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Review article

Quality-by-design (QbD) approach for HPLC analytical method development: principles, methodology, regulatory perspectives, and future directions

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ABSTRACT

Quality-by-Design (QbD) has transformed pharmaceutical analytical development by moving from empirical, trial-and-error approaches to systematic, science-based frameworks. This review presents a comprehensive review on the application of QbD principles, also known as Analytical Quality by Design (AQbD), to the development of high-performance liquid chromatography (HPLC) methods. This article is about the basic concepts of AQbD, such as Analytical Target Profile (ATP), Critical Method Attributes (CMAs), Critical Method Parameters (CMPs) and importance of risk assessment and Design of Experiments (DoE) in the development of robust methods. The methodological workflow is described from initial scoping through to the establishment of the Method Operable Design Region (MODR) and the implementation of lifecycle control strategies. Detailed coverage of regulatory views such as the enhanced approach in ICH Q14 and the USP Chapter <1220> lifecycle framework. Recent applications in pharmaceutical dosage forms, bioanalytical and stability indicating methods are reviewed to show the versatility and effectiveness of AQbD. The combination of AQbD and Green Analytical Chemistry (GAC) principles as a new paradigm for sustainable method development is discussed. Emerging trends like integration of artificial intelligence, green analytical chemistry principles and continuous manufacturing paradigms are explored as future directions. AQbD is an indispensable paradigm for modern HPLC method development, providing improved robustness, regulatory flexibility, and analytical efficiency, while guaranteeing consistent product quality and patient safety, the review concludes.

Keywords: Analytical quality by design, Design of experiments, Method operable design region, ICH Q14.

INTRODUCTION

High-performance liquid chromatography (HPLC) is still one of the most powerful and versatile analytical tools in pharmaceutical development and quality control, because it can separate, identify and quantify chemical components in complex matrices. It is applied in drug substance and product testing, impurity profiling, stability studies and bioanalytical investigations. However, the development of robust, reliable and regulatory-compliant HPLC methods has traditionally been a challenging task, often relying on trial-and-error or one-factor-at-a-time (OFAT) approaches that are time-consuming, resource-intensive, and often result in methods with limited understanding of the critical parameter interactions.

The Quality by Design (QbD) paradigm was initially introduced in the manufacturing industry and subsequently adopted by the pharmaceutical industry as a paradigm shift to overcome these challenges. The International Council for Harmonisation (ICH) defines QbD as “a systematic approach to development that begins with predefined objectives and emphasises product and process understanding and process control, based on sound science and quality risk management”. This framework, when applied to analytical method development, is referred to as Analytical Quality

by Design (AQbD). AQbD is a systematic, risk-based, and data-driven approach to ensure that analytical methods are fit for purpose throughout the lifecycle of the method.

The historical evolution of QbD can be traced back to Shewhart's introduction of quality control concepts in 1924, and Juran's later establishment of the fundamental principle that "Quality should be designed into a product, and most quality problems are related to how a product was initially designed". Formal regulatory adoption of QbD in the pharmaceutical sector began in 2004-2005 with the introduction and publication of ICH Q8 (Pharmaceutical Development) by the US Food and Drug Administration (FDA). Since then, the framework has been progressively expanded by ICH guidelines Q9 (Quality Risk Management), Q10 (Pharmaceutical Quality System) and most significantly, for analytical method development, Q14 (Analytical Procedure Development) and Q2(R2) (Analytical Validation).

The application of AQbD in HPLC method development represents a fundamental paradigm shift. Unlike the acceptance of results from empirical screening, the AQbD approach emphasises the understanding of the cause-and-effect relationships between method parameters and performance attributes, the definition of design spaces where method performance is consistently guaranteed, and the development of lifecycle management strategies for continued improvement. This systematic approach has many benefits: increased robustness of the method, decreased incidence of out-of-trend or out-of-specification results, regulatory flexibility, cost benefits through reduced experimental efforts and better scientific understanding of method performance.

The pharmaceutical industry is facing growing pressure to deliver not only analytical reliability but also to address environmental sustainability. Traditional HPLC methods consume large volumes of organic solvents. The US pharmaceutical industry alone reports methanol and dichloromethane waste of 44.8 million kg/year and 22.3 million kg/year, respectively. The combination of AQbD with the principles of Green Analytical Chemistry (GAC) has become a game-changing approach to meet both analytical quality and environmental sustainability at the same time.

