile (ATP); identification of the analytical performance requirements implied by that ATP; identification of candidate critical method variables on the basis of prior knowledge and mechanistic understanding; structured risk assessment to prioritize which variables warrant experimental investigation; selection of an appropriate analytical technology capable of meeting the ATP; Design of Experiments to explore the prioritized variables and their interactions; method optimization using the resulting statistical models; establishment of a Method Operable Design Region (MODR) within which the method is expected to perform acceptably; development of an analytical control strategy that translates the design region into practical operating instructions and system suitability criteria; method validation against ICH Q2(R2); and, finally, lifecycle management and continual improvement once the method is in routine use.^[1,8,17]

The overall philosophy underlying this sequence is to establish quality during method development rather than attempting to demonstrate quality only after development has been completed. In this sense AQbD mirrors, for analytical procedures, the same shift in philosophy that Quality by Design brought to pharmaceutical manufacturing processes roughly two decades earlier^[18,19]: quality is built into the procedure by design, understood through the design space, and maintained through an explicit control strategy, rather than being tested into the procedure through a battery of validation experiments performed after the method has already been fixed.^[9,20]

4. Analytical Target Profile (ATP)

The Analytical Target Profile is considered the starting point of an AQbD-based analytical procedure development strategy and plays a role broadly analogous to the Quality Target Product Profile (QTPP) in process QbD. Where the QTPP defines what the finished drug product must achieve for the patient, the ATP defines what the analytical procedure is required to measure and the level of performance expected for its intended purpose, independent of any particular analytical technique.^[1,21]

The ATP can include the intended measurement (for example, quantitation of an active pharmaceutical ingredient, determination of a specified or unspecified degradation product, or assessment of dissolution behavior), the analyte or quality attribute of interest, the sample matrix, and the required analytical performance characteristics expressed as measurable targets rather than as a description of a specific method.

Typical ATP considerations include the intended purpose of the analytical procedure; the identity of the analyte or quality attribute being measured; the required measurement or reportable range; the required accuracy, expressed for example as percentage recovery within a defined interval; the required precision, expressed as an acceptable relative standard deviation; the required specificity or selectivity in the presence of expected interferences such as excipients or degradation products; the required detection or quantitation capability where trace-level measurement is relevant; and the general suitability of candidate analytical technologies for meeting these requirements. ICH Q14 describes the ATP as an important, though not obligatory, element of analytical procedure development and lifecycle management, and notes that a well-constructed ATP allows the analyst to evaluate alternative analytical technologies against a common, technology-independent standard before committing to a specific procedure.^[1,22]

As an illustrative example, the ATP for an assay method of an active pharmaceutical ingredient in a finished dosage form might be stated as: "The analytical procedure should quantitatively determine the drug substance in the pharmaceutical product over the specified reportable range with suitable accuracy, precision, specificity and robustness, and should remain fit for purpose across the range of manufacturing sites, instruments and analysts expected to use the method." The ATP therefore acts as a bridge between the underlying analytical requirement — what must be known about the product and why — and the final analytical procedure that will be used, in practice, to generate that knowledge. Because the ATP is defined independently of technique, it also provides a documented, defensible basis for later comparing an alternative analytical technology to the originally validated procedure, which becomes particularly useful when a laboratory wishes to introduce a newer platform, such as UHPLC in place of conventional HPLC, without

repeating the entire development exercise from first principles.^[21,22]

5. Critical Analytical Attributes and Performance Characteristics

The analytical procedure should be capable of producing reliable results for its intended purpose, and the specific performance characteristics that matter will depend on the nature of the analytical application. Depending on the analytical application, important performance characteristics may include accuracy, precision, specificity or selectivity, the reportable range over which the method is expected to be valid, the analytical response and its relationship to concentration, the detection limit, the quantitation limit, and robustness to small, deliberate variations in method parameters.^[4]

ICH Q2(R2) describes these performance characteristics in detail and provides a harmonized framework for evaluating analytical procedure performance during validation, extending and updating the earlier ICH Q2(R1) framework to explicitly cover a wider range of analytical technologies, including multivariate and spectroscopic procedures that were not well addressed by the original guideline.^[2]

In AQbD, these characteristics should be considered from the earliest stages of development rather than being treated exclusively as post-development validation requirements to be demonstrated once the method is already fixed. Framing accuracy, precision, specificity and robustness as design targets from the outset — rather than as hurdles to be cleared at the end changes the practical conduct of development work: instead of optimizing a method against a single response such as resolution and only later checking whether it happens to be robust, the AQbD analyst deliberately designs experiments that probe robustness alongside separation performance, so that the two are optimized together rather than sequentially.^[23]

6. Risk Assessment in AQbD

Risk assessment is used to identify, from among the many analytical variables that could in principle be varied, the smaller subset that may have a significant influence on method performance and therefore warrant experimental investigation. Because the number of candidate variables in a typical chromatographic or spectroscopic method is large, risk assessment functions as a filtering step that keeps the subsequent Design of Experiments manageable in size while still ensuring that scientifically important variables are not overlooked.^[7,24]

6.1 Ishikawa or fishbone diagrams are commonly used as a first, qualitative step in risk assessment. Potential sources of analytical variability are brainstormed by the development team and categorized under headings such as method, instrument, material, analyst, environment, and sample preparation, producing a visual map of where variability could plausibly enter the measurement

process.^[24] This step is deliberately inclusive at first, capturing a broad list of candidate variables before any attempt is made to prioritize among them.

