J Pharm Biopharm Res, 2025, 7(1): 511-524
DOI: 10.25082/JPBR.2025.01.001

REVIEW

Analytical Quality by Design (AQbD) for Quality and Risk Assessment of Pharmaceuticals to Immunomarkers

Ritu Tiwari[1*] **Gaurav Sanjay Mahalpure**[1] **Shaily Tyagi**[1] **Meenakshi Dahiya**[1*] **Vivekanandan Kalaiselvan**[1]

[1] Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad 201002, Uttar Pradesh, India

Correspondence to: Ritu Tiwari, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad 201002, Uttar Pradesh, India; Email: ritutiwari.ipc@gov.in
Meenakshi Dahiya, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad 201002, Uttar Pradesh, India; Email: meenakshi.ipc@gov.in

Received: June 5, 2025;
Accepted: October 10, 2025;
Published: October 17, 2025.

Citation: Tiwari R, Mahalpure GS, Tyagi S, et al. Analytical Quality by Design (AQbD) for Quality and Risk Assessment of Pharmaceuticals to Immunomarkers. *J Pharm Biopharm Res*, 2025, **7**(1): 511-524. https://doi.org/10.25082/JPBR.2025.01.001

Abstract: Using advanced scientific and risk-based approaches for analytical methods offers significant benefits. Quality by Design (QbD) is a systematic framework that emphasizes understanding and controlling both product and process elements. Adhering to the principles outlined in the ICH guidelines can considerably improve the quality of drug substances, vaccines, immune markers, and medicinal products. This compliance not only enhances product quality but also drives continuous improvement and innovation throughout the entire product lifecycle. Developing and regulating analytical methods are vital for maintaining high standards of product quality. By employing sophisticated scientific techniques and risk-based strategies, stakeholders can use various analytical methods to ensure the consistent production of high-quality active pharmaceutical ingredients (APIs). Techniques such as size exclusion chromatography (SEC), high-performance liquid chromatography (HPLC), gel electrophoresis, Western blotting, SDS-PAGE, and enzyme-linked immunosorbent assays (ELISA) are essential for boosting industrial efficiency and reliability in the pharmaceutical sector. Each technique supports the rigorous testing and validation required for API production, ultimately facilitating the development of safe and effective medical products. Understanding the impact of variability on the performance and results of analytical methods is crucial. The QbD framework adopts a systematic approach that underscores the need to thoroughly understand and effectively manage various aspects of the product and its manufacturing processes–laying the groundwork for ongoing improvements and innovation throughout the product lifecycle. Additionally, strict development and regulation of analytical methods are key to achieving the highest standards of product quality. Fully grasping how variability influences the performance and outcomes of analytical methods is essential, as this understanding optimizes results and maintains consistent quality. Recognizing these dynamics enhances result reliability and supports the goal of delivering safer, more effective healthcare solutions.

Keywords: analytical quality by design, quality risk management, pharmaceutical quality system, critical quality attributes, experiment design

1 Introduction

Many new drug applications (NDAs) that incorporate regulatory flexibility through a quality-by-design (QbD)-based analytical approach have recently received approval from the Food and Drug Administration (FDA) [1]. Analytical quality by design (AQbD) is the term used to describe this QbD concept specifically applied to developing analytical methods. Techniques like chromatography–specifically High-Performance Liquid Chromatography (HPLC), Size Exclusion Chromatography (SEC), and Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)–play a crucial role in the analysis and purification of compounds. Additionally, mass spectrometry (MS) and a variety of immunological testing methods, including Enzyme-Linked Immunosorbent Assay (ELISA), gel electrophoresis, Western blotting, and SDS-PAGE, are employed extensively. These methodologies are essential in the comprehensive processing of Active Pharmaceutical Ingredients (APIs), which can range from extracting medicinal compounds from plants to the intricate development of vaccines, tailored to meet the unique requirements of each product type. The analytical method can operate within the method operable design region (MODR) in this approach, enabling applications such as API drug candidate production, purification, formulation, fill, and finish product development. Compared to existing techniques, an analytical method developed using the AQbD approach reduces the frequency

of out- of- trend (OOT) and out-of-specification (OOS) results due to its robustness [2, 3]. In the pharmaceutical industry, AQbD is commonly utilised in the development of analytical methods. It forms part of the pharmaceutical quality system, encompassing development and risk management. Due to the scarcity of explanatory reviews, this study aims to explore different analytical scientists' perspectives on applying AQbD within the pharmaceutical quality system and its relationship to product QbD and pharmaceutical analytical technology (PAT). Product QbD *vs* Analytical QbD *vs* Conventional approach in Table 1 [4].

Table 1 Product QbD versus Analytical QbD versus Conventional Approach – cont'd

Sr. No.	Parameter	Product QbD	AQbD	Traditional
1.	Specifications	Specifications are based on product performance requirements	based on method performance to analytical target profile (ATP) criteria	Specifications are based on batch history
2.	FDA Submission	Submission with product knowledge and process understanding	Submission with product knowledge and assurance by the analytical target profile	Including only data for submission
3.	Targeted Response	Focusing on robustness, which involves understanding control variation	Focus on a robust and cost-effective method	Focusing on reproducibility, ignoring variation
4.	Process	Flexible process with design space allows continuous improvement	Method flexibility with MODR and enabling continuous improvement	The process is frozen and discourages changes
5.	Benefit	It is an expanded process analytical technology (PAT) tool that replaces the need for end-product testing.	Replacing the need for revalidation and minimising OOT and OOS	Limited and simple

Quality by design is a systematic approach that integrates quality risk management (QRM) and utilizes various design tools in conjunction with statistical analysis to ensure the production of high-quality products (Figure 1) [5].