This review presents a thorough assessment of the AQbD approach for the development of analytical methods in HPLC. It introduces the basic principles and key concepts, explains the methodological process from initial design to lifecycle management, addresses the regulatory background for AQbD implementation, reviews recent applications in different analytical fields, and discusses emerging trends and future perspectives [1, 2].

Principles of analytical quality by design

Definition and scope of AQbD

Analytical Quality by Design is a systematic approach to analytical method development that starts with predefined objectives and focuses on method understanding and method control, based on sound science and quality risk management, to ensure a method's fitness for purpose throughout its lifecycle. In contrast to traditional Quality by Testing (QbT) methods that depend on testing the final product, AQbD builds quality into the method from the start through systematic planning, experimentation and knowledge management.

The AQbD approach is based on the basic principles of the general QbD framework, as described in ICH guidelines Q8-Q12. But it is precisely tailored to the analytical context, addressing the specific challenges of developing separation methods, especially HPLC, with the complex multiparameter interactions between mobile phase composition, stationary phase characteristics, flow rate, temperature and detection parameters.

Recent regulatory developments have validated the AQbD framework. The ICH Assembly adopted the ICH Q14 guideline (Analytical Procedure Development) in 2023, harmonising the guidance on the advanced approach to method development incorporating AQbD principles. Simultaneously, ICH Q2 (R2) was released, which contained a great deal more information on the expectations for analytical procedure validation studies than the previous version. USP general chapter <1220> (Analytical Procedure Life Cycle) outlines a three-stage framework for analytical procedure control: design and development, performance qualification, and ongoing performance verification.

Core concepts and terminology

Analytical target profile (ATP)

The backbone of the AQbD approach is the ATP (a prospective summary of the intended purpose and performance criteria of the method). It specifies what is to be measured and the performance parameters required (precision, accuracy, range and detection limits) but not the analytical technique to be used. For example, an ATP for impurity profiling might state: "The analytical procedure should be capable of quantifying all specified related substances and degradation products from the reporting limit to 120% of their specification limit with an accuracy of ±20% of the true value". Regulatory flexibility is supported by regulatory authorities' ATP approval to allow continuous method improvement.

The analytical procedure is based on the ATP and determines the analytical technology to be used. Several analytical techniques may be available that meet the performance criteria. Technology selection should consider the operating environment (e.g., at-line, in-line, off-line).

Critical method attributes (CMAs)

CMAs are the measured responses that control the performance of the method and provide a strong link between the intended use of the method and the performance criteria defined in the ATP. These attributes can be considered as method specifications and are a function of the analytical technique, working principle and purpose of the method.

For impurity-profiling HPLC methods, the critical CMA is generally the resolution of the critical peak pair (Rs), which can be expressed as time differences between peaks or their selectivity factors (α). In assay methods where quantitative accuracy is of the utmost importance, CMAs are often related to peak shape parameters, namely tailing factor (T) or theoretical plate count (N). Retention time, peak area and signal-to-noise ratio are other CMAs.

Critical method parameters

CMPs, the method parameters that have a strong impact on the CMAs and therefore need to be controlled to ensure consistent method performance. In developing the HPLC method, the CMPs are generally mobile phase composition, organic modifier ratio, buffer concentration, pH, flow rate, column temperature, injection volume, and detection wavelength.

The definition of CMPs is an important step in the AQbD workflow as these parameters are systematically studied by Design of Experiments. The relationship between CMPs and CMAs is defined by establishing mathematical models that allow prediction of method performance under different conditions [3].

Method operable design region

The MODR is a multidimensional combination of CMPs that has been shown to assure method quality. It describes the design space within which the method will be robustly performing and within which method parameters may be varied without resubmission to the regulatory authority. The MODR is defined using systematic experimentation and statistical analysis, generally through response-surface methodology, to establish the boundaries where CMAs satisfy predefined acceptance criteria.

The MODR offers a large amount of regulatory freedom, because changes within the established design space are not considered regulatory changes, which is a major advantage over traditional fixed-condition approaches.