6.2 Failure Mode and Effects Analysis (FMEA) can then be used to move from a qualitative list of candidate variables to a semi-quantitative prioritization. FMEA identifies potential failure modes associated with each candidate variable and prioritizes them based on three factors: severity, meaning how serious the consequence would be if the failure occurred; occurrence, meaning how likely the failure is to occur given current controls; and detectability, meaning how likely the failure is to be detected before it affects the result. A Risk Priority Number (RPN) is calculated as the product of these three factors, $RPN = \text{Severity} \times \text{Occurrence} \times \text{Detectability}$, and variables associated with the highest RPN values are treated as the highest priority for experimental investigation, since they combine a high likelihood of impact with a low likelihood of being caught by existing controls.^[7,24]

6.3 Risk ranking and filtering provides a complementary, often simpler approach in which variables are classified qualitatively into low-, medium- and high-risk categories on the basis of team judgment and prior knowledge, without necessarily calculating a numerical RPN for every candidate. High-risk parameters identified by either FMEA or risk ranking and filtering can then be investigated experimentally using Design of Experiments, while low-risk parameters are typically fixed at a convenient operating value and are not carried forward into the formal experimental program, though they may still be documented in the method's control strategy.^[7]

Used together, these tools allow the development team to move efficiently from an unmanageably large list of theoretically relevant variables to a focused, scientifically justified set of critical method parameters^[25], and to document the reasoning behind that narrowing in a form that can be reviewed by quality assurance staff and regulatory assessors.^[7,24]

7. Critical Method Parameters (CMPs)

Critical Method Parameters are analytical procedure parameters that, on the basis of risk assessment and prior knowledge, can significantly influence analytical performance and are therefore selected for detailed experimental study.

For an RP-HPLC method, potential parameters include mobile-phase composition, mobile-phase pH, flow rate, column temperature, injection volume, detection wavelength, gradient conditions, sample concentration, and sample preparation conditions such as extraction time or sonication duration.^[26] For example, mobile-phase composition and pH may significantly influence retention time, resolution and peak shape by altering the ionization state of an analyte or by changing its

interaction with the stationary phase, while flow rate and column temperature more typically influence efficiency, back-pressure and analysis time without necessarily altering selectivity to the same degree.^[26,27]

The purpose of AQbD is not necessarily to classify every method variable as critical; on the contrary, a well-conducted risk assessment is expected to eliminate the majority of candidate variables from further consideration, leaving only those that scientific understanding and preliminary experimentation suggest are genuinely capable of moving the method outside its acceptable performance envelope. The goal is to scientifically identify the smaller number of parameters that require close experimental characterization and, ultimately, tight control, rather than to treat every conceivable variable as equally important. This distinction matters practically, because it keeps the subsequent DoE program to a size that is experimentally and financially feasible while still directing effort toward the variables that matter most.^[6,26]

8. Design of Experiments (DoE)

Design of Experiments is one of the most important statistical tools in AQbD and is the mechanism by which critical method parameters, once identified through risk assessment, are experimentally characterized. Unlike

one-factor-at-a-time experimentation, DoE allows simultaneous evaluation of multiple factors and their interactions within a single, statistically planned set of experiments, so that the influence of each factor, and of combinations of factors, can be estimated from a comparatively small number of runs.^[28,29,30]

A number of DoE approaches are used at different stages of AQbD, summarized in Table 2. Factorial and fractional factorial designs, and Plackett–Burman designs in particular, are typically used early, during screening, when the goal is to identify which of a larger number of candidate factors have a statistically significant effect on the chosen responses, without yet attempting to model the response surface in detail. Once the significant factors have been narrowed down, response-surface designs such as Central Composite Design or Box–Behnken Design are used to model the relationship between the significant factors and the response with enough resolution to locate an optimum and to characterize curvature in the response surface. Mixture designs are used specifically where the factors being studied are proportions of a mixture, such as the components of a mobile phase, that are constrained to sum to a fixed total, a situation that ordinary factorial designs do not handle correctly.^[28,29]

Table 2: Common Design of Experiments (DoE) approaches used in AQbD.

DoE Type	Application
Factorial design	Screening and interaction studies
Fractional factorial design	Screening large numbers of variables
Plackett–Burman design	Identification of significant factors
Central Composite Design	Response surface optimization
Box–Behnken Design	Optimization of multiple factors
Mixture design	Optimization of mobile-phase composition

The typical experimental workflow proceeds from risk assessment, to factor screening using a screening design, to a more detailed DoE using a response-surface design, to statistical modelling of the resulting data, to response surface analysis, and finally to optimization of the method parameters against the fitted model. Important analytical responses evaluated during this process may include resolution between critical peak pairs, retention time, tailing factor, theoretical plate count, peak area or response, recovery, precision, and selectivity, depending on which of these are most relevant to the ATP for the method in question.^[23,31]

