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A COMPREHENSIVE REVIEW ON ADVANCING PHARMACEUTICAL ANALYSIS THROUGH ANALYTICAL QUALITY BY DESIGN (AQBD)

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ABSTRACT

Background: Pharmaceutical analytical methods are very important for ensuring drug quality. However traditional methods often rely on trial and error. This can lead to methods that're not very robust or flexible. Analytical Quality by Design (AQbD) is an approach. It uses science-based principles for analytical methods. **Objective:** This review looks at the basics, regulations, tools and uses of in reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC is widely used in analysis. **Methods:** We searched databases like PubMed and regulatory websites for information on AQbD, Quality by Design and RP-HPLC. We found publications from 2010 to 2025. **Results:** AQbD combines the Analytical Target Profile (ATP) risk assessment tools and Design of Experiments (DoE). This helps create a defined Method Operable Design Region (MODR).RP-HPLC methods created with AQbD are more robust. They are also more acceptable to regulators. These methods are used for: Assay of pharmaceutical ingredients (APIs), Impurity profiling, Dissolution testing, Stability-indicating methods, Bioanalytical quantification. AQbD is a big change in pharmaceutical analytical science. It follows ICH Q8–Q10 principles. With new technologies like artificial intelligence and automated platforms will become the standard for analytical method development. It helps create methods that're compliant, with regulations and aware of the whole lifecycle.

KEYWORDS: Analytical Quality by Design; RP-HPLC; Design of Experiments; Analytical Target Profile; Method Operable Design Region; ICH guidelines.

1. INTRODUCTION

1.1 Background of Pharmaceutical Analysis

Pharmaceutical analysis involves techniques to check the identity, purity, potency and safety of medicines throughout their development, manufacturing and storage.[1] Among the chromatographic methods used in pharmaceutical quality control reversed-phase high-performance liquid chromatography (RP-HPLC) is very important because it is versatile has high resolving power and works well with a wide range of substances.[2,3]

The United States Food and Drug Administration (US FDA) the European Medicines Agency (EMA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) require that analytical methods be thoroughly validated and monitored to ensure they remain effective throughout the product lifecycle.[4] In the past developing methods relied mainly on changing one variable at a time (OVAT) where one chromatographic parameter, such as mobile phase composition, pH, flow rate or column temperature was changed while keeping all other factors the same.[5] This approach is simple. Has limitations, such as not detecting interactions between parameters being inefficient and producing methods that only work well in narrow conditions.

As a result; these methods often lack robustness. Need to be revalidated when analytical conditions or reagent lots change, which increases the time-to-market and regulatory complexity.[6] Pharmaceutical analysis and RP-HPLC are crucial in ensuring the quality of medicines. The use of RP-HPLC, in analysis helps to ensure that medicines are safe and effective. Pharmaceutical analysis involves a range of techniques, including RP-HPLC to evaluate the quality of medicines.

1.2 The Imperative for Analytical Quality by Design

The idea of Quality by Design or QbD for short was first made official in the pharmaceutical manufacturing field with the help of ICH Q8, Q9 and Q10. These guidelines support an science-based approach to designing products and processes. People started to think that the same ideas could be used for procedures. So the pharmaceutical science community started working on Analytical Quality by Design or AQbD in the 2000s.[7] Vogt and Kord[8] and later Borman and his team were some of the people to clearly explain the framework of Analytical Quality by Design. They played a role in shaping the concept of Quality by Design and Analytical Quality, by Design.[9] AQbD defines method performance requirements a priori, systematically identifies sources of variability, and constructs a multidimensional design space; the Method Operable Design Region (MODR) ; within which method performance is guaranteed.[10]

The rules for AQbD work well with what the FDA wants to do with Process Analytical Technology. This is also in line with what the EMA says about using QbD principles when developing methods.[11] The USP has a chapter on the life cycle of analytical procedures and the new version of ICH Q14 will also support AqbD.[12] AQbD is the way for regulations. This review looks at how AQbD's used for RP-HPLC from the basics to the latest developments and what is coming next. It gives an honest opinion of the AQbD framework; AQbD framework is important, for the future of methods, including RP-HPLC. This review covers everything about AQbD, including what it's how it is used and what the future holds for it.