Risk assessment in AQbD

Risk assessment is an important part of the AQbD methodology and helps to make decisions from the early development of a method to its lifecycle management. Risk assessment aims to identify and prioritise the possible factors that could adversely affect the performance of the method such that the investigation can be focused and appropriate control strategies can be implemented. Several risk assessment tools are commonly employed in AQbD for HPLC method development:

Ishikawa (Fishbone) diagrams

This tool provides a systematic approach to identify potential factors that influence method performance and organises those factors into areas including instrumentation, materials, methods, measurements, laboratory environment, and human factors.

Failure mode and effects analysis (FMEA)

FMEA is a step-by-step method for gathering knowledge about the ways a system might fail and the consequences of failures on system performance. Every potential parameter is evaluated by three criteria: probability of occurrence, severity of Impact on CMAs and detectability of the failure mode. These are multiplied to form a Risk Priority Number (RPN) which prioritises parameters for further investigation.

In a practical bioanalytical method development exercise, FMEA was applied to screen eleven method parameters for alectinib determination in rat plasma. The parameters with RPN values above 20, such as injection volume, ratio of organic phase, pH of mobile phase, buffer concentration, flow rate, temperature of column and sample pretreatment method, were selected for further investigation with DoE.

In the risk assessment step, those variables that are most likely to impact method performance are systematically identified and ranked. All possible method variables, including mobile phase composition, pH, column temperature, flow rate and injection volume, are screened by means of tools such as the Ishikawa diagram and FMEA and ranked in terms of likelihood of occurrence, severity of impact and detectability. Such a systematic assessment enables the analyst to identify critical method parameters (CMPs) and non-influential parameters. The significant variables are then carried forward to the Design of Experiments (DoE) optimisation step, which reduces the experimental load and enhances method robustness and regulatory compliance [4].

AQbD methodology for HPLC method development

Systematic workflow overview

The AQbD approach to HPLC method development is a systematic, step-wise process that combines risk assessment, experimentation, and statistical analysis. The workflow generally consists of five defined stages:

Stage 1: Definition of the analytical target profile

Defining the objective and performance criteria of the method, such as analytes to be measured, selectivity, sensitivity, accuracy, precision and range. The ATP drives the analytical technology selection and is a basis for deriving analytical procedure attributes and performance criteria for validation.

Stage 2: Risk assessment and selection of method parameters

Identification and prioritisation of potential CMPs through the use of prior knowledge and risk assessment tools such as FMEA or Ishikawa diagrams, establishing CMAs describing the method

performance. Such an exhaustive analysis allows to distinguish the crucial parameters from the non-influencing ones, considerably decreasing the experimental effort.

Stage 3: Method development and optimization via design of experiments

Screening designs to identify important factors from a large set of variables, followed by response-surface experimental designs (e.g., Box-Behnken, Central Composite) to optimise CMPs and develop mathematical models that describe the relationship between parameters and CMAs. Multivariate experiments can be performed to obtain a complete picture of the method with minimum experimentation.

Stage 4: Establishment of MODR and control strategy

Defining the multidimensional space of design in which method performance is consistently assured and developing a control strategy that ensures continued method performance within the MODR. The MODR is the multidimensional combination of CMP settings that reliably meets the ATP requirements, while allowing flexible regulation and supporting lifecycle management.

Stage 5: method validation and lifecycle management

Method validation according to ICH Q2(R2) and ongoing performance verification to ensure method suitability throughout the method's lifecycle. Development studies data (e.g. robustness data from DoE studies) may be included as part of validation data, and there is no need to repeat studies [5].

Experimental designs in AQbD

The statistical backbone of the AQbD methodology is the Design of Experiments (DoE), which enables systematic investigation of CMPs and their impact on CMAs. DoE looks at several factors and their interactions together, giving a full picture with fewer experiments than traditional OFAT methods that change one parameter at a time.

Screening designs

Screening designs are employed in the early stages to efficiently identify significant factors from a potentially large set of variables. Common screening designs include:

Plackett-burman design

A two-level, highly efficient design for screening a large number of factors with minimal runs. This design is particularly useful when many potential factors must be evaluated.

Factorial designs

Full or fractional factorial designs allow estimation of main effects and interactions. A 2^k factorial design investigates k factors at two levels, while fractional designs reduce the experimental burden while maintaining the ability to identify significant factors.

The Taguchi method has also been employed for screening, as demonstrated in the development of an HPLC-FLD method for alectinib, where an L8 orthogonal array efficiently screened seven CMPs at two levels.