The statistical models generated during DoE — typically polynomial equations relating the response to the studied factors, including linear, interaction and quadratic terms as appropriate — are evaluated for goodness of fit using measures such as the coefficient of determination (R^2), the adjusted R^2 , and analysis of variance (ANOVA), with lack-of-fit tests used to confirm that the chosen model adequately describes the experimental data before it is used for optimization or for defining a design space.^[28,31]

9. Method Operable Design Region (MODR)

One of the major advantages of AQbD, relative to conventional method development, is the possibility of establishing a Method Operable Design Region rather than a single fixed set of operating conditions. The MODR represents a multidimensional region within which analytical procedure parameters can be varied, in combination, while maintaining predefined analytical performance requirements, in direct analogy to the design space concept used in process QbD.^[1,9]

For example, a MODR for a chromatographic assay might be expressed as a flow rate of 0.8 to 1.2 mL/min, an organic phase proportion of 55 to 65 percent, and a column temperature of 25 to 35 degrees Celsius, such that any combination of values drawn from within these ranges, considered jointly rather than one at a time, is expected to satisfy the method's predefined performance criteria for resolution, tailing and analysis time. This is a materially stronger and more useful statement than the equivalent conventional robustness claim, which typically only demonstrates that the method tolerates

small deviations from a single nominal set point taken one parameter at a time.^[23]

If the combination of parameters actually used during routine analysis remains within the scientifically established design region and satisfies the predefined performance criteria, the method is expected to maintain acceptable performance, and a change made within the boundaries of the MODR does not, in principle, require the same regulatory scrutiny as a change that falls outside it, since the change has already been scientifically justified as part of the original development work. The concept of parameter ranges and robustness, and their relationship to the design region, is specifically addressed in ICH Q14, which also introduces the related concept of "established conditions" the subset of the analytical procedure's parameters and their approved ranges that are formally reported to and approved by regulatory authorities, as distinct from other procedural details that a laboratory may adjust under its own change-control system without prior regulatory notification.^[1,32]

10. Analytical Control Strategy

An analytical control strategy is developed to ensure that the analytical procedure remains under appropriate control during routine use, translating the scientific understanding generated during development into practical instructions and acceptance criteria that laboratory analysts can apply without needing to repeat the underlying development work.

It may include the identified critical method parameters and their acceptable ranges as defined by the MODR; system suitability requirements, such as minimum resolution, tailing factor or theoretical plate count, that must be demonstrated before sample analysis proceeds; requirements for reference standards, including their source, purity and any system-suitability role they play; controls on sample preparation, such as extraction time, solvent choice or filtration; requirements for instrument qualification and, where relevant, column or equipment-specific requirements; ongoing monitoring requirements intended to detect drift in method performance over time; and change-control procedures that specify how proposed changes to the method are to be evaluated, and whether they fall inside or outside the established MODR.^[1,3]

The control strategy is, in effect, the operational memory of the development program: it connects the method development knowledge captured during risk assessment, DoE and MODR establishment with routine laboratory operation, so that an analyst running the method years later, and possibly at a different site, has access to the same understanding of which parameters matter and why, without needing to reconstruct that understanding from first principles.^[3]

11. AQbD and Analytical Method Validation

AQbD and analytical validation are complementary rather than independent activities, and one of the more significant conceptual contributions of ICH Q14 and Q2(R2), issued as a linked pair, is to make this complementarity explicit at the regulatory level rather than leaving it as an informal understanding within individual laboratories.^[1,2]

The information generated during development can support the validation strategy: DoE data collected to characterize the MODR, for instance, will typically already contain information relevant to^[33] demonstrating linearity, range and, to some extent, precision and robustness, so that validation becomes substantially a matter of formally confirming, under defined protocol conditions, what development work has already indicated rather than an entirely separate exercise conducted from a blank slate. ICH Q2(R2) explicitly describes analytical procedure validation as part of the analytical procedure lifecycle and allows suitable development data, generated with appropriate rigor and documentation, to contribute to the validation package.^[2,3]

Important validation characteristics addressed by ICH Q2(R2) include specificity or selectivity, meaning the method should be capable of measuring the intended analyte in the presence of potential interferences such as excipients, degradation products or other actives in a combination product; accuracy, which demonstrates closeness between the measured value and the accepted reference value, typically expressed as percentage recovery; precision, which can include repeatability (within-run variability), intermediate precision (variability across analysts, days or equipment within a single laboratory) and, where applicable, reproducibility across laboratories; linearity or response, meaning the analytical response should appropriately represent analyte concentration across the intended range, whether by a linear or a suitably justified non-linear model; range, meaning the reportable range should support the intended analytical application at both its lower and upper bounds; detection and quantitation limits, which are particularly relevant for impurity and trace-level analytical procedures; and robustness, which evaluates the ability of the procedure to remain reliable under deliberate, small variation of relevant parameters, and which, in an AQbD context, is already substantially characterized through the DoE work used to establish the MODR rather than being assessed for the first time during formal validation.