2. Principles of Analytical Quality by Design

2.1 Definition and Regulatory Perspective

Analytical Quality by Design may be formally defined as a systematic, science- and risk-based approach to analytical method development that begins with predefined objectives, and that identifies the multivariate relationships between method parameters and analytical performance, to deliver a robust, reproducible, and regulatorily flexible analytical procedure.[8] The AQbD paradigm draws extensively on three cornerstone ICH guidelines: ICH Q8(R2), which introduced the concept of design space and its regulatory implications; ICH Q9, which provided a structured framework for pharmaceutical risk management; and ICH Q10, which articulated the expectations for a comprehensive pharmaceutical quality system including change management and continual improvement.[7]

The draft ICH Q14 guideline, released for public consultation in 2022, specifically addresses analytical procedure development, establishing that enhanced approaches; wherein developers provide scientific justification for parameter ranges, define a design space, and demonstrate analytical understanding; warrant regulatory recognition comparable to that offered to product design spaces under Q8.[13] The US Pharmacopeia has made some changes to a part called chapter <1220> at the time. They want to introduce a way of doing things called the Analytical Procedure Lifecycle model. This model is about creating something checking that it works and making sure it keeps working. The US Pharmacopeia is doing this to make sure that people always think about quality, which's a big part of Analytical Quality, by Design or AqbD.[14]

2.2 Key Elements of AQbD

2.2.1 Analytical Target Profile (ATP)

Analogous to the Quality Target Product Profile (QTPP) in formulation QbD, the Analytical Target Profile (ATP) serves as a prospective summary of the minimum performance requirements that an analytical procedure must achieve to be fit for its intended purpose.[9] For RP-HPLC methods, the ATP typically specifies: the analyte identity and matrix; the analytical measurement range; required accuracy expressed as percentage recovery (e.g., 98.0–102.0%); precision thresholds (%RSD); specificity criteria (e.g., resolution ≥ 2.0 from all relevant impurities); limits of detection (LOD) and quantitation (LOQ); and permissible measurement uncertainty. The ATP thereby provides the guiding framework against which all subsequent development decisions are evaluated, ensuring that the method is designed explicitly to meet its intended regulatory and quality purpose.[10]

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

Critical Analytical Attributes (CAAs) are measurable properties of an analytical procedure output; such as resolution, peak symmetry (asymmetry factor), retention factor, signal-to-noise ratio, or percentage recovery; that are critical to ensuring the method meets its ATP.[15] Critical Method Parameters (CMPs) are the input variables whose variation within operationally realistic ranges may cause significant impact on one or more CAAs. In RP-HPLC, typical CMPs include organic modifier concentration and type, mobile phase pH, buffer concentration, column temperature, flow rate, injection volume, and gradient slope.[16] The systematic identification of CMPs and CAAs, and the elucidation of their interactions, constitutes the scientific core of the AQbD approach and distinguishes it fundamentally from empirical method optimisation.

2.2.3 Risk Assessment Tools

Risk assessment in AQbD employs formal quality risk management tools as mandated by ICH Q9. Ishikawa (fishbone or cause-and-effect) diagrams are widely used as a first-pass screening tool to map all conceivable sources of analytical variability in a structured, visual format, categorised across instrument, method, analyst, environment, reagent, and sample dimensions.[17] Following Ishikawa analysis, Failure Mode and Effects Analysis (FMEA) provides a semi-quantitative risk prioritisation by assigning numerical scores to the severity, occurrence probability, and detectability of each identified failure mode, computing a Risk Priority Number (RPN = Severity × Occurrence × Detectability) to guide experimental design.[18] High-RPN CMPs are prioritised for DoE investigation, while low-risk parameters may be fixed at nominal values, thereby optimising experimental resource allocation.

