REVIEW ARTICLE

# A Review on Application of Artificial Intelligence and Machine Learning in Liquid Chromatography-Tandem Mass Spectrometry Method Development and Validation of Anti-cancer Agents by Analytical Quality by Design

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## Abstract

The development of robust and reliable analytical methods is essential for the quality assessment of anticancer drugs. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become a preferred analytical technique due to its high sensitivity, selectivity, and capability for accurate quantitative analysis. However, conventional method development approaches often rely on trial-and-error experimentation, resulting in increased time, cost, and variability. Analytical quality by design (AQbD) has emerged as a systematic and science-based approach that enhances method understanding and robustness throughout the analytical lifecycle. AQbD involves the establishment of an analytical target profile, identification of critical analytical attributes and critical method parameters, risk assessment, and optimization using design of experiments. These elements facilitate the development of reliable LC-MS/MS methods with improved performance, reproducibility, and regulatory compliance. The establishment of a method operable design region and appropriate control strategies further ensures consistent analytical performance during routine application. This review provides a comprehensive overview of AQbD-based stability-indicating LC-MS/MS method development and validation for anticancer drugs. Key aspects, including risk assessment, experimental design, optimization strategies, design space establishment, method validation, and lifecycle management are discussed. The review also highlights the emerging role of artificial intelligence (AI) and machine learning in chromatographic optimization, predictive modeling, and analytical data interpretation. The integration of AQbD, LC-MS/MS, and AI offers a modern framework for developing efficient, robust, and regulatory-compliant analytical methods. These advancements are expected to improve analytical efficiency, enhance method reliability, and support future innovations in pharmaceutical analysis.

**Key words:** Analytical quality by design, anticancer drugs, artificial intelligence, design of experiments, liquid chromatography-tandem mass spectrometry

## INTRODUCTION

The development of reliable analytical methods is a critical component of pharmaceutical research and quality assurance. Analytical procedures are required throughout the drug development process for the identification, quantification, and characterization of pharmaceutical compounds.[1] In the case of anticancer drugs, analytical requirements are particularly

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**Received:** 21-05-2-026
**Revised:** 23-06-2026
**Accepted:** 30-06-2026

demanding due to the structural complexity of these molecules, their narrow therapeutic indices, and stringent regulatory expectations regarding product quality and safety.[2] Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become one of the most widely employed analytical platforms in pharmaceutical analysis.[3] The technique combines efficient chromatographic separation with highly selective and sensitive mass spectrometric detection.[4] As a result, LC-MS/MS is extensively used for assay determination, impurity profiling, pharmacokinetic studies, bioanalysis, and stability assessment of anticancer agents. The ability to provide both quantitative and structural information makes LC-MS/MS a preferred tool during drug development and quality control.[5] Conventional analytical method development often relies on empirical experimentation, where individual variables are optimized sequentially. Such approaches can be labor-intensive and may fail to identify interactions among method parameters.[6] To address these limitations, the pharmaceutical industry has increasingly adopted the principles of analytical quality by design (AQbD). AQbD provides a systematic and science-based framework for method development by defining analytical objectives, identifying critical method variables, performing risk assessments, and establishing robust operating conditions through statistical experimental designs.[7] The application of AQbD to LC-MS/MS method development enables a comprehensive understanding of the relationship between critical method parameters (CMPs) and analytical performance attributes.[8] Parameters, such as mobile phase composition, pH, flow rate, column temperature, ionization conditions, and mass spectrometric settings can be systematically optimized using design of experiments (DoE) approaches. This strategy facilitates the establishment of a method design space and improves method robustness, reproducibility, and lifecycle management.[9]

Recent advances in artificial intelligence (AI) and machine learning (ML) have further expanded the capabilities of analytical method development. AI-based models are increasingly being employed for chromatographic optimization, prediction of analytical responses, automated interpretation of mass spectral data, and multivariate data analysis.[10] These technologies complement AQbD principles by enhancing process understanding and supporting data-driven decision-making during method development.[9]

## AQBD: PRINCIPLES AND REGULATORY FRAMEWORK

### Evolution of quality by design (QbD) in pharmaceutical analysis

QbD has emerged as a systematic and scientific approach to pharmaceutical development. The concept was initially introduced to enhance product quality through a thorough understanding of formulation and manufacturing processes.[11] Over time, its principles have been extended to analytical sciences, giving rise to AQbD. Unlike conventional analytical method development, which frequently relies on trial-and-error experimentation, AQbD emphasizes pre-defined objectives, risk-based decision-making, and a comprehensive understanding of analytical variables and their interactions.[12,13] Across systematic optimization and risk assessment, AQbD enables the development of robust LC-MS/MS methods capable of consistently meeting pre-defined analytical performance requirements.[14] The framework is presented in Figure 1.