ICH Q2(R2) emphasizes throughout that validation should demonstrate that the analytical procedure is fit for its intended purpose language that deliberately echoes the ATP concept introduced in ICH Q14, reinforcing that validation is best understood as the formal confirmation of a fitness-for-purpose claim that was defined at the very start of development, not as an independent checklist applied at the end.^[2]

12. Applications of AQbD in Pharmaceutical Analysis

AQbD has been applied across several analytical platforms used routinely in pharmaceutical development and quality control^[34], with the specific critical parameters and design tools varying somewhat by technique.^[8]

12.1 HPLC and UHPLC. AQbD is particularly suitable for chromatographic methods because several variables can simultaneously influence separation, and because chromatographic responses such as resolution and tailing are well suited to quantitative modelling by DoE. Important variables include mobile-phase composition, pH, flow rate, temperature, gradient program, and column chemistry, and recent literature identifies AQbD as an increasingly systematic and increasingly expected approach for liquid chromatography method development, particularly for stability-indicating and impurity methods where separation of closely related substances is analytically demanding.^[20,23,27,35,36]

12.2 HPTLC. In high-performance thin-layer chromatography, AQbD can be applied to optimize the mobile phase composition, chamber saturation time, development distance, sample application parameters such as band width and application volume, and detection wavelength, with the relatively short analysis time and comparatively low solvent consumption of HPTLC also making it a natural fit for green-analytical-chemistry considerations discussed later in this review.^[8]

12.3 UV-Visible Spectrophotometry. For UV-Visible methods, AQbD may be used for optimizing wavelength selection, solvent choice, working concentration range, sample preparation procedure, and spectral processing conditions such as baseline correction or derivative order, particularly for methods intended to quantify an analyte in the presence of a spectrally overlapping matrix component.^[17]

12.4 LC-MS/MS. For liquid chromatography coupled with tandem mass spectrometry, critical variables may include mobile-phase composition and flow rate as in conventional LC, together with ionization conditions, source parameters such as spray voltage and desolvation temperature, collision energy for each monitored transition, and sample preparation steps such as protein precipitation or solid-phase extraction, all of which can materially affect sensitivity and matrix-effect behavior and are therefore natural candidates for risk assessment and DoE.^[37]

12.5 Dissolution Methods. AQbD can support optimization of dissolution methods by systematically evaluating dissolution medium composition and pH, agitation speed, temperature, sampling time points and technique, and apparatus-related parameters, an application that is particularly important given how directly dissolution methods can influence perceived in vitro-in vivo performance and, ultimately, biowaiver and bioequivalence arguments.^[38]

12.6 Multivariate Analytical Procedures. AQbD principles are increasingly relevant to multivariate analytical methods involving chemometric models and spectroscopic measurements, such as near-infrared or Raman-based methods used for real-time or at-line testing. ICH Q14 includes additional considerations specific to multivariate analytical procedures, including sample selection strategy, the design of calibration and validation sets, and assessment of model robustness, recognizing that these procedures raise questions^[39] such as how representative a calibration set must be, and how model performance should be monitored over time — that do not arise in the same way for single-analyte chromatographic or spectrophotometric methods.^[1,40]

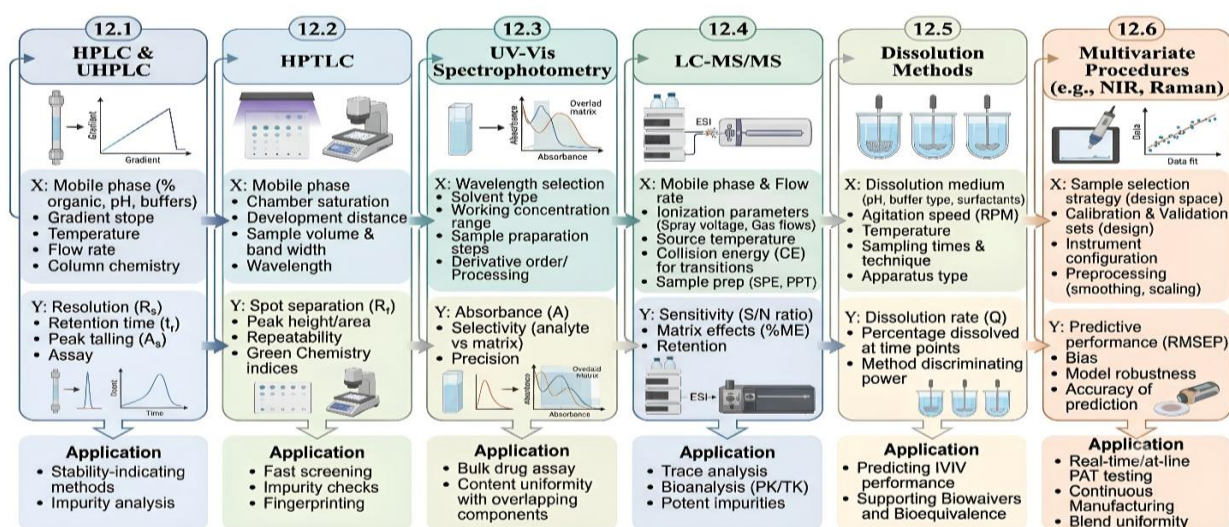


Fig. 1: Systematic application of Analytical Quality by Design (AQbD) principles, critical method parameters (CMPs), and performance attributes across key pharmaceutical analysis platforms (HPLC/UHPLC, HPTLC, UV-Vis, LC-MS/MS, Dissolution, and Multivariate Procedures).