3. AQbD Approach in RP-HPLC Method Development

3.1 Method Scouting

The first step in developing a method for RP-HPLC using AQbD is called method scouting. This is when you start looking at the parts of the process to see what works best. You try out stationary phases, mobile phase systems organic modifiers and pH conditions to find a good starting point for making the method better.[19] Nowadays people use machines to help with method scouting. These machines can try out a lot of things very quickly like different types of columns, such, as C18, C8, phenyl-hexyl, biphenyl and pentafluorophenyl phases. They can also change the conditions of the phase very fast. This helps to get an idea of how well the method can separate the different parts of the mixture.[20] Column screening databases and retention prediction software (e.g., DryLab, ACD/LC Simulator) further enable in silico scouting, reducing the number of experimental runs required before systematic optimisation begins.

For pharmaceutical impurity profiling methods, scouting must evaluate selectivity not only for the API but also for structurally related impurities, process-related residuals, and forced degradation products, as specificity under stressed conditions is a fundamental ATP requirement.[21] The outcomes of method scouting directly inform the selection of factors and ranges to be studied in subsequent DoE experiments, ensuring that the design space investigation is both scientifically justified and experimentally efficient.

3.2 Risk Assessment and CMP Identification

Following method scouting, a formal risk assessment is conducted to identify and prioritise CMPs for DoE investigation. The Ishikawa diagram is constructed for the RP-HPLC system under development, cataloguing sources of variability across the six M categories (Man, Machine, Method, Material, Measurement, Mother Nature).[17]

FMEA is then applied, drawing upon prior knowledge from the method scouting phase, mechanistic understanding of chromatographic retention, and published literature for structurally analogous compounds. Parameters assigned RPN values exceeding a pre-defined threshold (commonly RPN $\geq$ 100 on a 1000-point scale) are classified as CMPs and included as factors in the DoE.[18] Parameters with intermediate RPN values may be evaluated in a screening design, while low-risk parameters are set at fixed, operationally convenient levels.

3.3 Design of Experiments (DoE)

3.3.1 Screening Designs

When numerous potential CMPs are identified by risk assessment, screening designs are employed to efficiently identify the subset that exerts significant main effects on CAAs. Plackett-Burman designs, capable of evaluating up to (N–1) factors in N experiments, are particularly popular for initial screening in pharmaceutical method development.[22]

Fractional factorial designs at two levels (2^(k-p)) similarly offer resolution of main effects with minimal experimental runs, though they may suffer from aliasing of two-factor interactions at low resolution levels. Screening designs are not intended to provide detailed response surface modelling; rather, they identify the 'vital few' CMPs that account for the majority of response variation, which are then carried forward to optimisation designs.

3.3.2 Response Surface Designs

Response surface methodology (RSM) designs are employed during the optimisation phase to model the functional relationships between CMPs and CAAs across a defined experimental region. The most frequently applied RSM designs in AQbD-based RP-HPLC development include Central Composite Designs (CCD), which augment full or fractional factorial designs with axial (star) points and centre replicates to enable quadratic modelling; Box-Behnken Designs (BBD), which avoid extreme vertex combinations and are therefore preferred when corner conditions are operationally undesirable; and Doehlert (uniform shell) designs, which allow efficient re-use of experimental points during sequential optimisation.[23,24] The resulting polynomial regression models describe the CAA response surfaces mathematically, enabling prediction of chromatographic performance at any combination of CMP levels within the experimental space.

3.3.3 Optimal Designs and Mixture Designs

D-optimal, I-optimal, and other computer-generated optimal designs have gained traction in AQbD applications where the experimental region is constrained, where a combination of mixture and process variables must be studied simultaneously, or where the number of available experimental runs is limited.[25] Mixture designs; including simplex lattice and simplex centroid approaches; are specifically applicable when mobile phase composition is studied as a mixture variable (e.g., water, acetonitrile, methanol, and buffer proportions that must sum to unity), as classical factorial designs are theoretically inappropriate for constrained composition spaces.[26] The combination of mixture and process variable designs provides a comprehensive model of both compositional and operational CMPs in a single unified framework.