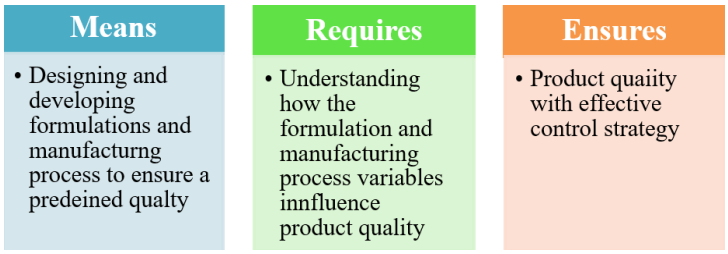

Figure 1 Quality by Design (QbD)

The FDA linked risk management to the analytical method in 2011 (1CH Q9) [6]. The severity of a drug's effects on patients due to ineffectiveness, the unpredictability of new procedures or products that increase the risk of failure, and poor detectability caused by inadequate analytical techniques all influence a product's risk profile. Additionally, the FDA has provided regulatory flexibility for certain AQbD-based analytical procedures. QbD (Figure 2) is now a crucial part of process and product design, as well as in the optimisation of analytical techniques within the pharmaceutical industry [7].

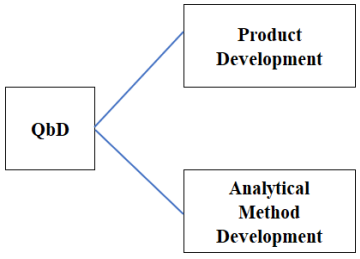

Figure 2 QbD in the pharmaceutical industry

According to the International Council on Harmonisation (ICH) quality guidelines, the Analytical Quality by Design (AQbD) approach for analytical method development in the industry is a crucial part of pharmaceutical development (Q8), quality risk management (Q9), and pharmaceutical quality systems (Q10). The primary aim of implementing AQbD is to

create a robust method, minimise the risk of method failure due to out-of-specification (OOS), out-of-trend (OOT), and out-of-control (OOC) results, speed up product quality, gain regulatory flexibility in adjusting method parameters within the method operable design region (MODR), and reduce analysis costs. Although literature on QbD for drugs is limited, some authors incorrectly interpret the use of design of experiments (DOE) as an AQbD approach. It is important to remember that DoE is merely one element of the QbD framework [8–10].

In the pharmaceutical industry, chromatographic techniques such as high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) are frequently used for testing pharmaceuticals because of their precision and accuracy. However, developing new analytical methods with these techniques is difficult due to the complexity of the variables. The Analytical Quality by Design (AQbD) process offers a solution by minimising the influence of critical method variables (CMVS) on method performance. By establishing a scientific relationship between CMVs and method responses, AQbD enables the design of methods that meet regulatory requirements, making it an essential tool for creating highly robust analytical methods in the industry [11–13].

In the pharmaceutical industry, QbD is a concept introduced to develop robust manufacturing processes, improve product quality, and produce products in accordance with 'Six Sigma'. The method of understanding control and capability (PUCC) was implemented as a continuous cycle for ongoing improvement. 'Six Sigma" is a system of practices designed to systematically enhance processes and eliminate defects with statistical significance. Since its development, Six Sigma has become a vital part of many total quality management (TQM) initiatives. The numerous reports on OOT results, OOS results, OOC, and out-of-statistical-control (OOSC) highlight that the current system in the pharmaceutical industry is not immune to these issues. At this stage, QbD implementation has become mandatory in some countries, particularly by the European Medicines Agency (EMA) and other ICH member countries. The International Conference on Harmonisation (ICH) Q8 (R1) guideline defines QbD as a systematic development approach that begins with pre-defined objectives and emphasises product and process understanding and control based on sound science and quality risk management. It indicates that scientifically designed product and process performance characteristics are necessary to meet specific objectives, but not solely based on test performance or quality control of batches for release. QbD concepts are clearly outlined in ICH guidelines Q8 (R1): Pharmaceutical Development, Q9: Quality Risk Management, and Q10: Pharmaceutical Quality System [14–16]. (Figure 3)

Figure 3 QbD bases

The text emphasises the growing focus on the Quality by Design (QbD) concept in analytical method development within the pharmaceutical industry. It discusses how QbD is integrated into developing efficient and high-quality analytical methods, known as Analytical Quality by Design (AQbD). The text highlights that analytical researchers often have limited experience or exposure to the AQbD approach, emphasising the need for more discussion and guidance on its application within pharmaceutical quality systems. It also identifies key areas lacking industry knowledge, such as the analytical target profile, method performance characteristics, risk assessment, the selection of Design of Experiments (DoE) tools, and optimisation of the MODR region. Additionally, it references recent updates by the USP and European Pharmacopeia regarding increased flexibility in analytical methods when implementing Aqbd. The implications of system suitability testing (SST) and its relationship with AQbD are also discussed.

1.1 The Regulatory Perspective of QbD

Process Analytical Technology (PAT) is emphasised as a framework for pharmaceutical development, production, and quality assurance in the International Council for Harmonisation's (ICH) Q8, Q9, and Q10, which also impose strict requirements for product quality. ICH Q8, Q9, and Q10 provide an overview of QbD concepts, including science- and risk-based product development, life cycle approach, risk assessment, and product strategy (Figure 4) [17].

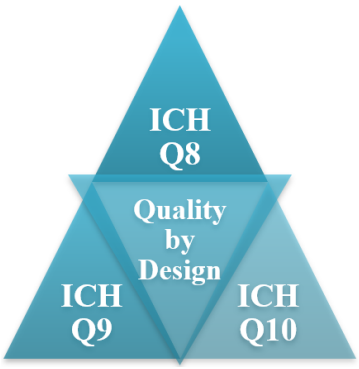

Figure 4 Principles of QbD

The pharmaceutical industry has embraced Quality by Design (QbD) through initiatives such as the FDA's cGMP for the 21st century and process analytical technology (PAT). Analytical methods are essential in guaranteeing specified performance and product quality. The FDA has approved numerous NDAs supported by QbD, emphasising its importance in method development and product quality monitoring.

The pharmaceutical industry is increasingly adopting Analytical Quality by Design (AQbD) as a means to enhance Quality Control (QC) processes and reduce the risk of method failure [18,19]. This approach aims to improve product quality and ensure high confidence in pharmaceutical development and manufacturing. Applying Quality by Design (QbD) principles to analytical methods results in more robust and reliable analytical data, thereby improving processes and product quality throughout their lifecycle. The regulatory perspective of Product and Analytical QbD is illustrated in Figure 5 [10, 20].