13. AQbD in Stability-Indicating Method Development

Stability-indicating analytical procedures are required to distinguish the active drug from degradation products and other potential impurities that may form during manufacture or storage, and to do so with sufficient resolution and specificity that the true assay value and degradation profile of the product are not distorted by co-elution or spectral overlap.^[35]

AQbD can improve stability-indicating method development by systematically evaluating degradation pathways identified through forced-degradation studies conducted under acidic, basic, oxidative, thermal, and photolytic stress conditions; the choice of stress conditions and their intensity; the chromatographic separation achieved between the parent drug and each degradation product; critical peak resolution, particularly between structurally similar degradants that are prone to co-elution; mobile-phase variables that influence this resolution; detection conditions, including wavelength selection where degradation products absorb differently from the parent compound; and overall method specificity in the presence of the full set of expected degradants.^[7,41]

A scientifically designed, DoE-based method can provide a considerably better understanding of the relationship between chromatographic parameters and separation quality than a conventional OFAT approach^[42], because it allows the analyst to identify not just a single set of conditions that separates the degradants observed in one forced-degradation batch, but a design region within which separation is expected to remain adequate even if the relative proportions or identities of minor degradation products shift somewhat between batches or over the course of a long-term stability study.^[35,41]

14. AQbD and Chemometrics

Chemometrics involves the application of mathematical and statistical methods to chemical data, and its integration with AQbD extends the framework beyond univariate responses such as a single peak area or absorbance value toward the full information content of a spectrum or chromatogram.^[40]

The integration of AQbD with chemometrics can support multivariate data analysis of complex analytical signals; pattern recognition used to classify samples or detect atypical results; spectral interpretation, including resolving overlapping bands that cannot be separated by wavelength selection alone; calibration modelling, most commonly using partial least squares or principal component regression; process monitoring in real time; and method optimization where the response of interest is itself a multivariate model output, such as a root-mean-square error of prediction, rather than a single scalar measurement.^[40]

This integration is particularly relevant to spectroscopy, to Process Analytical Technology (PAT) applications more broadly, and to multivariate analytical procedures of the kind explicitly addressed in ICH Q14, where the risk-assessment and DoE logic of AQbD is applied not only to instrumental parameters such as scan range or resolution but also to chemometric modelling choices such as the number of latent variables, the pretreatment method applied to spectra, and the composition of the calibration and validation sample sets.^[1,40]

15. AQbD and Green Analytical Chemistry

Modern analytical development increasingly considers environmental sustainability alongside the traditional performance characteristics of accuracy, precision and specificity, reflecting both broader institutional sustainability commitments and, in some regions, emerging regulatory and publication expectations around solvent use and waste generation.^[43]

AQbD can support green analytical method development by incorporating solvent consumption, waste generation, analysis time and energy requirements directly into method optimization, treating them as additional responses to be considered alongside conventional chromatographic or spectroscopic performance rather than as an afterthought addressed only once a method has already been finalized on performance grounds alone.^[44,45]

Potential strategies include reduction of organic solvent volumes through smaller-bore columns or reduced flow rates; use of safer, less hazardous solvents in place of more toxic alternatives wherever chromatographic performance permits; shorter chromatographic run times achieved through gradient optimization; miniaturized sample preparation techniques that reduce solvent and reagent consumption; reduced sample quantities, which is particularly valuable where sample material is limited, as in early-phase formulation work; and energy-efficient instrumentation and operating modes. Thus, future AQbD approaches may combine quality, robustness and sustainability rather than optimizing analytical performance alone, treating greenness as one more critical quality attribute of the analytical procedure to be balanced against the others during DoE-based optimization, for example using multi-response optimization or desirability-function approaches that weigh a green-chemistry metric alongside resolution and analysis time.^[45,46,47]

16. Current Advances in AQbD

16.1 Integration with Artificial Intelligence and Machine Learning. Artificial intelligence and machine learning can potentially assist AQbD-based development in several ways, including prediction of chromatographic retention behavior from molecular structure or from a limited set of experimental runs, optimization of analytical conditions using algorithms that can search a design space more efficiently than a fixed experimental

design, identification of significant variables from historical or high-dimensional data, prediction of method performance under conditions not directly tested, and automated interpretation of complex analytical data such as spectra or chromatograms. However, appropriate model validation, data quality and scientific justification remain essential, since a machine-learning model that has not been properly validated against independent data, or that has been trained on a data set that does not represent the intended operating range, risks reproducing the same kind of unjustified extrapolation that AQbD is intended to prevent, only obscured behind a more sophisticated mathematical apparatus.^[48]

16.2 Automated Method Development. Automated chromatographic systems, incorporating automated mobile-phase blending, column switching and injection sequencing, can perform large numbers of experiments with limited analyst intervention, running overnight or over a weekend to complete a DoE program that would take considerably longer if executed manually. The combination of automation, DoE, AQbD and data analytics may substantially accelerate analytical method development by removing much of the manual labor associated with preparing and running large numbers of experimental conditions, while still preserving the statistical rigor of a properly designed experiment.^[49]