3.4 Optimisation Strategies and MODR Establishment

Following DoE execution and response surface modelling, multivariable optimisation is performed using desirability function approaches, wherein individual CAA responses are transformed into dimensionless desirability scores (d, ranging from 0 to 1) reflecting the extent to which each response meets its ATP target, and an overall composite desirability (D = geometric mean of all individual desirabilities) is maximised.[27] Graphical overlaid contour plot (OCP) analysis provides a visual representation of the feasible operating region ; the Method Operable Design Region (MODR) ; defined as the multidimensional space within which all CAAs simultaneously satisfy their ATP-derived specifications.[28]

The MODR is distinct from the nominal optimal point in that it explicitly quantifies the extent of permissible parameter variation, thereby enabling analytical laboratories to operate at any point within the MODR without compromising method performance. Monte Carlo simulations and Bayesian probability-of-success calculations may be overlaid on the MODR to provide probabilistic assurance that method performance requirements will be met under realistic operational variability.[29] This probabilistic dimension of design space characterisation is increasingly expected by regulatory reviewers as evidence of analytical robustness and method understanding.

4. Method Validation Under AQbD

4.1 Integration of Validation with the Design Space

In the conventional paradigm, method validation as prescribed by ICH Q2(R1) is conducted at a single, fixed set of analytical conditions that represents the developer's best-guess optimum, with no explicit consideration of the method's behaviour across a range of conditions.[30] Under AQbD, validation is conceptualised as a multi-point, lifecycle-integrated exercise wherein performance characteristics are demonstrated not merely at a nominal operating point but across the full extent of the MODR. This approach provides far stronger regulatory assurance that the method will remain valid as operating conditions fluctuate within the defined design space during routine use.[31] The validation plan is derived directly from the ATP, ensuring that each validation parameter directly demonstrates fitness-for-purpose as pre-defined.

4.2 Validation Parameters and AQbD Enhancement

Table 2 summarises the principal validation parameters specified under ICH Q2(R1) alongside the specific enhancements that AQbD introduces to each parameter.

Table 1: Comparison of ICH Q2(R1) Validation Requirements and AQbD-Enhanced Validation Approaches for RP-HPLC Methods.

Validation Parameter	ICH Q2(R1) Requirement	AQbD Enhancement
Accuracy	%Recovery: 98–102% (drug substance); 98–105% (formulations)	Evaluated across full design space; bracketing at extremes of optimised variables
Precision (Repeatability)	RSD ≤ 2.0% for ≥6 determinations at 100% level	Intra-day and inter-day assessed within and at edges of design space
Intermediate Precision	Different analysts, instruments, days	Embedded within DoE; variance components modelled statistically
Linearity	$R^2 \geq 0.999$ over 50–150% of target concentration	Assessed over full ATP-defined concentration range; residuals examined
Specificity	Baseline resolution from impurities, matrix, degradants	Demonstrated under worst-case design space conditions; peak purity by PDA/MS
Robustness	Deliberate variation of critical parameters	Defined systematically via Plackett-Burman or full factorial; edge-of-failure identified
LOD / LOQ	Signal-to-noise (3:1; 10:1) or residual std dev	Risk-driven; benchmarked against ATP reportable threshold
Range	80–120% of test concentration	ATP-based; extended where justified by design space

4.3 Comparison with Conventional Validation

The fundamental conceptual distinction between conventional and AQbD-integrated validation lies in its directionality and scope. Conventional validation proceeds from a fixed method to a set of statistically defined performance characteristics, offering no insight into how those characteristics would change under parameter perturbation. AQbD-

integrated validation, by contrast, proceeds from a characterised design space to a set of performance characteristics demonstrated to be robust across operationally relevant parameter ranges.[32] A seminal comparative study by Mantelingu et al. demonstrated that RP-HPLC methods developed and validated under AQbD exhibited a three-fold improvement in robustness index relative to conventionally validated counterparts, with no out-of-specification results observed across 120 Monte Carlo simulations within the MODR.[33]

Furthermore, AQbD validation is inherently prospective in its risk management dimension. Because CMPs and their acceptable ranges are explicitly defined in the design space, any proposed change in analytical conditions during the product lifecycle can be evaluated a priori against the MODR boundaries. Changes within the design space may be implemented without regulatory notification, whereas changes exceeding design space boundaries trigger a managed change control process ; a significant regulatory advantage over conventionally validated methods, where even minor parameter changes frequently require formal variation submissions.[34]