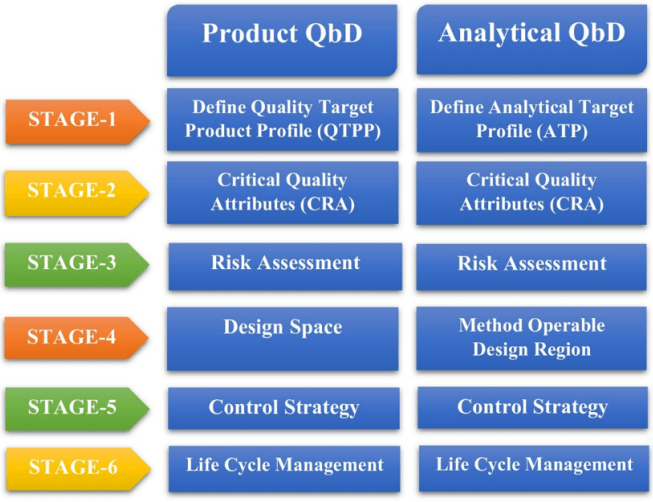

Figure 5 Product QbD *vs* Analytical QbD: Regulatory Viewpoints

1.2 One Factor at a Time *vs* QbD in Analytical Method Development

There is a growing trend of method failure during method transfer and within quality control departments. This is attributed to the lack of robust test compliance as outlined in the ICH Q2 guidelines. Chromatographic methods such as HPLC, UPLC, and RRLC are frequently

employed for content uniformity, assay, impurity profiling, and stability-indicating assays [21, 22]. However, the complexity of method development combined with low sensitivity and selectivity often necessitates revalidation protocols. The traditional one-factor-at-a-time (OFAT) approach to method development results in limited robustness and higher costs. The Analytical Quality by Design (AQbD) approach, including the Design of Experiments (DoE), is recommended for improving method robustness and cost-efficiency, while also reducing the incidence of out-of-specification (OOS) results.

1.3 Implementation of AQbD

The Quality by Design (QbD) concept applies to analytical methods because many variables can significantly affect results. Implementing QbD offers an opportunity to obtain regulatory flexibility, but it requires high robustness, product quality, and understanding of the analytical method. AQbD implementation is shown in Table 2. Applying QbD in analytics is essential for achieving overall quality improvement, as shown in Figure 6 [23].

Table 2 AQbD implementation

Sr. No.	Stage by stage Implementation	Description
1.	Target measurement	Determine what to measure and where/when to measure it. Define ATP and develop measurement requirements based on product QTPP and CQA.
2.	Select technique	Select the appropriate analytical technique for the desired measurement defined in ATP. Define method performance criteria.
3.	Evaluation of risks	Evaluate the risks of sample variation, environmental factors, and method input variables. Tools for risk assessment (such as FMEA) can be employed.
4.	Method development and validation	To comprehend the method's robustness and ruggedness, define MODR and examine possible multivariate interactions by DoE.
5.	Strategy for control	Describe the control space and system suitability; satisfy the ATP method performance requirements.
6.	Ongoing development	Analyzers proactively detect and correct the method's out-of-trend performance by monitoring method performance that complies with ATP criteria. Update with new analytical tools and procedures.

Figure 6 Implementing AQbD in a pharmaceutical quality system

2 Stages of Quality by Design

AQbD starts with an analytical target profile (ATP), similar to QTPP, which outlines the method's objective. Once approved by regulatory authorities, ATP supports the continuous improvement of analytical techniques. The internal change control management system ensures effective ATP implementation, offering regulatory flexibility in the pharmaceutical sector [24,25]. For example, Table 3 demonstrates the CQA for a drug product [10].

Table 3 Performance of the necessary analytical method for the Critical Quality Attributes (CQA) of the drug product

Sr. No.	Critical Quality Attributes (CQA)	Methods of detection	Detectability methodology	Performance of the required method
1.	Appearance	Visual	Qualitative	Specificity, LOD
3.	Assay	Analytical method	Quantitative	Specificity, Precision, accuracy, linearity
2.	Identification	Analytical method	Qualitative	Specificity, LOD
4.	Heavy metals	Analytical method	Quantitative	Specificity, LOD, LOQ Precision, accuracy, linearity
5.	Impurity	Analytical method	Quantitative	Specificity, LOD, LOQ Precision, accuracy, linearity
6.	Disintegration	Disintegration apparatus	Quantitative	Reproducibility, accuracy
7.	Dissolution	Analytical method	Quantitative	Specificity, Precision, accuracy, linearity
8.	Friability	Friability apparatus	Quantitative	Reproducibility, accuracy
9.	Moisture content	Analytical methods	Quantitative	Specificity, LOD, Precision, accuracy, linearity
10.	Hardness	Hardness tester	Quantitative	Reproducibility, accuracy

2.1 Characteristics of Analytical Method Performance

The method performance characteristics in (ATP) include bias, variance, accuracy, precision, specificity, linearity, and robustness.25 It is essential to consider a combination of two or more method performance characteristics. Linearity and specificity may not need to be included in the ATP, as they are not directly related to evaluating how well a measurement agrees with the true value. For example, although linearity and specificity are not always included, an assay ATP should feature a statement of accuracy and precision in Table 4 [26, 27].

Table 4 Method performance characteristics according to ICH Q2 (R1) and USP

Sr. No.	According to ICH and USP	Features of Method Performance
1.	Systematic variability	Accuracy, linearity, & specificity
2.	Inherent random variability	Precision, detection limit, & quantification limit
3.	Not applicable (NA)	Range & robustness

2.2 Selection of Analytical Techniques

The requirements outlined in the ATP must be followed. Additionally, the chosen analytical method must comply with the regulatory requirements for method validation parameters. Although the ATP might not be specific, the analytical method should meet the criteria.

Therefore, the chromatographic method can meet the ICH validation requirements and the performance criteria specified in ATP [28]. Alternatively, the UV spectrophotometric method might not satisfy ICH Q2 but can fulfil ATP requirements. For example, Table 5 displays the performance characteristics of the analytical method and potential techniques for the dosage form [29, 30].