16.3 Multivariate Analytical Techniques. Multivariate models can use multiple analytical signals simultaneously, extracting more information from a single measurement than a univariate method could obtain, and ICH Q14 specifically recognizes multivariate analytical procedures and provides additional development considerations for them, reflecting their growing use in areas such as near-infrared-based content uniformity testing and Raman-based polymorph monitoring.^[1,40]

16.4 Real- Time Analytical Testing. AQbD principles can support analytical procedures intended for real-time release testing (RTRT) by establishing scientifically justified analytical performance and control strategies that give regulators and manufacturers confidence that a rapid, in-process or at-line measurement can substitute for a traditional end-product test, provided that the underlying method has been developed and characterized with the same rigor as a conventional laboratory method.^[50,51]

16.5 Digital Analytical Lifecycle Management. Digital systems can facilitate method knowledge management by capturing risk assessments, DoE data and MODR definitions in a structured, searchable form; experimental data storage that preserves the full data set behind a method rather than only its summary conclusions; risk assessment tools that standardize FMEA scoring across a laboratory or organization; statistical modelling software integrated with laboratory information systems; change management workflows that automatically check

whether a proposed change falls within an established MODR; and continuous monitoring of method performance indicators over the method's working life, allowing early detection of drift before it results in an out-of-specification result.^[3,32]

17. Regulatory Perspective

The regulatory significance of AQbD has increased substantially with the finalization of ICH Q14 and Q2(R2), which together represent the first harmonized, tripartite regulatory guidance specifically addressing analytical procedure development as a discipline in its own right^[52], rather than treating development only implicitly through the lens of validation requirements.^[1,2,53,54]

ICH Q14 provides harmonized scientific approaches for analytical procedure development and includes concepts such as the Analytical Target Profile; knowledge management, meaning the systematic capture and reuse of information generated during development; risk management, drawing on the principles already established in ICH Q9; robustness, addressed as a development consideration rather than solely a validation exercise; analytical procedure parameter ranges, formalized through the MODR concept; analytical control strategy; established conditions, meaning the specific subset of parameters and ranges reported to regulators; lifecycle management, covering the method's entire working life rather than only its initial development and validation; multivariate analytical procedures, addressed with dedicated considerations; and real-time release testing, recognizing analytical procedures used for in-process or at-line measurement in place of traditional end-product testing.^[1,24]

ICH Q2(R2) provides the corresponding framework for validation and emphasizes demonstrating that an analytical procedure is fit for its intended purpose, extending the validation characteristics and statistical approaches of the earlier ICH Q2(R1) guideline to more explicitly accommodate a wider range of analytical technologies. The two guidelines were finalized and adopted together, and the U.S. Food and Drug Administration finalized both the Q14 and Q2(R2) guidance documents in March 2024, formally adopting the harmonized ICH text into the U.S. regulatory framework and signalling to industry that both new submissions and, over time, existing analytical procedures would increasingly be expected to demonstrate the kind of scientific understanding that AQbD is designed to generate.^[53,54]

This represents an important shift from traditional analytical method validation, understood as a fixed, largely retrospective checklist applied once development is complete, toward a more integrated analytical procedure lifecycle approach in which development, validation, routine use and post-approval change are treated as connected stages of a single continuum, each

informed by the scientific understanding generated at the stages before it.^[3,32]

18. Advantages of AQbD

Major advantages include a more scientific and systematic approach to method development, in which decisions are documented and justified rather than made ad hoc; improved method robustness, arising directly from the deliberate characterization of the design space rather than a single operating point; a better understanding of method variables and, in particular, of interactions between them that OFAT approaches cannot detect; identification of parameter interactions that would otherwise remain hidden until they caused a failure during routine use;^[4,5,6] a reduction in unnecessary experiments once a properly designed screening study has eliminated variables that do not matter; better control of analytical variability through a documented control strategy; improved transferability of analytical procedures between laboratories, sites or instruments, since the MODR defines explicitly how much variation the method can tolerate; facilitated lifecycle management, since development knowledge is captured in a form that can be reused when changes are proposed; scientific justification for method changes, allowing many changes to be assessed against the existing MODR rather than triggering a full new validation; a potential reduction in regulatory burden when changes are appropriately justified within an established design space; improved reproducibility across analysts, instruments and time; and compatibility with automation and advanced analytical technologies, since a well-characterized design space is naturally suited to automated monitoring and control.

A recent review described AQbD as a framework that shifts analytical development from reactive troubleshooting, in which problems are addressed only once they have already caused an atypical or out-of-specification result, toward proactive reduction of analytical failure, in which the conditions likely to cause such a failure have already been identified and either avoided or explicitly controlled for during development.