### Regulatory perspective of AQbD

The implementation of AQbD is strongly supported by international regulatory agencies. Regulatory authorities encourage the application of scientific and risk-based approaches throughout pharmaceutical development to ensure product quality, patient safety, and process understanding.[15]

More recently, ICH Q14 has specifically addressed analytical procedure development and highlights the importance of systematic method development, knowledge management, and control strategies. In parallel, ICH Q2 (R2) outlines requirements for analytical method validation and encourages the incorporation of enhanced method understanding during validation activities.[16]

The integration of these regulatory guidelines has shifted analytical development from a compliance-oriented activity toward a knowledge-driven process. Consequently, analytical methods developed using AQbD principles are increasingly recognized as being more reliable, flexible, and suitable for lifecycle management.[17-19]

### Critical analytical attributes (CAAs)

In LC-MS/MS method development, CAAs commonly include chromatographic resolution, retention time reproducibility, peak symmetry, signal intensity, signal-to-noise ratio, sensitivity, and quantitative accuracy. The selection of appropriate CAAs depends on the analytical objectives and the nature of the analyte being investigated.[20]

For anticancer drugs, sensitivity is often considered a particularly important attribute because many compounds require quantification at trace concentrations. Similarly, specificity becomes critical when analyzing complex matrices or evaluating stability-indicating methods. Monitoring CAAs throughout method development allows researchers to systematically assess the impact of experimental variables on analytical performance.[21]

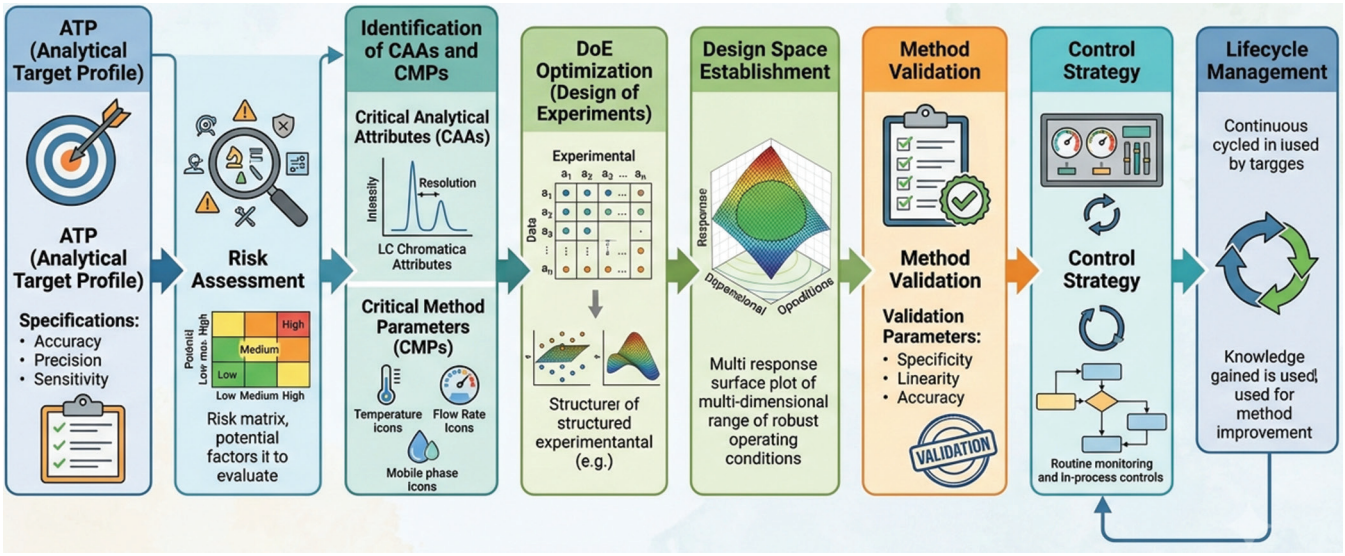


**Figure 1:** Frame work of analytical quality by design for liquid chromatography-tandem mass spectrometry method development

### CMPs

In LC-MS/MS analysis, chromatographic parameters, such as mobile phase composition, buffer concentration, pH, organic solvent ratio, flow rate, column temperature, and injection volume can significantly affect analytical performance. Similarly, mass spectrometric parameters, including ionization mode, capillary voltage, source temperature, gas flow rates, collision energy, and cone voltage influence sensitivity and signal stability.[22]

### Knowledge management in AQbD

Knowledge management begins with literature review and prior scientific understanding. Experimental data generated through screening studies, optimization experiments, and validation activities further contribute to the analytical knowledge base. This accumulated knowledge facilitates efficient troubleshooting, method transfer, and future method improvements.[23]

### Advantages of AQbD in LC-MS/MS method development

An important advantage is enhanced regulatory flexibility. Since AQbD-generated methods are supported by extensive scientific understanding, post-approval modifications can often be managed more efficiently through established control strategies and lifecycle management principles.[24]

Finally, AQbD supports continual improvement throughout the analytical lifecycle. Knowledge generated during routine application can be incorporated into future optimization efforts, ensuring sustained method performance and regulatory compliance. The key components are presented in Table 1.