Table 5 Analytical target profile for tablet assay

Targeted CQA	Possible Analytical Methods	Performance Requirements for the Method according to ICH Q2	Method Response Expected
Simultaneous Assay of PRO and ETZ	1. UV-Visible method 2. HPLC 3. UPLC 4. LC-MS/MS 5. HPTLC	1. Specificity (Pass) 2. Linearity ($r^2 > 0.99$) 3. Precision (% RSD < 2) 4. Accuracy (98%–102%) 5. Robustness (Pass)	1. Resolution > 2 2. Plate count > 3000 3. Tailing factor < 2 4. tR (PRO) = 3–5 min 5. tR (ETZ) $>$ 7-10 min

2.2.1 Critical quality attributes for monoclonal antibodies and vaccine development technologies

Critical quality attributes are crucial in developing monoclonal antibodies and bacterial or viral vaccines, significantly influenced by Critical Process Parameters (CPP) and Critical Material Attributes (CMAs). These attributes are essential for ensuring the safety, efficacy, and overall quality assessment of vaccine candidates and drug products. To support robust development, a comprehensive range of immunological and analytical test methods have been carefully optimised. This rigorous process aims to thoroughly characterise the physico-chemical properties of the drug candidates or Active Pharmaceutical Ingredients (API). These analytical methods not only provide insights into the structural and functional integrity of the candidates

but also serve as a basis for establishing their therapeutic potential and regulatory compliance. CE-SDS-PAGE, SRID, and RP-LC, among others, deliver precise and accurate results for the development of human antibodies and vaccine variants. All API drug candidates for vaccines must undergo an enhanced manufacturing process that scales up the initial crude cell lines of the vaccine candidates. This includes upstream processing, which involves the bulk production of cell cultures in a bioreactor, as well as the purification of the drug product from its API substance. Additionally, downstream processing of the drug candidates involves their formulation, filling, and the overall development and manufacturing of vaccines or monoclonal antibodies [31–36].

3 Establishing CQA(S) by Risk Assessment

The risk assessment process concentrates on method development and identifies critical variables within the method. It involves assessing the risks linked to various factors, including environmental conditions, sample preparation, measurement and method parameters, analyst techniques, instrument configuration, and sample characteristics. Usually carried out after the method development phase, the assessment approach should adhere to the ICH Q9 guideline.36 It is essential for routine laboratory operations and the transfer of methods. Three risk assessment techniques are knowledge-based, cause-and-effect analysis, and failure mode and effects analysis, as shown in Figure 7 and 8. Crucial method variables are systematically optimised using a Box-Behnken design, with different tools employed for risk management [37, 38].

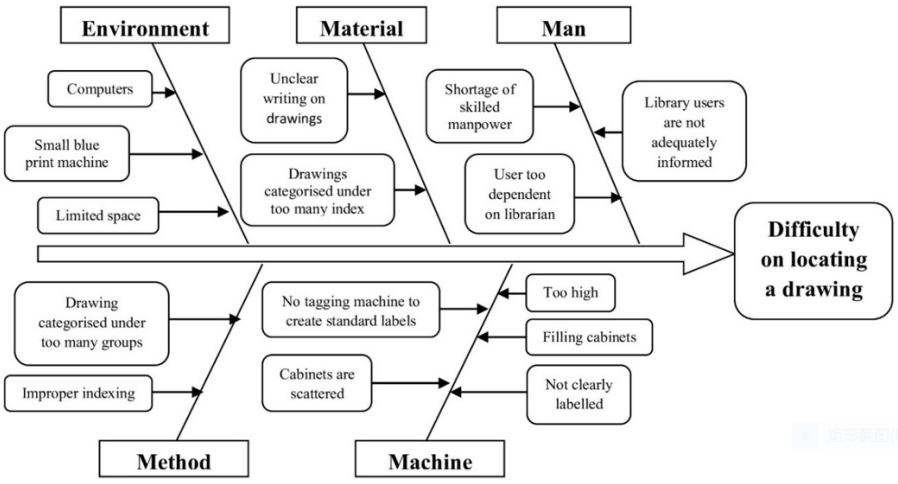

Figure 7 Diagram of the Ishikawa fishbone (Analysis of causes and effects

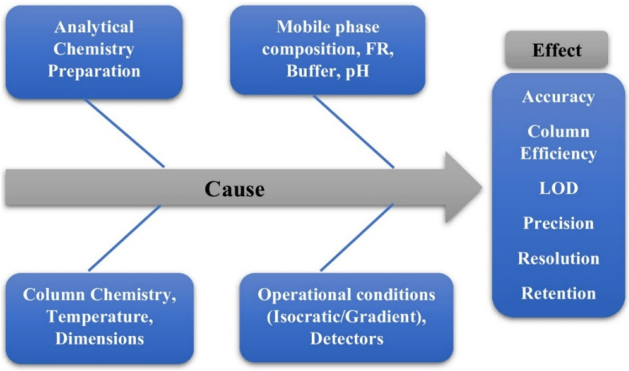

Figure 8 An illustration of the analysis of causes and effects

Because it helps identify potential failure modes and their effects, this analysis is crucial for reliability, safety, and quality engineering. It is often used to minimise failures and manage risks in manufacturing and development. One design tool that systematically examines hypothetical component failures and their impact on system operations is the Failure Modes and Effects (FME) Analysis. Failure Modes and Effects Analysis (FMEA) and Criticality Analysis (CA) are its two sub-analyses [39]. FMEAs can be performed at various levels, from part to system. Completing the FMECA on schedule is vital for guiding design decisions and reducing or

eliminating critical failure modes. Additionally, for more detailed scenario modelling that considers multiple failures and external influences, a fault tree analysis (FTA) might be employed [40–43].

3.1 Functional Analysis

The analysis starts at the functional level and moves up to the hardware level as the design develops. The hardware-level FMECA assumes that interfacing hardware operates within specified parameters. Multiple FMEAs are conducted to evaluate how lower-level failures impact system operation and to avoid irreversible damage at interfaces caused by failures. FMECA combines FMEA and CA, with the latter requiring identification of system-level critical deficiencies in the former.