19. Challenges and Limitations

Despite its advantages, implementation of AQbD presents several challenges that have, to date, limited its adoption to a subset of organizations and analytical programs rather than making it a universal default.^[10,11,12]

19.1 Requirement for Statistical Expertise. Successful implementation requires knowledge of DoE, statistical modelling and data interpretation that goes beyond the training traditionally provided to analytical chemists, and organizations without ready access to statistical support may find the learning curve a significant barrier, particularly for smaller laboratories or academic groups working with limited personnel.^[10,55]

19.2 Increased Initial Development Effort. AQbD may require more planning and experimentation during the early development stage compared with simple trial-and-error methods, since risk assessment, screening designs and response-surface studies all represent additional, structured work that is not required under a conventional approach, even though this additional up-front investment is generally intended to reduce total effort and rework over the full lifecycle of the method.^[11,55]

19.3 Software and Infrastructure. Advanced DoE, chemometric and modelling approaches may require specialized software, together with personnel trained to use it correctly, and organizations may face a real cost, both financial and in terms of training time, in acquiring and maintaining this infrastructure.^[55]

19.4 Lack of Standardized Implementation Practices. Although ICH Q14 provides a harmonized conceptual framework, practical implementation may vary considerably among organizations, for example in how formally risk assessment is documented, how large a screening design is considered sufficient, or how a MODR is translated into a filed established condition, and this variability can make it harder for regulators and industry to develop a fully shared understanding of what a well-executed AQbD program should look like in practice.^[11,12]

19.5 Data Quality. Poor-quality experimental data arising from inadequate instrument qualification, inconsistent sample preparation, or insufficient replication can produce unreliable statistical models, and because AQbD places considerable weight on the statistical models generated from DoE data, errors introduced at the data-collection stage can propagate into an incorrectly defined MODR if they are not detected.^[7]

19.6 Organizational Resistance. Transitioning from conventional development to lifecycle-based analytical development may require changes in laboratory culture, documentation practices and training that go beyond the technical content of AQbD itself, and analysts and managers accustomed to a validated, fixed-method paradigm may be reluctant to adopt a framework that requires more up-front statistical rigor and more extensive documentation.^[12,55]

Recent industry research indicates that technical, operational and regulatory barriers still affect broader implementation of AQbD, and that even where organizations recognize its scientific merits, practical adoption often lags behind the conceptual acceptance of the framework, particularly in resource-constrained settings and in regions where regulatory expectations around AQbD are still developing.^[11,12]

20. Future Perspectives

The future of AQbD is expected to move beyond statistical optimization of a fixed set of chromatographic

or spectroscopic responses toward a more intelligent, automated and sustainable model of analytical development, in which the framework is extended by, rather than replaced by, emerging digital and computational tools.^[48,49,56]

20.1 AI-Driven AQbD. Machine learning may increasingly be used to predict analytical method performance from a reduced set of experiments and to recommend experimental conditions for the next round of DoE, potentially reducing the total number of physical experiments required to characterize a design space while preserving the statistical justification that regulators expect.^[48]

20.2 Autonomous Analytical Development. Integration of automated instruments, DoE and AI may allow analytical systems to perform iterative experiments with minimal human intervention, in a closed loop where the outcome of one set of experiments directly informs the design of the next, moving progressively toward an optimum or toward a fully characterized design space with limited manual planning at each iteration.^[49]

20.3 Digital Twins. Digital representations of analytical procedures, built from mechanistic and statistical models of how a given technique behaves, may eventually help predict method performance before physical experimentation is undertaken at all, allowing at least an initial screening of the variable space to be conducted in silico before committing laboratory time and material to physical DoE runs.^[48,49]

20.4 AQbD–PAT Integration. Integration of AQbD with Process Analytical Technology may strengthen real-time monitoring and release strategies by ensuring that the analytical procedures used for in-process measurement are themselves developed and characterized with the same rigor as conventional end-product test methods, rather than being adopted with a lighter development burden simply because they operate in a process rather than a quality-control setting.^[50,51]

20.5 Green AQbD. Future method development may simultaneously optimize analytical performance, robustness, cost and environmental impact as a single multi-response problem, using desirability functions or similar multi-criteria optimization approaches to balance traditional performance characteristics against solvent consumption, waste generation and energy use.^[45,46]

20.6 Lifecycle-Based Method Management. Analytical procedures are increasingly expected to be managed throughout their lifecycle, from initial development through routine use, periodic review and eventual retirement or replacement, rather than being treated as static validated methods that are revisited only when a problem occurs or a change is proposed.^[3,32]

20.7 Advanced Multivariate Methods. Chemometrics, spectroscopy and machine learning may become increasingly important for complex matrices and multicomponent pharmaceutical products, where a single univariate measurement is often insufficient to characterize the product adequately and where the additional information content of a full spectrum, analyzed by an appropriately validated chemometric model, offers real analytical advantages over conventional single-wavelength or single-peak approaches.^[40]

21. Proposed AQbD Workflow

The individual elements discussed in the preceding sections can be assembled into a single generalized AQbD workflow, presented in Figure 2, that begins with definition of the Analytical Target Profile, proceeds through identification of analytical performance requirements, selection of an analytical technique, knowledge gathering, risk assessment, identification of critical method parameters, Design of Experiments, method optimization, establishment of the MODR or parameter ranges, development of an analytical control strategy, method validation against ICH Q2(R2), routine implementation, and finally lifecycle monitoring and continual improvement, which in turn feeds back into the knowledge base available for future methods and future changes to the same method. This workflow reflects the lifecycle-oriented principles described in ICH Q14, in which the arrow from lifecycle monitoring back toward the earlier stages of the workflow is deliberate: AQbD is presented as a cycle rather than a strictly linear sequence, since knowledge generated during routine use and during the investigation of any atypical results is expected to feed back into the understanding of the method and, where appropriate, into the justification for future changes.