## LC-MS/MS IN PHARMACEUTICAL ANALYSIS OF ANTICANCER DRUGS

### Overview of LC-MS/MS technology

LC-MS/MS has become one of the most powerful analytical tools in pharmaceutical research and quality control. The growing complexity of modern anticancer drugs has further increased the importance of LC-MS/MS because conventional chromatographic techniques may not always provide adequate sensitivity or selectivity for comprehensive analysis.[25] The widespread adoption of targeted therapies and small-molecule kinase inhibitors has introduced new analytical challenges. Many anticancer agents possess complex molecular structures and diverse physicochemical properties that require advanced analytical approaches for accurate quantification.[26,27]

In recent years, LC-MS/MS has been increasingly incorporated into AQbD-based analytical development programs. The technique is particularly suitable for systematic optimization because numerous chromatographic and mass spectrometric variables can be investigated through statistical experimental designs. This capability supports a more comprehensive understanding of method performance and contributes to the development of robust analytical procedures.[28]

### Principle of LC-MS/MS analysis

The first mass analyzer selects a pre-cursor ion corresponding to the analyte of interest. This selected ion undergoes collision-induced dissociation within a collision cell, generating characteristic product ions. The second mass analyzer then detects these fragments, producing highly selective analytical signals. The resulting data provide both qualitative and quantitative information regarding the analyte.[29,30]

This dual-stage mass analysis significantly improves selectivity compared with single-stage detection systems. The probability of interference is substantially reduced because both pre-cursor and product ions must satisfy predefined selection criteria.[31] Consequently, LC-MS/MS methods demonstrate excellent specificity even when analyzing complex pharmaceutical formulations. The schematic representation is presented in Figure 2.

## Major components of an LC-MS/MS system

An LC-MS/MS system consists of several integrated components that collectively determine analytical performance. The liquid chromatography module includes solvent reservoirs, pumps, autosamplers, and chromatographic columns. These components are responsible for achieving efficient separation of analytes before mass spectrometric detection.[32,33]

Within the tandem mass spectrometer, precursor ions are selected, fragmented, and detected. Data acquisition software subsequently processes the resulting signals to generate chromatograms and mass spectra. Each component contributes to overall method performance and must therefore be carefully evaluated during AQbD-based method development.[34]

## Ionization techniques used in pharmaceutical analysis

Efficient ionization is essential for achieving high sensitivity in LC-MS/MS analysis. Among available ionization techniques, electrospray ionization (ESI) is the most frequently employed for anticancer drug analysis.[35] The ionization efficiency of ESI is influenced by several factors, including mobile phase composition, pH, flow rate, source temperature, and solvent volatility. Because these parameters can significantly affect analytical performance, they are often identified as CMPs during AQbD studies.[36]

Atmospheric pressure chemical ionization (APCI) represents another commonly utilized ionization technique. APCI is

**Table 1:** Key components of AQbD in LC-MS/MS method development

| AQbD element | Purpose | Typical examples |
|---|---|---|
| ATP | Defines analytical objectives | Sensitivity, specificity |
| CAAs | Measures analytical performance | Resolution, peak area |
| CMPs | Variables affecting performance | pH, flow rate, CE |
| Risk assessment | Identifies critical factors | FMEA, Ishikawa |
| DoE | Systematic optimization | BBD, CCD |
| Design space | Acceptable operating region | Optimized CMP ranges |
| Control strategy | Maintains performance | System suitability tests |
| Lifecycle management | Continuous improvement | Trending and monitoring |

AQbD: Analytical quality by design, LC-MS/MS: Liquid chromatography-tandem mass spectrometry, ATP: Analytical target profile, CAAs: Critical analytical attributes, CMPs: Critical method parameters, DoE: Design of experiments, FMEA: Failure mode and effects analysis, BBD: Box-Behnken design, CCD: Central composite design, CE: Collision energy