3.2 Ground Rules

The ground rules for FMEA include project procedures, analysis assumptions, included and excluded hardware, rationale for exclusions, indenture level, hardware status, and system success criteria. Establishing these rules before beginning is vital, but they can be expanded as the analysis progresses. Typical assumptions involve one failure mode at a time, all inputs at nominal values, sufficient consumables, and available nominal power.

3.3 Benefits

The key advantages of properly executed FMECA efforts include:
(1) Selecting a design that has a high chance of successful operation and safety.
(2) Assessing potential failure mechanisms and their impact on system operation.
(3) Identifying issues related to single-point failures and system interface problems.
(4) Evaluating proposed design changes and operational procedures.
(5) Developing in-flight troubleshooting procedures and identifying fault detection devices.
(6) Planning tests ahead.

The most important benefits are early detection of single-failure points, support for troubleshooting procedures, and the identification of performance monitoring and fault-detection devices.

4 Design of Experiments

According to ICHQ8 guidelines, MODR can be established as a reliable and cost-effective source for methods during the development phase. MODR defines the operating range for critical method input variables that consistently meet the goals set in the ATP. It is science-based and allows flexibility in method parameters, ensuring expected performance without resubmission to the FDA [44]. The FDA recommends conducting MODR alongside method validation. Once defined, appropriate method controls can be implemented [45]. This approach requires a thorough understanding of input variables and output response selection.

4.1 Selection of DoE Tools

Various approaches can be employed during optimisation to establish a mathematical relationship (model). The selection of a design of experiments (DoE) tool depends on the number of input variables, knowledge of controlled parameters, and the relationship between variables and outcomes [46]. Statistical understanding is vital to interpret variable interactions and their influence on method responses. Depending on the specific needs of the study, different methods, such as factorial design, response surface methodology (RSM), Taguchi method, and Plackett-Burman design, can be utilised. Table 6 presents the specific requirements of the study [38,47].

John Bennet Lawes and Joseph Henry Gilbert used factorial designs in the 19th century. In 1926, Ronald Fisher contended that factorial designs were more efficient than studying OFAT.

4.2 Advantages of Factorial Designs

Factorial experiments offer several benefits over OFAT experiments, including improved efficiency, the capability to investigate additional factors at no extra cost, the ability to detect interactions between factors, and the estimation of factor effects at different levels [48, 49]. Furthermore, to achieve specific goals such as setting each factor at three evenly spaced values, fitting a quadratic model, maintaining a suitable ratio of experimental points to coefficients, and

Table 6 Selecting the design of experiment (DOE) tools in analytical quality by design (AQbD)

Sr. No.	Design	Number of variables and usage	Advantage	Disadvantage
1.	Fractional factorial design or Taguchi methods	Variables for optimisation or screening	Reducing the number of experimental runs	Resolving interaction confounding effects is a challenging task
2.	Full factorial design	Two to five variables or optimisation	Identifying the main and interaction effects without any confounding	As the number of variables increases, so does the number of experimental runs
3.	Pseudo-Monte Carlo sampling (pseudorandom sampling) method	Quantitative risk analysis or optimisation	Behaviour and model modifications can be quickly and easily examined. This is the preferred method when a precise calculation can be made.	This sampling method can be more difficult to employ for nonconvex design spaces. A random number-generating algorithm can generate random numbers.
4.	Plackett-Burman Method Screening/identifying	Screening/ identifying essential factors from a large number of variables	Requiring very few runs for a large number of variables	The interaction effect is not revealed.
5.	Doehlert design	Variable optimization	Utilised in response to surface analysis	-
6.	Box-Behnken design	Each factor had three levels (-1, 0, +1)	The design region contains the design points	There was no two-factor design provided

ensuring that estimation variance mainly depends on the distance from the centre, box-Behnken designs–experimental designs for response surface methodology (RSM)–were developed in 1960.

The design combines an incomplete block design with a two-level factorial design. While keeping the other factors at their central values, each block changes a specific number of factors across all possible combinations for the factorial design [50]. Centre points are also included. An 81-run design is created using the 9-factor design by removing one column, but the design for eight factors was not included in the original paper. At least up to 21 designs have also been developed for other factors. Plackett-Burman designs can be used to construct a 16-factor design with only 221 points, whereas a 16-factor design with just 256 factorial points already exists.

The advantages of a spherical experimental domain, including uniform space-filling, the ability to explore the entire area, and the possibility of employing the Doehlert design for sequential experiments, are the reasons it was chosen [22]. It offers benefits over alternative designs in response surface analysis and is straightforward to apply to optimised variables. The number of experiments required depends on the number of variables and centre points. During the optimisation phase, the design allows for the inclusion of quantitative measurements for important method variables and the exclusion of qualitative input variables [10, 51].

5 Model Validation

Before choosing between contour and graph, the predicted values for the method response must be confirmed by actual experimental runs. After that, regression analysis must be conducted to validate the model statistically.

Method validation should be performed in accordance with ICHQ2 (R1) guidelines under normal operating conditions or optimised conditions with fixed variables at a single point [52]. Additionally, method verification can be verified through accuracy and precision assessments at multiple points within the chromatographic separation space [53]. This multi-point verification should go beyond standard robust test limits to confirm the method's capability to meet requirements. The experiments should demonstrate robustness across parameter ranges, such as verifying column temperature between 35°C and 45°C, the percentage of aqueous or organic components in the mobile phase, and pH levels [54]. The operable range of the method can be determined based on validation and verification results, provided that the performance characteristics are satisfactory and meet the acceptance criteria.

6 Control Strategy/Conformance to Atp

In product QbD, the control strategy ensures rapid production while maintaining the required quality. It is developed from data collected during method development and verification and

forecasts the method's capacity to meet ATP criteria and the control strategy. The implemented control strategy considers parameters and their influence on product quality for changes both within and outside the design space. The method control strategy of the AQbD approach is similar to that of the traditional control strategy [55].