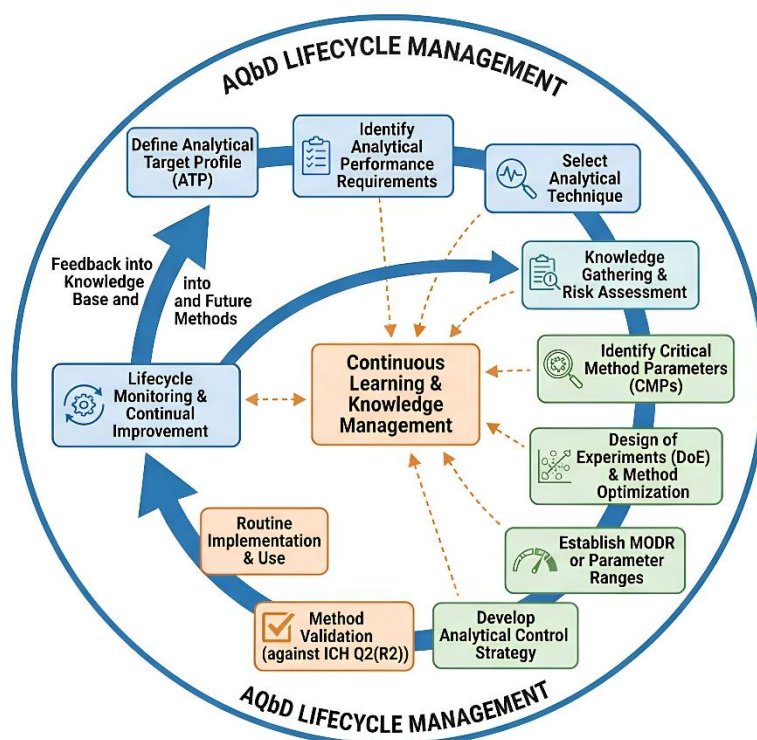


Fig. 2: Generalized analytical Quality by design (AQbD).

22. AQbD Compared with Traditional Analytical Method Development

Table 3 summarizes the principal differences between traditional analytical method development and the AQbD approach across a range of dimensions, including development philosophy, experimental strategy, treatment of risk, treatment of variable interactions,

timing of robustness assessment, presence or absence of a formally defined design space, nature of the control strategy, relationship between development and validation, extent of lifecycle management, approach to change management, and the basis on which changes are regulatorily justified.

Table 3: AQbD compared with traditional analytical method development.

Parameter	Traditional Approach	AQbD Approach
Development philosophy	Trial-and-error	Systematic
Experimental strategy	OFAT	DoE
Risk assessment	Limited	Integrated
Variable interactions	Often overlooked	Systematically evaluated
Robustness	Usually evaluated later	Considered during development
Design space	Generally absent	MODR / design region
Control strategy	Limited	Scientifically established
Validation	Separate activity	Connected to development
Lifecycle management	Limited	Integral component
Change management	Reactive	Science / risk-based
Regulatory justification	Often condition-specific	Knowledge-based

23. CONCLUSION

Analytical Quality by Design represents an important evolution in pharmaceutical analytical method development, moving the discipline away from a largely empirical, trial-and-error tradition and toward a systematic, science- and risk-based framework grounded in an explicit statement of purpose. By integrating the Analytical Target Profile, structured risk assessment, Design of Experiments, statistical modelling, robustness evaluation conducted as part of development rather than only at validation, and an explicit analytical control

strategy, AQbD enables development of analytical procedures that are scientifically justified, demonstrably robust, and fit for their intended purpose across the range of conditions they are likely to encounter over their working life.

The finalization of ICH Q14 and ICH Q2(R2) as a linked pair of guidelines has further strengthened the regulatory foundation for modern analytical procedure development and validation, giving industry a harmonized vocabulary and a harmonized set of expectations that were

previously addressed only unevenly across regions and organizations. This regulatory clarity is likely to accelerate adoption of AQbD principles even among organizations that have not previously invested heavily in statistical method development.

Future AQbD implementation is expected to increasingly incorporate artificial intelligence, machine learning, chemometrics, automation, green analytical chemistry, real-time analytical technologies and digital lifecycle management, each of which extends the reach of the framework without displacing its central logic of defining purpose first, understanding variables through structured experimentation, and maintaining that understanding through an explicit control strategy across the full lifecycle of the method. Although challenges remain regarding statistical expertise, resource availability, standardization of practice, and organizational readiness, the transition toward AQbD-based analytical lifecycle management is likely to become increasingly important, and increasingly expected by regulators, in pharmaceutical analysis over the coming years.

Overall, AQbD should not be viewed merely as a statistical optimization technique applied to squeeze slightly better resolution out of a chromatographic separation, but as a comprehensive scientific framework for designing, understanding, controlling and continuously improving analytical procedures throughout their entire lifecycle, from the earliest definition of what the procedure is intended to measure through to its eventual retirement or replacement.

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