5. Applications of AQbD in Pharmaceutical Analysis

5.1 Assay of Drug Substances and Formulations

The use of AQbD in creating RP-HPLC assay methods for pharmaceutical ingredients and their dosage forms is well known.[35] People have written a lot about it in papers. For example; Sahu and his team used a method called Box-Behnken Design to improve the RP-HPLC assay of metformin hydrochloride and sitagliptin phosphate. These two ingredients are found in a tablet that people take to get a fixed dose of medicine. They looked at how different things affected the assay such as the amount of acetonitrile used the pH of the buffer and the flow rate of the liquid. They wanted to see how these things changed the way the ingredients separated and were retained during the assay. They tested concentrations between 15 and 25 percent phosphate buffer pH between 2.8 and 3.4 and flow rates between 0.8 and 1.2 milliliters per minute. AQbD was used to develop the RP-HPLC assay methods, for these pharmaceutical ingredients.The resulting MODR demonstrated a wide zone of acceptable operation, and the validated method met all ICH Q2(R1) criteria with %RSD values not exceeding 0.45% for repeatability ; a performance standard substantially superior to methods developed by OVAT optimisation for the same combination.

Similarly, Kaur et al.[36] employed a Central Composite Design to develop an AQbD-guided stability-indicating RP-HPLC method for canagliflozin, demonstrating through Ishikawa analysis that column temperature, gradient slope, and mobile phase pH were the dominant CMPs influencing peak resolution and asymmetry. The validated method resolved the drug from five specified impurities and eight forced-degradation products with resolution factors exceeding 2.0 under all conditions within the MODR, confirming that the AQbD framework effectively embeds specificity assurance into the design phase rather than treating it as an afterthought during validation.

5.2 Impurity Profiling

Impurity profiling represents a particularly demanding application for RP-HPLC method development, as the simultaneous resolution of an API from multiple structurally related impurities, genotoxic impurities, and process-related residuals at ICH Q3A/Q3B-specified threshold concentrations requires exquisite chromatographic selectivity.[37]

AQbD's systematic exploration of the multivariate separation space confers a decisive advantage in this context by identifying CMP combinations that maximise the critical pair resolution across the entire impurity profile simultaneously. Pagire et al.[38] demonstrated this through a D-optimal design applied to the impurity profiling of

amlodipine besylate, wherein the simultaneous optimisation of five CMPs across a 25-experiment design yielded an MODR within which all eight monitored impurities were resolved at critical resolution factors ≥ 1.5. The probabilistic confirmation of MODR validity through Monte Carlo simulation (n = 10,000) showed a 97.8% probability of achieving all resolution criteria simultaneously; a compelling demonstration of the method's analytical robustness.

5.3 Stability-Indicating Methods

The development of stability-indicating analytical procedures, mandated by ICH Q1A(R2) for the characterisation of drug degradation products under stress conditions, imposes the dual requirement of specificity for known and unknown degradants alongside the quantitative accuracy of the parent drug assay.[39] AQbD uniquely addresses this challenge by incorporating forced degradation studies into the risk assessment phase, using the resulting degradant profile to define a stringent specificity criterion within the ATP. Blessy et al.[40] outlined a systematic AQbD workflow for the development of stability-indicating RP-HPLC methods that integrated forced degradation screening (acid, base, oxidative, photolytic, and thermal conditions) as a prerequisite to DoE factor selection, ensuring that the MODR is defined with direct reference to the worst-case degradant mixture.

5.4 Bioanalytical Methods

The idea of using principles for bioanalytical method development is getting more attention. This is about measuring drugs and what they break down into in things like blood, urine and tissue. The FDA and EMA have said this is an idea in their guidelines from 2018 and 2011.[41] When we use a kind of chromatography called RP-HPLC with tandem mass spectrometric detection, which is also known as bioanalytical RP-HPLC with tandem mass spectrometric detection or LC-MS/MS for short we need to think about a few important things. These things include how we prepare our samples like what solvent we use to precipitate proteins and how we wash and elute during solid-phase extraction. We also need to think about the pH of the phase and how much organic modifier we use.. Then there are the source parameters that affect how well ions are made.[42] For example Zhu and his team[43] used a design to develop a good LC-MS/MS method for a drug called imatinib and its major metabolite N-desmethyl imatinib in human plasma. They first used a Plackett-Burman screening design and then a CCD optimisation. This showed that using AQbD principles the MODR, can help make methods that work well even when different labs are used. This is a problem with methods that are developed in the usual way. They often do not work as well when used in different labs. Bioanalytical methods like these are very important for measuring drugs and their byproducts, in samples and using AQbD principles can help make these methods more robust.