Response surface designs

Following screening, response surface methodology is employed to optimize CMPs and develop predictive mathematical models. These designs provide estimates of quadratic effects that describe curvature in the response surface:

Box-behnken design

A widely used three-level design requiring fewer runs than Central Composite designs while efficiently fitting quadratic response surfaces. BBD has been extensively employed in recent AQbD applications, including the optimization of pH, organic modifier concentration, and flow rate for simultaneous estimation of Deflazacort and Tamsulosin. In a study for aloe-emodin estimation, Box-Behnken design explained the relationships between flow rate, buffer concentration, and column temperature at three distinct levels, with responses monitored including retention time (Rt), tailing factor (Tf), and number of theoretical plates [6].

Central composite design

A robust design that allows estimation of quadratic effects and provides good prediction capability across the design space. CCD was employed for Lumateperone Tosylate method development, using two independent factors—buffer pH and mobile phase composition—to evaluate critical method attributes including retention time, peak area, and symmetry factor.

Mixture designs

Employed when the composition of the mobile phase components is the primary focus of investigation.

The application of BBD in HPLC method optimization typically involves establishing the experimental matrix, conducting experiments in randomised order, and fitting a second-order polynomial model to the data. Statistical analysis, including ANOVA, validates the model's adequacy and provides insights into significant factor effects and interactions.

Key CMPs investigated in HPLC method optimization

The selection of CMPs for investigation depends on the analytical context and the nature of the separation challenge. Recent AQbD applications have investigated a range of parameters:

Mobile phase composition

Including organic modifier percentage (e.g., acetonitrile or methanol), buffer concentration, and pH. For reversed-phase HPLC, the choice of organic modifier and its proportion significantly impact separation selectivity due to differences in solvatochromic properties. Acetonitrile possesses a higher dipole moment and acidity, while methanol is less polar and more basic, with slightly weaker elution strength.

Flow rate

Affecting both separation efficiency and analysis time. Optimization typically balances resolution with run time.

Column temperature

Influencing retention and selectivity, with temperature control providing additional method robustness.

Detection parameters

Including detection wavelength and injection volume, particularly relevant for sensitivity optimisation.

Establishment of MODR and control strategy

The Method Operable Design Region represents the culmination of the experimental phase of AQbD development. The MODR is the multidimensional combination of the CMPs that ensures method quality and is typically represented as the region of the design space where the CMAs meet predefined acceptance criteria.

Response-surface experiments-based mathematical models are used to predict the behaviour of CMA in the design space. The MODR is determined by computer-aided optimisation taking into account the confidence levels and tolerance intervals of the predictions using these models. The MODR assures method robustness by defining boundaries, within which acceptable method performance is reliably attained, despite the presence of expected variability in method conditions.

An analytical control strategy is developed after the MODR is established. The control strategy includes planned controls based on the knowledge of the method to ensure process performance and method quality. It usually consists of:

System Suitability Requirements: Defined operating ranges for critical parameters.

Quality Control Procedures: Procedures for ensuring continued method performance.

Lifecycle Management Plans: Processes for method transfer, monitoring, and continuous improvement.

The control strategy includes set points and/or ranges for relevant analytical procedure parameters. These could include Proven Acceptable Ranges and/or Method Operable Design Regions (MODRs). Applying elements of the enhanced approach to development can lead to better understanding of the impact of analytical procedure parameters on performance and more flexibility for lifecycle management [7].

Regulatory perspectives and guidelines

ICH Guidelines

The regulatory framework for AQbD implementation in HPLC method development is established through a series of ICH quality guidelines that provide harmonised approaches for pharmaceutical quality and analytical development.

ICH Q8 (Pharmaceutical development)

Introduces the QbD concept and emphasizes product and process understanding. While primarily focused on drug products, it establishes the foundational principles that underpin AQbD.

ICH Q9 (Quality risk management)

Provides principles and tools for risk assessment, which are integral to the AQbD approach. The systematic risk-based approach to method development is guided by the principles of ICH Q9.

ICH Q10 (Pharmaceutical quality system)

Establishes a lifecycle approach to quality management, supporting the continuous improvement and lifecycle management principles of AQbD.

ICH Q14 (Analytical procedure development)

This guideline, endorsed by the ICH Assembly in November 2023, specifically addresses analytical procedure development and describes two approaches: the minimal approach (traditional) and the enhanced approach that incorporates AQbD principles. The enhanced approach involves comprehensive method understanding, risk-based development, DoE, and establishment of a MODR—providing the framework for AQbD implementation. The guideline also describes additional considerations for the development of multivariate analytical procedures and for real-time release testing.