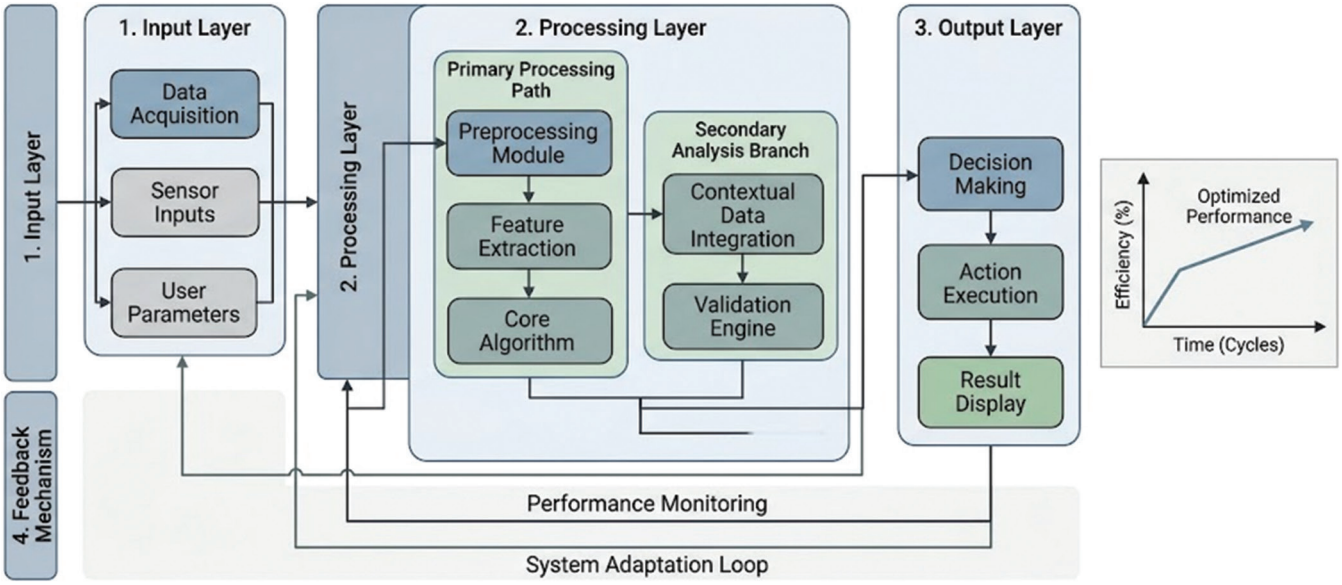


**Figure 2:** Schematic representation of a liquid chromatography-tandem mass spectrometry system

generally preferred for less polar compounds and may offer improved robustness for certain analytes.[37] Systematic evaluation of ionization mode is therefore an important component of method optimization.[38]

### Tandem mass spectrometric detection

The primary advantage of tandem mass spectrometry lies in its exceptional selectivity. By monitoring specific precursor-to-product ion transitions, analytical interference can be substantially minimized. This feature is particularly important for pharmaceutical analysis, where accurate quantification is required even in the presence of structurally related compounds and formulation components.[39]

Multiple reaction monitoring (MRM) has become the most widely employed acquisition mode in quantitative LC-MS/MS analysis. In MRM, predefined precursor and product ion pairs are continuously monitored throughout the chromatographic run. The resulting method demonstrates excellent sensitivity, reproducibility, and quantitative performance.[40]

### Application of LC-MS/MS to anticancer drug analysis

LC-MS/MS has become an indispensable analytical platform in oncology research and pharmaceutical quality control.[41]

### Role of LC-MS/MS in AQbD-based method development

The integration of LC-MS/MS with AQbD principles therefore represents a modern analytical strategy that emphasizes scientific understanding rather than empirical optimization. This approach facilitates efficient method development, reduces variability, and improves overall analytical reliability.[42]

## ANALYTICAL TARGET PROFILE (ATP), CAAs, AND CMPS IN AQBD-BASED LC-MS/MS DEVELOPMENT

### ATP: The foundation of AQbD

In LC-MS/MS method development for anticancer drugs, the ATP describes the characteristics required for successful analysis. These characteristics generally include specificity, sensitivity, accuracy, precision, linearity, robustness, and an appropriate analytical range.[43]

For stability-indicating LC-MS/MS methods, the ATP must ensure that the analytical procedure can accurately quantify the target anticancer drug without interference from related substances, excipients, or other components present within the sample matrix.[44]

### Relationship between ATP and method development

The relationship between ATP and method development can be viewed as a systematic progression from analytical objectives to operational conditions. Once the ATP is established, critical performance characteristics are identified. These characteristics subsequently guide risk assessment activities and experimental design strategies. The information generated during optimization studies is then used to establish operating conditions capable of consistently delivering the desired analytical performance.[45]

In LC-MS/MS analysis, several analytical responses may be considered critical depending on the intended application. These responses typically include chromatographic behavior, signal quality, and quantitative performance. The selection of relevant CAAs depends upon the objectives defined within the ATP.[46]

For anticancer drug analysis, chromatographic resolution often represents an important attribute because adequate separation is required to ensure accurate quantification. Similarly, peak symmetry contributes to reliable integration and improved analytical precision. Retention time reproducibility is another important consideration because consistent analyte elution supports method robustness and routine applicability.[47]

### CMPs

CMPs are operational variables that influence one or more CAAs. The identification and control of CMPs are central objectives of AQbD because method variability is largely governed by these factors.[48]

Mass spectrometric variables also play a significant role in determining analytical performance. Parameters, such as source temperature, desolvation gas flow, capillary voltage, cone voltage, collision energy, and dwell time can substantially influence ionization efficiency and detector response. Because LC-MS/MS methods depend upon efficient ion generation and fragmentation, optimization of these parameters is essential for achieving acceptable sensitivity and reproducibility.[49]

### Identification of CAAs and CMPs through risk assessment

The identification of CAAs and CMPs is typically performed through structured risk assessment exercises. Risk assessment provides a scientific framework for evaluating the potential impact of analytical variables on method performance and prioritizing factors for further investigation.[50]

For LC-MS/MS methods, risk assessment frequently reveals that mobile phase composition, pH, flow rate, source

temperature, and collision energy exert significant influence on analytical outcomes. These parameters are therefore commonly selected for subsequent experimental optimization studies.[51]

### Interrelationship between ATP, CAAs, and CMPs

The concepts of ATP, CAAs, and CMPs are closely interconnected and collectively form the foundation of AQbD-based analytical development. The ATP defines the desired analytical objectives. CAAs represent measurable indicators of analytical performance, while CMPs represent operational variables that influence those indicators.[52]