6. Advantages and Challenges of AQbD

6.1 Advantages Over Traditional Methods

The advantages of AQbD over traditional analytical method development are multifaceted and span scientific, operational, and regulatory dimensions. Scientifically, AQbD generates a mechanistic understanding of the analytical procedure that enables intelligent troubleshooting, rational transfer, and evidence-based optimisation, in contrast to the black-box empiricism of OVAT development. The design space provides an explicit, quantitative definition of method robustness, which traditional validation characterises only qualitatively through the Youden ruggedness test or limited Plackett-Burman perturbation studies.[5] Operationally, the efficiency gains from DoE ; typically requiring 30–50% fewer experiments than comprehensive OVAT investigation of the same parameter space ; translate directly to reduced development timelines and resource expenditure.[44]

Regulatorily, the AQbD framework offers substantial post-approval flexibility. The rules say that if you make changes inside an approved design space like the ones talked about in ICH Q14 you do not have to get a marketing authorisation.[13] This makes things a lot easier because you do not have to deal with a lot of paperwork every time you make changes to how you test your products. This is really helpful for companies that make medicines and sell them in different countries because they have to follow many different rules. If they can use the design space everywhere it is easier for them to sell their products in all those places at the same time. Table 1 shows how AQbD is different, from the way of developing methods and it compares them in ten important areas.

Table 2: Comparative Analysis of Traditional and AQbD-Based Approaches to Analytical Method Development in RP-HPLC.

Parameter	Traditional Method Development	AQbD-Based Method Development
Approach	One-variable-at-a-time (OVAT); empirical and sequential	Systematic, multivariate; risk-based and science-driven
Regulatory philosophy	Compliance-centric; method locked post-approval	Quality-centric; built-in flexibility via design space
Risk assessment	Informal or absent; reactive identification of failures	Formal (FMEA, Ishikawa); proactive identification of CMPs/CAAs
Experimental efficiency	High number of experiments; resource-intensive	Reduced experimentation through DoE; resource-efficient
Method understanding	Limited mechanistic insight; empirical optimisation	Deep mechanistic understanding; predictive models
Design space	Not defined; single operating point	Explicitly defined; enables post-approval flexibility
Validation strategy	Performed after development; often narrow scope	Integrated throughout development; broader, robustness-focused
Change management	Requires regulatory filing for most changes	Changes within design space may not require refiling
Documentation	Protocol-driven; limited scientific justification	Science and risk-based; traceable lifecycle documentation
Method lifecycle	Static; limited continual improvement	Dynamic; supports continual improvement framework

6.2 Practical Limitations and Challenges

There are some challenges that make it hard to use AQbD in pharmaceutical labs even though it has some great benefits. One of the hurdles is that it requires a lot of resources upfront. This includes things, like doing a risk assessment testing different conditions running multiple experiments creating complex models validating results and keeping detailed records. All these steps take time require special skills and need more equipment and staff.[45] For early-phase pharmaceutical development; where compound availability is limited, timelines are compressed, and method requirements may change substantially as the programme advances, the full AQbD framework may be disproportionate to operational needs.

A second challenge concerns the statistical expertise required to design, execute, and correctly interpret DoE experiments and response surface models.

Design of experiments can go wrong if we do not set the ranges for factors. We also need to pick the model and make sure it fits the data. If we do not do this. If we misunderstand the interaction plots we can end up with a bad design space. This bad design space can make us think that our method is more robust than it really is.[46]

The software we use for design of experiments like JMP, Minitab, Design-Expert and MODDE can also cause problems. These programs are all different. Have different default settings, ways of picking models and ways of showing the design space. This means that different companies can get results, which can be confusing.