ICH Q2(R2) (Analytical validation)

Guides method validation requirements, including the validation attributes relevant to AQbD-developed methods. Released in December 2023, this revision provides significantly more detail regarding expectations for analytical procedure validation studies, including illustrative examples of analytical procedures for testing products and appropriate validation criteria for each.

The ICH Q14 document emphasises that knowledge gained from application of an enhanced approach to analytical procedure development can provide better assurance of the performance of the procedure, serve as a basis for the analytical procedure control strategy, and provide an opportunity for more efficient regulatory approaches to related post-approval changes. Among ICH Q14 recommendations, the need for knowledge assessment, multivariate models for proven acceptable ranges as method operable regions, sample suitability assessment in robustness, and real-time release testing with product critical quality attribute specifications are highlighted as challenging components for pharmaceutical industries.

USP framework: analytical procedure lifecycle

USP general chapter <1220>, entitled "Analytical Procedure Life Cycle," provides a complementary framework for AQbD implementation. It establishes a three-stage lifecycle approach:

Stage 1

Procedure Design and Development: Encompasses defining the ATP, selecting the analytical technique, identifying CMPs and CMAs, risk assessment, method development and optimization, and establishing the MODR. This stage ensures that quality is built into the procedure from its inception.

Stage 2

Procedure Performance Qualification: Involves demonstrating that the method meets its predefined performance criteria as defined in the ATP. This is equivalent to traditional method validation but is framed within the lifecycle approach.

Stage 3

Ongoing Performance Verification: Ensures continued method suitability through risk-based monitoring of performance-related data, facilitating early detection of performance drift. This stage recognises that method performance should be monitored throughout its lifecycle, not just at the point of validation.

The USP framework emphasises the importance of knowledge management and change management throughout the lifecycle [8].

FDA guidance

The FDA has actively encouraged the implementation of AQbD principles. In 2015, the FDA recommended systematic robustness studies starting with risk assessment and multivariate experiments like DoE, emphasizing the importance of managing analytical procedure lifecycles post-validation. FDA guidance documents provide specific recommendations for HPLC method validation, including performance characteristics, validation study methodologies, and robustness evaluation requirements. These emphasize that robustness studies should be performed as part of analytical procedure development, involving deliberate variation of relevant parameters such as mobile phase composition, pH, column temperature, and flow rate.

The FDA adopted ICH Q14 in March 2024 as guidance for industry, recommending the science- and risk-based approaches for developing and maintaining analytical procedures suitable for evaluating the quality of drug substances and drug products.

ICH Q2 (R2) and Q14 integration

The ICH Q2 (R2) and Q14 guidelines are closely related designed to be implemented together. ICH Q2 (R2) provides the validation framework, while Q14 provides the development and lifecycle management framework. A comprehensive set of training materials has been delivered by the ICH Q2 (R2)/Q14 Implementation Working Group (IWG) to ensure proper interpretation and aligned understanding of the new guidelines by industry and regulators.

The integration of these guidelines supports a holistic approach to analytical procedure lifecycle management, where development, validation, and ongoing performance verification are viewed as a cohesive and interconnected progression. This integration encourages effective knowledge and risk management as crucial facilitators throughout the analytical procedure lifecycle.

Integration of AQbD with green analytical chemistry

Rationale for integration

The integration of AQbD with Green Analytical Chemistry (GAC) principles represents a transformative approach in HPLC method development and validation. Traditional HPLC methods are resource-intensive and ecologically challenging, consuming large volumes of organic solvents and generating substantial waste. The traditional HPLC technology produces about 500 litres of organic solvent waste per instrument per year.

The AQbD framework provides a structured approach to method optimization, while GAC focuses on minimising hazardous solvent use, energy consumption, and waste production. When combined, these approaches create a philosophy of "Quality and Green by Design" where analytical performance criteria and sustainability metrics are optimized together in one continuous design and control framework.

Integration framework

Within this multidisciplinary integrated system, green objectives are directly embedded within the Analytical Target Profile (ATP); parameters of eco-impact are considered when composing the Design of Experiments (DoE); and greenness measures such as AGREE and the Eco-Scale are reflected in the control strategy as well as the lifecycle management of the analytical method.