### Significance of ATP-driven development in LC-MS/MS methods

ATP-driven development offers several advantages over traditional analytical approaches. By focusing on analytical objectives from the outset, development activities become more systematic and scientifically justified. Experimental studies are designed to address specific analytical questions rather than relying on empirical optimization.[53]

## RISK ASSESSMENT STRATEGIES IN AQBD-BASED LC-MS/MS METHOD DEVELOPMENT

### Role of risk assessment in AQbD

Risk assessment is a fundamental component of AQbD. It provides a systematic approach for identifying variables that may influence analytical performance and for prioritizing factors requiring further investigation. In contrast to conventional method development, where experimental variables are often selected based on experience or trial-and-error experimentation, AQbD relies on scientific risk evaluation to guide method optimization.[54]

The information generated during risk assessment subsequently guides the selection of CMPs for experimental optimization studies [Figure 3].

### Objectives of risk assessment

Risk assessment also contributes to resource optimization. By focusing experimental efforts on parameters with the highest potential impact, researchers can reduce unnecessary experimentation while improving the overall efficiency of method development. Furthermore, the knowledge generated during risk assessment supports regulatory expectations regarding scientific justification and method understanding.[55-57]

### Ishikawa diagram as a risk assessment tool

The primary advantage of the Ishikawa diagram lies in its ability to facilitate comprehensive brainstorming and knowledge gathering during the early stages of method development. By encouraging consideration of multiple sources of variability, the diagram reduces the likelihood that critical factors will be overlooked [Figure 4]. Although the Ishikawa diagram does not provide quantitative estimates of risk, it serves as an effective starting point for identifying variables that require further evaluation through more formal risk assessment techniques.

### Failure mode and effects analysis (FMEA)

The FMEA process begins by identifying potential failure modes that may affect method performance. Each failure mode is then evaluated according to three criteria: Severity, occurrence, and detectability. Severity reflects the potential impact of the failure on analytical quality.[58]

In LC-MS/MS method development, parameters, such as mobile phase pH, flow rate, source temperature, collision energy, and column temperature often receive relatively high risk rankings because of their significant influence on analytical performance. FMEA therefore provides a rational basis for selecting variables for DoE investigations.[59]

### Application of risk assessment to LC-MS/MS optimization

For example, risk assessment may indicate that mobile phase pH, organic solvent composition, flow rate, source temperature, and collision energy exert significant influence on CAAs. These variables can subsequently be incorporated into screening and optimization studies to establish their individual and interactive effects on analytical performance.[60]

## DoE IN AQBD-BASED LC-MS/MS METHOD DEVELOPMENT

DoE represents one of the most important tools within the AQbD framework. Following the identification of CMPs through risk assessment, DoE provides a systematic approach for studying the effects of these variables on CAAs. The methodology enables simultaneous evaluation of multiple factors and their interactions, thereby generating a comprehensive understanding of method behavior.[29]

DoE overcomes these limitations by employing structured experimental designs that allow multiple variables to be investigated simultaneously. The resulting mathematical models describe the relationship between analytical factors

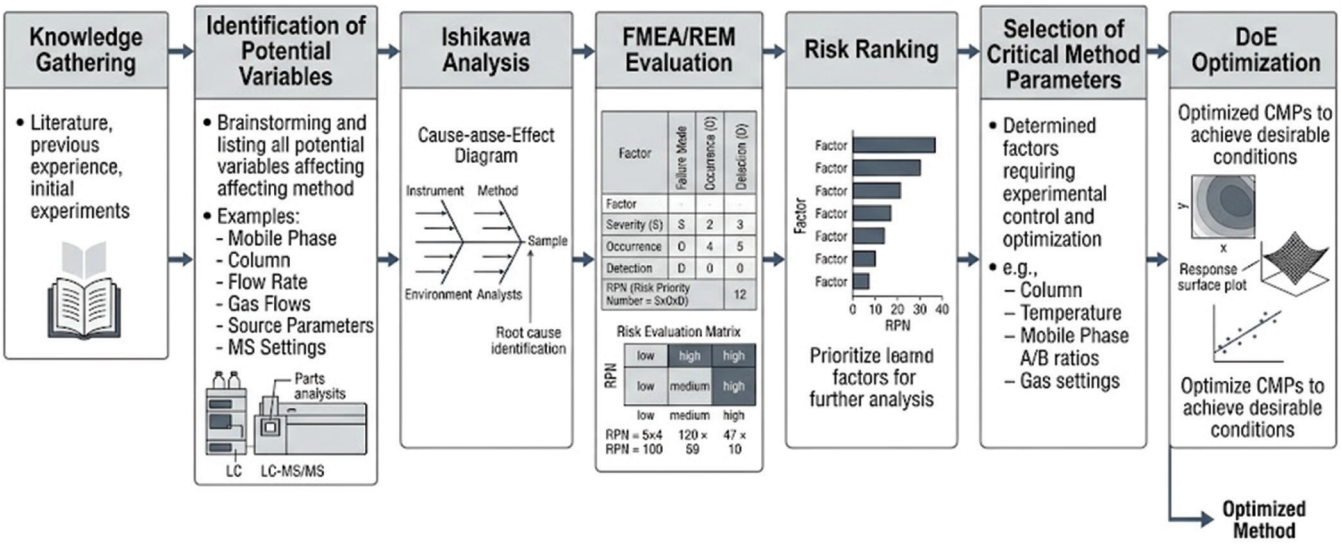


**Figure 3:** Risk assessment workflow in analytical quality by design-based liquid chromatography-tandem mass spectrometry method development