7 Continuous Monitoring/Life-Cycle Management

Establishing an analytical method for quality control involves monitoring its performance over time to ensure it meets established criteria. Control charts and other tools are employed in the pharmaceutical industry to track system suitability data. This ongoing monitoring enables the detection and correction of abnormal or OOT performance of the method, which is crucial from raw material testing to stability testing (Table 7) [56, 57].

Table 7 The role of analytical methods in pharmaceutical control and testing

Sr. No.	Control	Strategy
1.	Testing of raw material	Specification based on product Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQA). Effects of variability, including supplier variations, on process and method development are understood.
2.	Testing for In-process	Real-time (at, on–, or in-line) measurements. Active control of the process to minimise product variation. Criteria based on multivariate process understanding.
3.	Testing for release	Quality attributes are predictable from process inputs (design space). Specification is only part of the quality control strategy. Specification based on patient needs (quality, safety, efficacy, and performance).
4.	Testing for stability	Predictive models at release minimise stability failures. The specification is set on the desired product performance over time.

8 PAT and AQbD

To measure critical process parameters that influence Critical Quality Attributes (CQA), the USFDA developed the Process Analytical Technology (PAT) system, which involves designing, analysing, and managing the manufacturing process [58]. Its objectives are to reduce production cycle time, prevent batch rejections, enable continuous processing, improve material utilisation, promote automation, and mitigate risks. The FDA recommends that pharmaceutical manufacturing processes adopt ongoing, real-time quality assurance to ensure product performance and quality. The Design of Experiments (DOE) and Multivariate Data Analysis (MVDA) are based on PAT initiatives [59]. Pharmaceutical companies are working on developing a specific understanding of processes and designing analytical control strategies to enhance the effectiveness of the PAT approach [60].

9 Conclusion

The case study successfully achieved its aims, highlighting the substantial benefits of using an Analytical Quality by Design (AQbD) approach to improve both the quality and efficiency of drug candidate development. The insights shared in this study offer a valuable framework for assessing whether a published pharmacopoeial method for the active pharmaceutical ingredient (API) drug product under review is suitable, ensuring it meets the specific criteria for its intended use.

The development of AQbD methods, along with the Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQA), has become an essential part of the characterisation processes for various medicinal drug products, including bacterial and viral vaccines, as well as monoclonal antibodies. These approaches have the transformative potential to improve precision and accuracy in the bulk manufacturing of medicinal and vaccine drug candidates, ultimately supporting the prevention and control of infectious diseases through targeted therapeutic strategies.

Furthermore, the principles and methodologies outlined in this study not only offer comprehensive guidance but also have the potential to improve the efforts of analysts and organisations alike substantially. This encourages wider adoption and application of these innovative strategies in the pharmaceutical development landscape.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgement

The authors are grateful to the Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, for providing the platform to develop the manuscript.

Transparency Statement

The lead author, Dr Ritu Tiwari, confirms that this manuscript provides an honest, accurate, and transparent account of the study reported, with no crucial aspects omitted, and any discrepancies from the original (and if relevant, registered) study have been explained.

References

[1] Zagalo DM, Sousa J, Simões S. Quality by design (QbD) approach in marketing authorization procedures of Non-Biological Complex Drugs: A critical evaluation. European Journal of Pharmaceutics and Biopharmaceutics. 2022, 178: 1-24. https://doi.org/10.1016/j.ejpb.2022.07.014

[2] Mahr AG, Lourenço FR, Borman P, et al. Analytical Target Profile (ATP) and Method Operable Design Region (MODR). Introduction to Quality by Design in Pharmaceutical Manufacturing and Analytical Development. Published online 2023: 199-219. https://doi.org/10.1007/978-3-031-31505-3_9

[3] Das P, Maity A. Analytical quality by design (AQbD): a new horizon for robust analytics in pharmaceutical process and automation. International journal of pharmaceutics and drug analysis, 2017, 5(8): 324-337.

[4] Mishra V, Thakur S, Patil A, et al. Quality by design (QbD) approaches in current pharmaceutical set-up. Expert Opinion on Drug Delivery. 2018, 15(8): 737-758. https://doi.org/10.1080/17425247.2018.1504768

[5] Patil AS, Pethe AM. Quality by Design (QbD): A new concept for development of quality pharmaceuticals. International Journal of Pharmaceutical Quality Assurance, 2013, 4(2): 13-9.

[6] Roizard F. 8th Middle East Regulatory Conference. Drug Information Journal. 2009, 43(3): 377-384. https://doi.org/10.1177/009286150904300318

[7] Park G, Kim MK, Go SH, et al. Analytical Quality by Design (AQbD) Approach to the Development of Analytical Procedures for Medicinal Plants. Plants. 2022, 11(21): 2960. https://doi.org/10.3390/plants11212960

[8] Phadke R, Gosar A, Mali R, et al. A review on quality by design approaches to analytical method development. Journal of Pharma Research. 2019, 9(7): 3044-55.

[9] Fukuda IM, Pinto CFF, Moreira C dos S, et al. Design of Experiments (DoE) applied to Pharmaceutical and Analytical Quality by Design (QbD). Brazilian Journal of Pharmaceutical Sciences. 2018, 54(spe). https://doi.org/10.1590/s2175-97902018000001006

[10] Ramalingam P, Jahnavi B. QbD Considerations for Analytical Development. Pharmaceutical Quality by Design. Published online 2019: 77-108. https://doi.org/10.1016/b978-0-12-815799-2.00005-8

[11] Panda SS, Ravi Kumar Bera VV, Beg S, et al. Analytical Quality by Design (AQbD)-oriented RP-UFLC method for quantification of lansoprazole with superior method robustness. Journal of Liquid Chromatography & Related Technologies. 2017, 40(9): 479-485. https://doi.org/10.1080/10826076.2017.1327442

[12] Ali J, Pramod K, Tahir Ma, et al. Pharmaceutical product development: A quality by design approach. International Journal of Pharmaceutical Investigation. 2016, 6(3): 129. https://doi.org/10.4103/2230-973x.187350

[13] Green FB. Six-Sigma and the Revival of TQM. Total Quality Management & Business Excellence. 2006, 17(10): 1281-1286. https://doi.org/10.1080/14783360600753711