Another problem is that there is no way of reporting the results of design of experiments when we submit them to regulatory agencies. Even though there are guidelines, like ICH Q14 there is no template that everyone uses. This can cause problems when the regulatory reviewers look at the results because they may not be familiar with the way the results are presented. Design of experiments results can be hard to understand. This can lead to problems, with regulatory approval. Design of experiments is a tool but we need to use it carefully and make sure that we are presenting the results in a clear and standard way.[47]

Additionally, the AQbD framework, as currently practised, primarily addresses chromatographic and instrumental CMPs but may insufficiently account for sample preparation variability, reference standard characterisation uncertainty, and biological matrix effects in complex bioanalytical applications.[42] Future developments in AQbD methodology must address these gaps to extend the framework's reach across the full analytical procedural system, rather than the chromatographic separation alone.

7. Recent Advances and Future Perspectives

7.1 Integration with Artificial Intelligence and Machine Learning

The convergence of AQbD with artificial intelligence (AI) and machine learning (ML) represents one of the most exciting frontiers in modern pharmaceutical analytical science. Retention prediction models based on quantitative structure-retention relationships (QSRR); trained on large databases of experimental retention data ; now enable ML-assisted method scouting that can predict optimal chromatographic conditions for new chemical entities with no prior experimental data, dramatically accelerating the early scouting phase.[48] Deep learning architectures, including convolutional neural networks applied to chromatogram pattern recognition, have demonstrated the ability to automatically detect, identify, and quantify co-eluting impurity peaks with superhuman accuracy, potentially augmenting human data review in both development and routine quality control.[49]

Bayesian optimisation algorithms have been integrated with DoE platforms to enable sequential, adaptive experimental design, in which the algorithm selects the next experiment based on the current posterior model of the response surface, concentrating experiments in the most informative regions and achieving MODR identification with as few as 30–40% of the runs required by conventional non-adaptive RSM designs.[50] These AI-augmented AQbD workflows are beginning to appear in published pharmaceutical method development reports and are expected to become mainstream within the coming decade as computational tools become more accessible and as regulatory guidance matures.

7.2 Process Analytical Technology (PAT) & Real-Time Monitoring

The integration of with PAT tools like in-line and at-line spectroscopic sensors real-time pH and conductivity monitoring and automated systems enables continuous monitoring of pharmaceutical manufacturing streams. This happens without needing to collect samples and do offline HPLC analysis.[51] The design space concept, central, to AQbD works well with PAT-enabled real-time release testing frameworks. Here the defined MODR provides boundaries for using real-time sensor readings to confirm product quality attributes. This helps make release decisions

without waiting for HPLC results.[52] The FDA supports RTRT as seen in the 2004 PAT Guidance and Quality Metrics initiative. This shows that AQbD works well with PAT-integrated manufacturing environments.

7.3 Automation and High-Throughput Platforms

Automated liquid handling systems and robotic sample preparation platforms and throughput analytical screening instruments have changed the way things work in pharmaceutical analytical laboratories. Automation and High-Throughput Platforms like these have made a difference.[53]

Platforms that can do things like 96-well plate-format HPLC screening or automated phase preparation and injection scheduling are very helpful. These Automation and High-Throughput Platforms have made it possible to do comprehensive Box-Behnken or CCD experiments in a few days. Before these experiments used to take weeks. This is really good for AQbD, in development programmes where time's very important. Coupled with laboratory information management systems (LIMS) that automatically capture, curate, and export experimental data in DoE-compatible formats, these automation platforms promise to remove many of the practical barriers to AQbD adoption cited by analytical scientists.[54]

7.4 Lifecycle Management and Continual Improvement

The AQbD framework's natural extension into the analytical lifecycle management phase ; supported by USP <1220>, ICH Q10, and the draft ICH Q14 ; positions it as the foundation for a continual improvement culture in pharmaceutical analytical science.[14] Ongoing performance verification (OPV) programmes, wherein statistically designed control charts monitor key CAAs (e.g., system suitability resolution, peak tailing, and %RSD of system precision injections) over time, enable early detection of performance drift attributable to column ageing, reagent lot variation, or instrument degradation, before out-of-specification results occur.[55] The AQbD design space provides the theoretical framework for interpreting OPV trends: drift towards MODR boundaries can trigger proactive investigation and corrective action, while drift within the interior of the MODR is acknowledged as normal operational variability requiring no regulatory response. This integrated lifecycle approach represents the fullest realisation of the AQbD philosophy and is expected to become standard practice as regulatory expectations evolve.