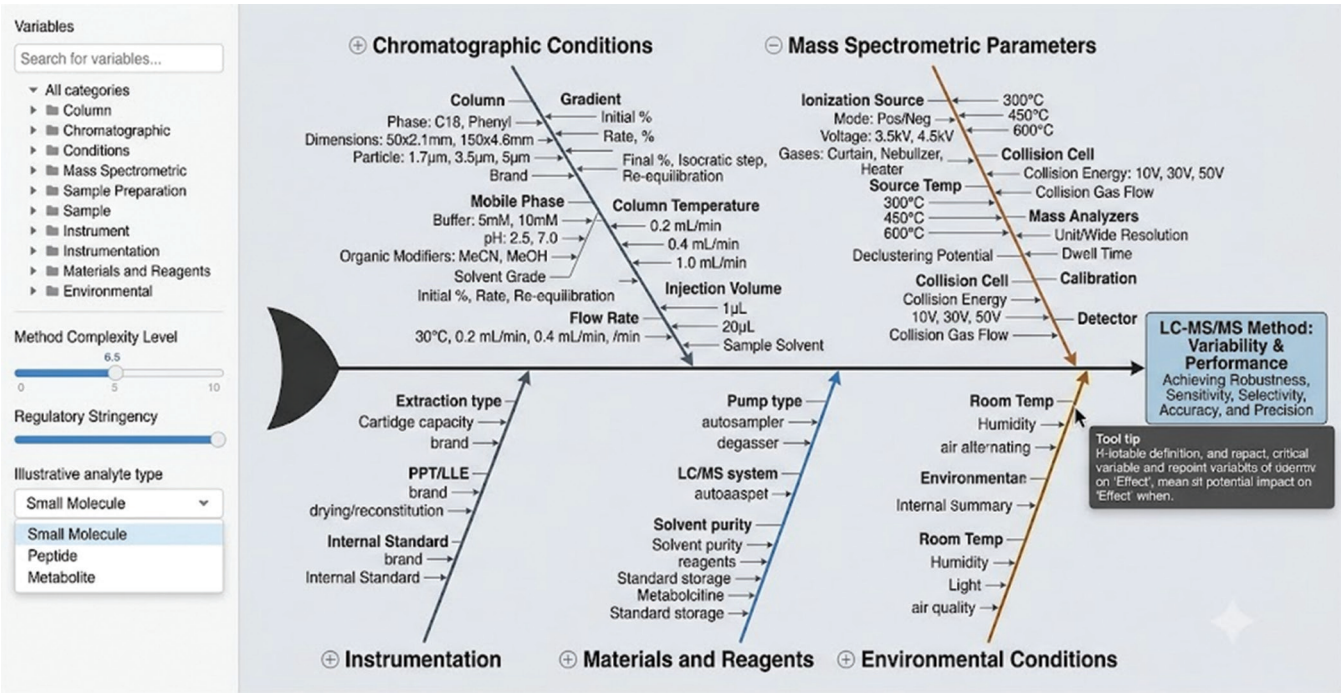


**Figure 4:** Representative Ishikawa diagram for liquid chromatography-tandem mass spectrometry method development

and analytical responses, facilitating optimization and supporting the establishment of a scientifically justified design space. Consequently, DoE has become an integral component of AQbD-based LC-MS/MS method development.[61]

For anticancer drug analysis, where numerous chromatographic and mass spectrometric variables influence analytical performance, DoE provides an efficient and reliable strategy for method optimization. The approach not only reduces the number of experiments required but also improves understanding of analytical processes and enhances method robustness.

## Experimental factors and analytical responses

The successful application of DoE requires careful selection of both experimental factors and analytical responses. Experimental factors correspond to the CMPs identified during risk assessment, whereas analytical responses represent the CAAs used to evaluate method performance.[62]

The selection of appropriate factors and responses is essential because the quality of the resulting model depends upon the relevance of the variables included in the experimental design.

### Screening designs

Screening studies are particularly useful in LC-MS/MS development because numerous chromatographic and mass spectrometric parameters may potentially affect analytical performance. Investigating all variables simultaneously through optimization designs would be impractical and resource intensive.[63]

### Optimization designs

Response surface methodology (RSM) is the most commonly employed optimization strategy in AQbD. RSM utilizes mathematical and statistical techniques to model relationships between experimental variables and analytical responses. The resulting response surfaces provide a visual representation of factor effects and facilitate identification of optimal conditions.[64]

Optimization studies are particularly valuable because analytical responses often exhibit non-linear behavior. Simple linear models may therefore be insufficient to describe the true relationship between experimental variables and method performance. RSM addresses this limitation by incorporating quadratic terms and interaction effects into the mathematical model.[65]

The application of response surface designs enables researchers to identify experimental regions where multiple analytical objectives can be simultaneously achieved.