[14] Peraman R, Bhadraya K, Padmanabha Reddy Y. Analytical Quality by Design: A Tool for Regulatory Flexibility and Robust Analytics. International Journal of Analytical Chemistry. 2015, 2015: 1-9. https://doi.org/10.1155/2015/868727

[15] Jain NK, Bajwa N, eds. Introduction to Quality by Design (QbD). Springer Nature Singapore, 2024. https://doi.org/10.1007/978-981-99-8034-5

[16] Tome T, Žigart N, Časar Z, et al. Development and Optimization of Liquid Chromatography Analytical Methods by Using AQbD Principles: Overview and Recent Advances. Organic Process Research & Development. 2019, 23(9): 1784-1802. https://doi.org/10.1021/acs.oprd.9b00238

[17] Hasnain MS, Ahmed SA, Khatoon A, et al. Pharmaceutical product development: A quality by design (QbD) approach. Advances and Challenges in Pharmaceutical Technology. Published online 2021: 131-146. https://doi.org/10.1016/b978-0-12-820043-8.00017-7

[18] Bondi RW, Drennen JK. Quality by Design and the Importance of PAT in QbD. Handbook of Modern Pharmaceutical Analysis. Published online 2011: 195-224.
https://doi.org/10.1016/b978-0-12-375680-0.00005-x

[19] Caro YS, Cámara MS, De Zan MM. Quality by Design Applications to Development of Analytical Methods for Quality Control of Pharmaceuticals. Introduction to Quality by Design in Pharmaceutical Manufacturing and Analytical Development. Published online 2023: 221-244.
https://doi.org/10.1007/978-3-031-31505-3_10

[20] Verch T, Campa C, Chéry CC, et al. Analytical Quality by Design, Life Cycle Management, and Method Control. The AAPS Journal. 2022, 24(1).
https://doi.org/10.1208/s12248-022-00685-2

[21] Tiwari R, Gulati P, Solanki P, et al. Analytical quality-by-design guided development of a novel and robust HPTLC method for quantifying plumbagin from Plumbago species. Journal of Liquid Chromatography & Related Technologies. 2024, 48(6-10): 144-151.
https://doi.org/10.1080/10826076.2024.2435651

[22] Chiarentin L, Gonçalves C, Augusto C, et al. Drilling into "Quality by Design" Approach for Analytical Methods. Critical Reviews in Analytical Chemistry. 2023, 54(8): 3478-3519.
https://doi.org/10.1080/10408347.2023.2253321

[23] Pratik B. Aru, Mayur S. Gulhane, Vinayak A. Katekar, Swati P. Deshmukh. Quality by Design (QbD) in pharmaceutical development: A comprehensive review. GSC Biological and Pharmaceutical Sciences. 2024, 26(1): 328-340.
https://doi.org/10.30574/gscbps.2024.26.1.0019

[24] Das V, Bhairav B, Saudagar RB. Quality by Design approaches to Analytical Method Development. Research Journal of Pharmacy and Technology. 2017, 10(9): 3188.
https://doi.org/10.5958/0974-360x.2017.00567.4

[25] Chadha H, Parmar R, Shah P, et al. Quality by Design approach for a planar chromatographic method for simultaneous estimation of Ivabradine Hydrochloride and Carvedilol in pharmaceutical formulation. SEPARATION SCIENCE PLUS. 2023, 6(6).
https://doi.org/10.1002/sscp.202200149

[26] Borman P, Elder D. Q2(R1) Validation of Analytical Procedures. ICH Quality Guidelines. Published online September 27, 2017: 127-166.
https://doi.org/10.1002/9781118971147.ch5

[27] Ermer J, Kleinschmidt G, Rignall A, et al. Method Performance Qualification. Method Validation in Pharmaceutical Analysis. Published online August 15, 2014: 303-376.
https://doi.org/10.1002/9783527672202.ch7

[28] Jackson P, Borman P, Campa C, et al. Using the Analytical Target Profile to Drive the Analytical Method Lifecycle. Analytical Chemistry. 2019, 91(4): 2577-2585.
https://doi.org/10.1021/acs.analchem.8b04596

[29] Jadhav ML, Tambe SR. Implementation of QbD Approach to the Analytical Method Development and Validation for the Estimation of Propafenone Hydrochloride in Tablet Dosage Form. Chromatography Research International. 2013, 2013: 1-9.
https://doi.org/10.1155/2013/676501

[30] European Medicines Agency. Guideline on process validation for finished products—Information and data to be provided in regulatory submissions, 2016.

[31] Geurink L. Analytical Quality by Design Method Development for Vaccine Characterization (Doctoral dissertation, Acta Universitatis Upsaliensis), 2022.

[32] Hakemeyer C, McKnight N, St. John R, et al. Process characterization and Design Space definition. Biologicals. 2016, 44(5): 306-318.
https://doi.org/10.1016/j.biologicals.2016.06.004

[33] Parenteral Drug Association. PDA Technical Report No. 60-3 (TR 60-3) Process Validation: A Lifecycle Approach, Annex 2: Biopharmaceutical Drug Substances Manufacturing. Parenteral Drug Association, ID: 9781945584244, 2021.
https://www.pda.org

[34] Kapteyn JC, Porre AM, de Rond EJP, et al. HPLC-based quantification of haemagglutinin in the production of egg- and MDCK cell-derived influenza virus seasonal and pandemic vaccines. Vaccine. 2009, 27(9): 1468-1477.
https://doi.org/10.1016/j.vaccine.2008.11.113

[35] KAPTEYN J, SAIDI M, DIJKSTRA R, et al. Haemagglutinin quantification and identification of influenza A&B strains propagated in PER.C6® cells: A novel RP-HPLC method. Vaccine. 2006, 24(16): 3137-3144.
https://doi.org/10.1016/j.vaccine.2006.01.046

[36] McMahon ME, Abbott A, Babayan Y, et al. Considerations for Updates to ICH Q1 and Q5C Stability Guidelines: Embracing Current Technology and Risk Assessment Strategies. The AAPS Journal. 2021, 23(6).
https://doi.org/10.1208/s12248-021-00641-6