The integration involves

Green objectives in ATP

Sustainability parameters are incorporated into the method's intended purpose and performance criteria

Green considerations in DoE

Environmental impact parameters are included alongside analytical performance parameters during method optimization

Greenness assessment in control strategy

Metrics such as Analytical Eco-Scale, AGREE, and GAPI are used to evaluate and monitor environmental sustainability throughout the method lifecycle

Greenness assessment metrics

Several quantitative metrics have been developed to assess the greenness of analytical methods:

Analytical eco-scale

Uses penalty points for waste, energy usage, and hazardous chemicals, starting at 100; methods with higher scores above 75 are deemed to be excellent in the context of green performance.

GAPI (green analytical procedure index)

Uses a pentagram with colour coding to assess the environmental impact of each step in the procedures, from sample collection through waste disposal.

AGREE (analytical greenness) metric

Synthesises all twelve GAC principles into a 0–1 scale, providing a comprehensive picture of the sustainability profile of a method.

BAGI (blue applicability grade index)

Considers elements associated with the scope of analysis and practicality.

White analytical chemistry (WAC)

Combines aspects of analytic performance, environmental quality, and practical use using an RGB system. In this model, red denotes the quality of the analysis (accuracy, precision, sensitivity), green denotes environmental and safety-oriented aspects (toxicity, waste, energy consumption), and blue measures practicality and economic efficiency (cost, time, simplicity). The synthesis of the three parameters results in a measurable value of whiteness, offering a properly calibrated analysis tool that balances performance, sustainability, and pragmatism.

This integration not only reinforces regulatory compliance but is also highly consistent with international initiatives, such as the United Nations Sustainable Development Goals (SDG) 12 (Responsible consumption and production) and 13 (Climate action), thus showing the extended relevance of such scientific developments to society [9].

Recent applications of AQbD in HPLC method development

Pharmaceutical dosage form analysis

AQbD has been extensively applied to the development of HPLC methods for quality control of pharmaceutical dosage forms. These applications demonstrate the approach's ability to deliver robust, efficient methods for simultaneous estimation of multiple analytes.

Lumateperone toluate in capsules

A QbD-driven HPLC method was developed for Lumateperone Tosylate determination in bulk drug and capsule dosage form. The AQbD methodology utilized an Ishikawa diagram for risk assessment, identifying buffer pH and mobile phase composition as critical method parameters. A central composite design was employed for optimization, evaluating the effects of these independent factors on critical method attributes including retention time, peak area, and symmetry factor.

The optimized method employed a Zorbax SB C18 column with mobile phase comprising 10 mM ammonium acetate buffer (pH 3.2): acetonitrile (80:20 v/v) at 1 mL/min flow rate, with UV detection at 230 nm. Lumateperone Tosylate showed linearity in the concentration range of 25-250 µg/mL (r^2 = 0.9921). % RSD for interday and intraday precision was found to be 0.25-0.52 and 0.12-0.32, respectively. The % assay of drug content was found to be 100.01 ± 0.06, and accuracy was found to be 100.30-100.65%. In compliance with ICH recommendations, the optimized assay conditions were validated.

Aloe-emodin estimation

A QbD-facilitated RP-HPLC technique was developed for efficient analysis of aloe-emodin. The chromatographic conditions were optimised with Design Expert software 11.0, evaluating flow rate, buffer concentration, and column temperature. A Box-Behnken design explained the relationships between these parameters at three distinct levels, with responses monitored including retention time (Rt), tailing factor (Tf), and number of theoretical plates (NTPs). The results showed R^2 = 0.9988 for linearity, with LOQ of 0.07949 µg/mL and LOD of 0.02623 µg/mL. According to ICH rules, the technique validation parameters were within the allowed range.

Deflazacort and tamsulosin hydrochloride

A QbD-driven RP-HPLC method was developed for the concurrent estimation of Deflazacort and Tamsulosin hydrochloride in capsule dosage form. A Box-Behnken design was employed to optimise critical method parameters including mobile phase pH, organic modifier concentration, and flow rate. The optimised method yielded retention times of 3.3 minutes for Tamsulosin and 7.4 minutes for Deflazacort. Validation per ICH Q2(R2) guidelines confirmed excellent linearity, accuracy, precision, specificity, LOD, and LOQ.