### Central composite design (CCD)

CCD is one of the most widely applied response surface designs in pharmaceutical analytical development. CCD combines factorial points, axial points, and center points to generate a comprehensive experimental dataset suitable for fitting quadratic models.

The design offers several advantages. It enables efficient estimation of curvature effects and provides detailed information regarding interactions among variables. Furthermore, CCD allows exploration of a relatively broad experimental region, making it suitable for analytical optimization studies where factor behavior is not yet fully understood.[66]

### Box-Behnken design (BBD)

BBD represents another widely used response surface design in AQbD-based analytical development. The design requires fewer experimental runs than CCD while still providing sufficient information to develop quadratic models.

### Mathematical modeling and response surface analysis

The data generated through DoE are used to develop mathematical models that describe the relationship between experimental factors and analytical responses. These models typically take the form of polynomial equations incorporating linear, interaction, and quadratic terms.

Statistical analysis is subsequently performed to evaluate model significance and adequacy. Parameters, such as regression coefficients, analysis of variance, coefficient of determination, and lack-of-fit tests are commonly used to assess model quality.[67]

Response surface plots provide graphical representations of these mathematical relationships. Such plots facilitate visualization of factor effects and interactions, allowing researchers to identify experimental regions associated with optimal analytical performance.[68]

### Multi-response optimization using desirability functions

Analytical method development frequently involves simultaneous optimization of multiple responses. For example, an LC-MS/MS method may require high sensitivity, adequate resolution, acceptable peak symmetry, and short analysis time. Since these objectives may not always be achieved under identical conditions, a strategy is required for balancing competing requirements.[69]

### Significance of DoE in AQbD-based LC-MS/MS development

DoE improves method understanding, facilitates efficient resource utilization, and supports the development of robust analytical procedures. Furthermore, the knowledge generated through DoE contributes directly to design space establishment, control strategy development, and lifecycle management.[70]

As regulatory agencies continue to encourage science-based analytical development, DoE is expected to remain a central component of AQbD implementation. Its ability to provide detailed process understanding makes it particularly valuable for the development of modern LC-MS/MS methods intended for complex pharmaceutical applications [Figure 5].

## DEVELOPMENT OF STABILITY-INDICATING LC-MS/MS METHODS USING AQbD

### Analytical method development within the AQbD framework

The development of stability-indicating LC-MS/MS methods has evolved considerably with the implementation of AQbD principles. Traditional approaches often focused on achieving chromatographic separation through repeated experimental

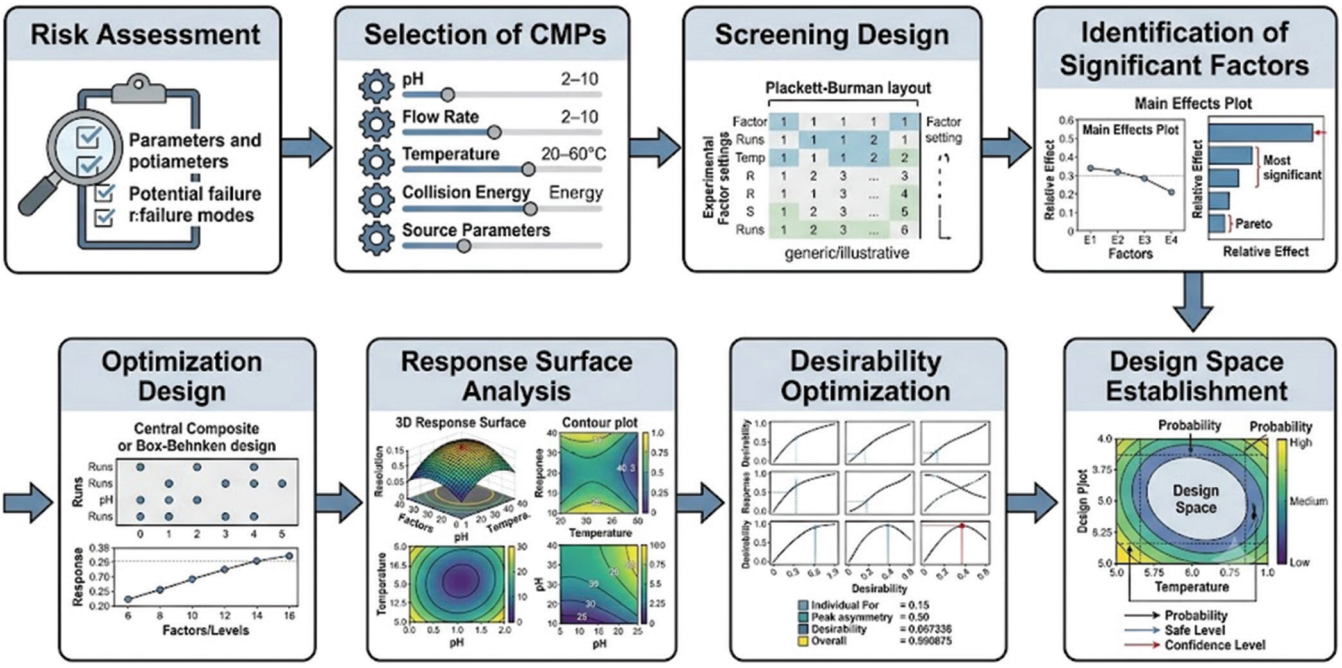


**Figure 5:** Application of design of experiments in analytical quality by design-based liquid chromatography-tandem mass spectrometry method development