[37] Sayyadi Tooranloo H, Ayatollah AS, Alboghobish S. Evaluating knowledge management failure factors using intuitionistic fuzzy FMEA approach. Knowledge and Information Systems. 2018, 57(1): 183-205.
https://doi.org/10.1007/s10115-018-1172-3

[38] Ettaboina SK, Nannapaneni S, Jeedimalla N, et al. Development, evaluation of critical method variables and stability assessment using a Box–Behnken design for the determination of organic impurities in a pharmaceutical dosage form of a centrally acting muscle relaxant drug chlorzoxazone. Biomedical Chromatography. 2024, 38(11). https://doi.org/10.1002/bmc.6001

[39] Onofrio R, Piccagli F, Segato F. Failure Mode, Effects and Criticality Analysis (FMECA) for Medical Devices: Does Standardization Foster Improvements in the Practice? Procedia Manufacturing. 2015, 3: 43-50. https://doi.org/10.1016/j.promfg.2015.07.106

[40] Boryczko K, Szpak D, Żywiec J, et al. The Use of a Fault Tree Analysis (FTA) in the Operator Reliability Assessment of the Critical Infrastructure on the Example of Water Supply System. Energies. 2022, 15(12): 4416. https://doi.org/10.3390/en15124416

[41] Carpitella S, Certa A, Izquierdo J, et al. A combined multi-criteria approach to support FMECA analyses: A real-world case. Reliability Engineering & System Safety. 2018, 169: 394-402. https://doi.org/10.1016/j.ress.2017.09.017

[42] BOUTI A, KADI DA. A STATE-OF-THE-ART REVIEW OF FMEA/FMECA. International Journal of Reliability, Quality and Safety Engineering. 1994, 01(04): 515-543. https://doi.org/10.1142/s0218539394000362

[43] Sharma KD, Srivastava S. Failure mode and effect analysis (FMEA) implementation: a literature review. Journal of Advance Research in Aeronautics and Space Science. 2018, 5(1): 1-17.

[44] Mahapatra A, Meyyanathan SN. Approach of analytical quality by design and regulatory need. International journal of health sciences. Published online June 18, 2022: 2572-2592. https://doi.org/10.53730/ijhs.v6ns5.9208

[45] Kollipara S, Bende G, Agarwal N, et al. International Guidelines for Bioanalytical Method Validation: A Comparison and Discussion on Current Scenario. Chromatographia. 2011, 73(3-4): 201-217. https://doi.org/10.1007/s10337-010-1869-2

[46] Beg S, Swain S, Rahman M, et al. Application of Design of Experiments (DoE) in Pharmaceutical Product and Process Optimization. Pharmaceutical Quality by Design. Published online 2019: 43-64. https://doi.org/10.1016/b978-0-12-815799-2.00003-4

[47] Kumar R, Reji M. Response surface methodology (RSM): An overview to analyze multivariate data. Indian Journal of Microbiology Research. 2023, 9(4): 241-248. https://doi.org/10.18231/j.ijmr.2022.042

[48] Sharma DK, Bhalla V. Medication Adherence Challenges in Visually Impaired Patients: Insights and Solutions from the National Capital Region of India. African Journal of Pharmaceutical Sciences. 2024, 6(15): 332-345. https://doi.org/10.48047/AFJBS.6.15.2024.332-345

[49] Frey DD, Wang H. Adaptive One-Factor-at-a-Time Experimentation and Expected Value of Improvement. Technometrics. 2006, 48(3): 418-431. https://doi.org/10.1198/004017006000000075

[50] Bezerra MA, Santelli RE, Oliveira EP, et al. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008, 76(5): 965-977. https://doi.org/10.1016/j.talanta.2008.05.019

[51] Azcarate SM, Teglia CM, Chiappini FA, et al. Fundamentals of Design of Experiments and Optimization: Experimental Designs in Response Surface Methodology. Introduction to Quality by Design in Pharmaceutical Manufacturing and Analytical Development. Published online 2023: 47-66. https://doi.org/10.1007/978-3-031-31505-3_3

[52] Pradhan R, Hejmady S, Alexander A, et al. Analytical quality by design for gas chromatographic method development. Handbook of Analytical Quality by Design. Published online 2021: 45-70. https://doi.org/10.1016/b978-0-12-820332-3.00002-9

[53] Ashritha N, Rajitha G. Analytical Quality by Design Approach for the Development and Validation of Liquid Chromatographic Procedure for the Estimation of Abrocitinib. Pharmaceutical Chemistry Journal. 2024, 58(2): 362-371. https://doi.org/10.1007/s11094-024-03153-7

[54] Dolan JW. Temperature selectivity in reversed-phase high performance liquid chromatography. Journal of Chromatography A. 2002, 965(1-2): 195-205. https://doi.org/10.1016/s0021-9673(01)01321-8

[55] Vogt FG, Kord AS. Development of Quality-By-Design Analytical Methods. Journal of Pharmaceutical Sciences. 2011, 100(3): 797-812. https://doi.org/10.1002/jps.22325

[56] Bargal S, Potdar M, Dey R, et al. Developing an Application Model for Planning, Controlling, Improving, and Assuring Quality for Pharmaceutical Industry: Covering Quality Planning and Quality Control. Modern Aspects of Pharmaceutical Quality Assurance. Published online 2024: 93-166. https://doi.org/10.1007/978-981-99-9271-3_6

[57] Ahuja S. Overview of Modern Pharmaceutical Analysis. Handbook of Modern Pharmaceutical Analysis. Published online 2011: 1-9. https://doi.org/10.1016/b978-0-12-375680-0.00001-2

[58] Hussain AS. Process Analytical Technology (PAT). Handbook of Validation in Pharmaceutical Processes. Published online August 30, 2021: 883-915. https://doi.org/10.1201/9781003163138-56

[59] Lui CA. An Investigation of Multivariate Process Control for Biomanufacturing. Massachusetts Institute of Technology, 2021.

[60] Simon LL, Pataki H, Marosi G, et al. Assessment of Recent Process Analytical Technology (PAT) Trends: A Multiauthor Review. Organic Process Research & Development. 2015, 19(1): 3-62. https://doi.org/10.1021/op500261y