Bioanalytical applications

AQbD has been successfully extended to bioanalytical method development, addressing the particular challenges of quantifying drugs in biological matrices such as plasma.

Alectinib quantification in rat plasma

A fluorescence-based HPLC method was developed for quantifying alectinib in rat plasma using AQbD principles. The study employed FMEA to prioritise potential parameters, identifying 11 method parameters for risk assessment. Seven parameters with RPN > 20 were screened using a Taguchi L8 orthogonal array, followed by optimization using a Box-Behnken design. Critical method parameters including organic phase ratio, buffer concentration, and flow rate were optimised. The final method demonstrated excellent linearity (R^2 > 0.99), high accuracy (95.6-102%), precision (RSD < 11%), consistent recovery (98.3-105%), and minimal matrix effects. The method was successfully applied in a pharmacokinetic study.

Stability-indicating methods

AQbD has been increasingly applied to the development of stability-indicating HPLC methods, where method robustness is particularly critical for assessing drug degradation over time. The integration of AQbD with stability studies enables the development of methods capable of resolving drug substances from their degradation products, ensuring accurate stability assessment and shelf-life determination. For impurity profiling applications, AQbD-optimized methods provide enhanced robustness within the MODR, reducing the occurrence of out-of-trend or out-of-specification results due to method-related factors.

Benefits and challenges of AQbD implementation

Advantages

Enhanced Method Robustness: AQbD-developed methods are inherently more robust, as the systematic investigation of CMPs and their interactions ensures method performance is maintained despite expected variability. This reduces the occurrence of out-of-trend or out-of-specification results due to method-related factors.

Regulatory Flexibility: Working within an established MODR provides significant regulatory flexibility, as changes within the design space are not considered regulatory changes. This facilitates continuous improvement, method transfer, and adaptation to new circumstances.

Reduced analytical costs

While requiring upfront investment in experimental design and statistical analysis, AQbD reduces overall analytical costs by minimizing experimental efforts in method development, reducing method failures, and decreasing revalidation requirements. It ensures optimal operation, robustness, easy validation, and cost-effectiveness by minimizing experimental efforts in the method development of chromatographic analysis.

Improved scientific understanding

AQbD provides a comprehensive understanding of method performance and the relationships between parameters and responses, enabling informed decision-making and problem-solving.

Lifecycle Management: The lifecycle approach inherent in AQbD ensures continued method suitability, supporting regulatory compliance and facilitating technology transfer.

Environmental Benefits: Integration with GAC principles enables development of methods that are both robust and environmentally sustainable, reducing solvent consumption, waste generation, and energy use.

Challenges

Statistical Expertise: AQbD implementation requires comprehensive understanding of statistical analysis and experimental design. The absence of standardized directives or regulatory requirements can hinder adoption. Its adoption can be hindered owing to the necessity for a comprehensive grasp of statistical analysis and experimental design, coupled with the absence of standardized directives or regulatory prerequisites.

Upfront investment

The methodology demands significant investment in time, resources, and expertise during the development phase, which can be challenging for organizations with limited resources.

Regulatory disparities

Despite increasing harmonization through ICH guidelines, differences in regulatory expectations between agencies can complicate AQbD implementation.

Cultural shift

AQbD requires a shift from traditional, experience-based method development to systematic, data-driven approaches, requiring changes in organizational culture and training.

Green chemistry integration challenges

Challenges persist in integrating AQbD with GAC, including inconsistent application of greenness metrics, limited availability of green solvent alternatives, and the need for integrated software tools that combine AQbD and GAC evaluation.

Emerging trends and future directions

Integration of artificial intelligence and machine learning

AI-driven approaches are emerging as transformative tools in AQbD implementation. Machine learning algorithms can identify complex relationships between method parameters and performance attributes that may not be apparent through traditional statistical approaches. Bayesian optimization and other AI methods are being explored to enhance design space exploration and optimize experimental designs.

Recent advances include model-based design of experiments (MBDoE) for isotherm model identification in HPLC chromatography. This methodology aims to identify the most suitable isotherm model, estimate its parameters, and evaluate its predictive capability. MBDoE has been shown to require fewer experiments than traditional factorial design of experiments while achieving comparable or superior results in reducing parametric uncertainty.