adjustments, with limited understanding of the relationship between method variables and analytical performance. In contrast, AQbD promotes a structured and knowledge-based approach in which analytical objectives are defined at the outset and method development proceeds through systematic investigation of critical variables.[71]

The AQbD framework provides a logical pathway from ATP definition to method validation and lifecycle management. This approach ensures that analytical methods are developed with a clear understanding of their intended purpose and performance requirements. The resulting methods are generally more robust, reproducible, and suitable for routine implementation.[72]

For LC-MS/MS analysis of anticancer drugs, AQbD-based development is particularly advantageous because multiple chromatographic and mass spectrometric variables influence analytical performance. The systematic evaluation of these variables allows the establishment of scientifically justified operating conditions and reduces the risk of unexpected method failure during routine use.

## APPLICATION OF AI AND ML IN LC-MS/MS METHOD DEVELOPMENT

### Emergence of AI in pharmaceutical analytics

AI has emerged as a transformative technology across pharmaceutical research and development. The increasing complexity of analytical data generated during LC-MS/MS studies has created opportunities for advanced computational approaches capable of identifying patterns and relationships that may not be readily apparent through conventional analysis.

The integration of AI into AQbD-based method development represents a natural progression toward data-driven analytical science. Both approaches emphasize systematic understanding, prediction, and optimization of analytical performance.[71]

### ML for method optimization

ML algorithms can analyze large experimental datasets and predict analytical responses based on historical observations. Algorithms, such as artificial neural networks, random forests, support vector machines, and gradient boosting models have demonstrated potential for predicting chromatographic and mass spectrometric behavior.[63]

These models can significantly reduce experimental burden by identifying promising operating conditions before laboratory experimentation.

### AI-assisted experimental design

AI has the potential to complement traditional DoE approaches. ML models can identify hidden relationships among variables and suggest optimized experimental conditions.

This capability may improve development efficiency and reduce the number of experiments required to achieve robust analytical performance.

### Prediction of chromatographic performance

Retention time prediction has become one of the most actively investigated applications of AI in chromatography. Predictive models can estimate chromatographic behavior based on molecular descriptors and analytical conditions. Such predictions facilitate method development and support efficient column and mobile phase selection.

### AI in mass spectral interpretation

Mass spectral interpretation often represents one of the most time-consuming aspects of LC-MS/MS analysis. AI-based algorithms can assist in identifying precursor ions, assigning fragmentation pathways, and recognizing spectral patterns. The application of deep learning approaches has shown promising results in automated spectral annotation and compound identification.

### AI and AQbD integration

The integration of AI with AQbD creates opportunities for enhanced analytical understanding. ML models can support risk assessment, experimental optimization, design space prediction, and lifecycle monitoring. This integration may ultimately lead to adaptive analytical systems capable of continuously improving performance based on accumulated data.[72]

## PRESENT CHALLENGES AND FUTURE OPPORTUNITIES

Despite significant advances, several challenges continue to influence the implementation of AQbD-based LC-MS/MS methods. Method complexity, extensive data requirements, software limitations, and resource demands can complicate development activities. The increasing adoption of AI, advanced chemometrics, cloud computing, and automated analytical platforms is expected to address many of these challenges. Future analytical laboratories may increasingly employ predictive models capable of recommending optimal conditions, monitoring performance in real time, and supporting autonomous decision-making. The convergence of AQbD, LC-MS/MS, and AI is expected to redefine analytical method development over the coming decade.[67]

## CONCLUSION

AQbD has fundamentally transformed the development of stability-indicating LC-MS/MS methods for pharmaceutical analysis. Across the systematic application of ATP-driven development, risk assessment, DoE, and design space establishment, AQbD provides a scientifically robust framework for analytical method development and lifecycle management. LC-MS/MS continues to play a central role in pharmaceutical analysis because of its exceptional sensitivity, selectivity, and versatility. The integration of AQbD principles enhances method robustness, reproducibility, and regulatory acceptability while reducing development uncertainty. Emerging advances in AI and ML are further expanding the capabilities of analytical science. These technologies offer opportunities for predictive modeling, automated optimization, and intelligent data interpretation. The combined implementation of AQbD, LC-MS/MS, and AI has the potential to create next-generation analytical platforms that are more efficient, adaptive, and knowledge-driven. As regulatory expectations continue to evolve toward science-based development and lifecycle management, the integration of these approaches is expected to play an increasingly important role in pharmaceutical research, quality assurance, and regulatory decision-making.

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**Source of Support:** Nil. **Conflicts of Interest:** None declared.