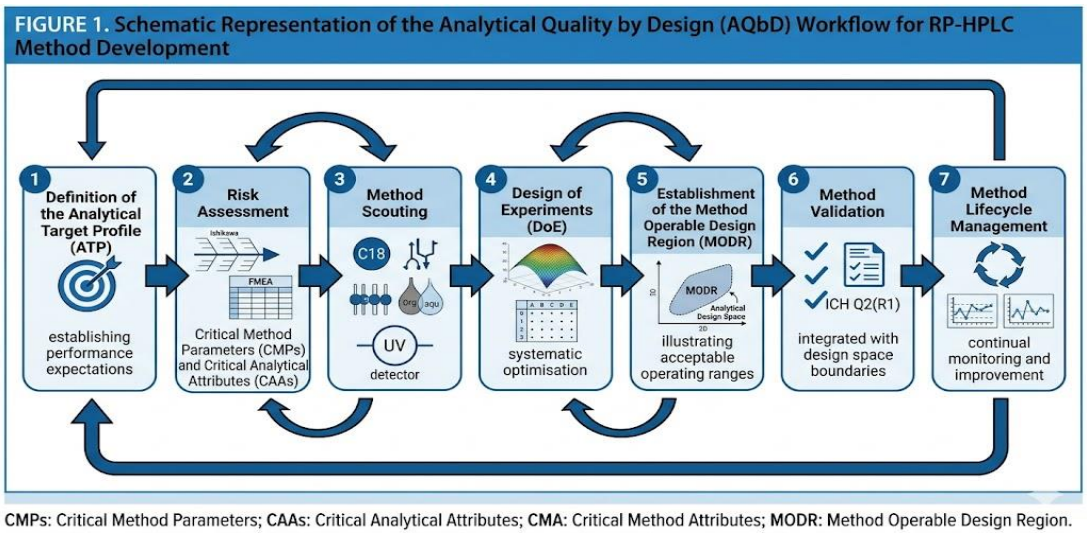

Figure 1: Schematic Representation of the Analytical Quality by Design (AQbD) Workflow for RP-HPLC Method Development.

8. CONCLUSION

This review has taken a look at the Analytical Quality by Design framework and how it is used to develop, validate and manage the lifecycle of RP-HPLC methods in pharmaceutical analysis. Analytical Quality by Design is based on the rules set by ICH Q8, Q9, Q10 and the new ICH Q14. It is a big change from the traditional way of developing methods, which was mostly trial and error. Analytical Quality by Design is an scientific way of building quality and robustness into analytical procedures from the start.

The Analytical Quality by Design workflow, which includes defining the Analytical Target Profile, assessing risks using Design of Experiments establishing the Method Operable Design Region and validating the method over its lifecycle has been used in different pharmaceutical analytical applications. These applications include testing the purity of a substance identifying impurities testing the stability of a substance and analyzing samples. The methods developed using Analytical Quality by Design are more robust, flexible and transparent than those developed using methods.

There are some challenges to using Analytical Quality by Design, such as the need for a lot of resources upfront the need for expertise in statistics and the lack of templates for reporting results to regulatory agencies. However the pharmaceutical analytical community has made a lot of progress in addressing these challenges. For example they have developed automated systems for Design of Experiments. Used artificial intelligence to model the retention of substances. They have also created guidance documents to help companies implement Analytical Quality by Design consistently.

The finalization of ICH Q14 is expected to lead to submissions using Analytical Quality by Design as regulatory agencies will provide clear guidance on what they expect. In the future combining Analytical Quality by Design with intelligence, Process Analytical Technology and automated high-throughput platforms will speed up the development of analytical methods and give us a deeper understanding of these methods. The idea of an integrated analytical lifecycle, from the first time a substance is tested in humans to after it is on the market is now possible with Analytical Quality, by Design. The pharmaceutical analytical community should adopt Analytical Quality by Design not to comply with regulations but as a way to improve the quality, reliability and longevity of analytical procedures, which will ultimately help keep patients safe.

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