Green analytical chemistry integration

The integration of AQbD with GAC principles is gaining significant momentum. The hybrid approach aims to develop methods that are both robust and environmentally sustainable. Greenness assessment tools such as AGREE, GAPI, AMGS, and Analytical Eco-Scale are increasingly applied alongside AQbD to evaluate environmental impact. The combination of AQbD and GAC not only ensures analytical reliability and regulatory compliance but also aligns with global sustainability goals and emerging eco-conscious scientific practices.

Case studies on pharmaceutical analytes such as metronidazole, nicotinamide, irbesartan, metformin, and empagliflozin illustrate the practical implementation of these approaches, with several studies reporting high green scores and regulatory alignment with ICH guidelines. Future directions include extending AQbD-GAC approaches to complex matrices and integrating Artificial Intelligence to enhance optimization and sustainability.

UHPLC method development

The application of AQbD to UHPLC method development represents a significant trend. UHPLC, with its sub-2 μm particle columns and high-pressure operation, provides enhanced throughput, resolution, and sensitivity, while AQbD ensures method robustness.

Recent reviews have documented the growing application of AQbD in UHPLC, examining stationary phase selection, mobile phase composition trends favouring water-acetonitrile combinations, and optimized flow rates (0.2-0.5 mL/min).

Biopharmaceutical applications

The extension of AQbD to biopharmaceutical method development is advancing rapidly. Analytical techniques for biologics encompassing molecular structural characterisation, purity and impurities analyses, and function and stability assessment benefit from AQbD's systematic framework. AQbD-based methods for biologics offer greater robustness within the MODR, reducing out-of-trend results and enhancing regulatory flexibility.

Continuous manufacturing and real-time release

The integration of AQbD with continuous manufacturing paradigms represents a significant frontier. AQbD principles provide the framework for understanding and controlling analytical methods in continuous processes, supporting real-time release testing and adaptive control. ICH Q14 describes additional considerations for the development of multivariate analytical procedures and for real-time release testing.

Model-based design of experiments

Model-Based Design of Experiments (MBDoE) is emerging as an advanced approach for efficient and accurate method optimization. MBDoE-based methodology aims to identify the most suitable mathematical models, estimate parameters, and evaluate predictive capability. This approach has been tested on in silico case studies where performance was compared to that of traditional factorial design of experiments, demonstrating that MBDoE requires fewer experiments to achieve comparable or superior results [10].

CONCLUSION

The Quality-by-Design approach to HPLC analytical method development, formalized as Analytical Quality by Design, represents a fundamental paradigm shift in pharmaceutical analysis. Moving beyond empirical, trial-and-error methods, AQbD provides a systematic, risk-based, and data-driven framework that ensures analytical procedures are fit for purpose throughout their entire lifecycle. The core elements—Analytical Target Profile, Critical Method Attributes, Critical Method Parameters, Method Operable Design Region, and lifecycle control strategies—provide a structured approach grounded in sound science and quality risk management.

Recent regulatory developments, particularly ICH Q14 and USP Chapter <1220>, have provided harmonized guidance and formal recognition of the enhanced AQbD approach. The implementation of AQbD in HPLC method development delivers substantial advantages: enhanced method robustness, regulatory flexibility, reduced analytical costs, improved scientific understanding, and effective lifecycle management. Applications spanning pharmaceutical dosage form analysis, bioanalytical studies, and stability-indicating methods demonstrate the versatility and effectiveness of the approach.

The integration of AQbD with Green Analytical Chemistry principles represents an important emerging paradigm, enabling the development of methods that are both analytically robust and environmentally sustainable. This integration aligns with global sustainability goals and addresses the growing imperative for eco-conscious scientific practices.

While challenges remain—including the need for statistical expertise, upfront investment, and organizational cultural shifts—the benefits of AQbD far outweigh these obstacles. Emerging trends, including artificial intelligence integration, model-based design of experiments, UHPLC applications, biopharmaceutical development, and continuous manufacturing, point toward an increasingly sophisticated future for AQbD.

Ultimately, AQbD represents not merely a regulatory requirement but a fundamental commitment to analytical quality that benefits patients through enhanced product safety and efficacy, manufacturers through improved efficiency and compliance, regulatory agencies through robust, science-based submissions, and the environment through sustainable analytical practices. As the pharmaceutical industry continues to evolve, the adoption of AQbD principles in HPLC method development will become increasingly standard practice, driving continuous improvement in analytical quality and patient outcomes.

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