INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Review Paper

Analytical Method Validation a Comprehensive Review of Principles, Parameters, Regulatory Frameworks, And Evolving Practices

Ankita Mohite*, Amit Kasabe

Meruling Shikshan Sanstha's College of Pharmacy, Medha..

ARTICLE INFO

Published: 09 June 2026
Keywords:
Analytical method validation, ICH Q2(R2), specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, Analytical Target Profile, lifecycle management, pharmaceutical quality assurance
DOI:
10.5281/zenodo.20610664

ABSTRACT

Analytical method validation lies at the very core of pharmaceutical quality assurance. Before any analytical procedure can be trusted to support regulatory decisions about product safety, efficacy, or quality, its performance must be rigorously and systematically characterized. The International Council for Harmonisation (ICH) has long provided the global pharmaceutical community with harmonized guidance for this purpose through its Q2 series of guidelines. The most recent iteration, ICH Q2(R2), was formally adopted in November 2023 alongside the companion guideline ICH Q14 on Analytical Procedure Development. Together, these documents represent the most far-reaching revision to analytical validation guidance in nearly three decades.This review examines the principles, validation parameters, and regulatory expectations that underpin ICH Q2, with close attention to the substantive differences between Q2(R1) and Q2(R2). The parameters covered include specificity, linearity, range, accuracy, precision in its three recognized forms—repeatability, intermediate precision, and reproducibility—as well as limit of detection (LOD), limit of quantitation (LOQ), and robustness. Beyond these classic parameters, the review also explores the lifecycle-based framework for managing analytical procedures over time, the formal introduction of the Analytical Target Profile (ATP), considerations for method transfer and revalidation, and the newly introduced guidance on multivariate analytical procedures.A comparative overview of complementary regulatory guidance from the US Food and Drug Administration, the European Medicines Agency, the World Health Organization, and the United States Pharmacopeia is also presented. The review concludes by examining the practical difficulties that pharmaceutical laboratories face when implementing Q2(R2) requirements and by reflecting on the future direction of method validation in an era increasingly defined by Quality by Design, advanced spectroscopic technology, and artificial intelligence.

***Corresponding Author:** Ankita Mohite
Address: *Meruling Shikshan Sanstha's College of Pharmacy, Medha...*
Email ✉: mohiteankita134@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INTRODUCTION

The pharmaceutical industry operates within one of the most rigorously regulated environments of any scientific discipline. Central to this regulatory framework is the concept of product quality—the assurance that a medicine delivers exactly what it promises: the correct amount of the stated active ingredient, free from harmful contaminants, and capable of producing the intended therapeutic effect in the patient. Throughout the product lifecycle, from early-stage development through commercial manufacturing and post-market surveillance, analytical methods serve as the essential instruments by which pharmaceutical quality is measured and confirmed.[9]

For any analytical method to be genuinely relied upon in quality decision-making, it must first be demonstrated that the method is suitable for its intended purpose. This demonstration is achieved through analytical method validation—a systematic series of experimental studies that collectively establish whether a given procedure will consistently yield reliable, accurate, precise, and specific results under the conditions in which it will be used. Without validated analytical methods, neither regulatory agencies nor manufacturers can have confidence that the data generated in quality testing reflects the true state of the product being tested.[1,2]

The ICH was established in 1990 as a collaborative initiative between regulatory bodies and pharmaceutical industry associations from the United States, Europe, and Japan, with the express purpose of reducing regulatory duplication and harmonizing technical requirements across these major markets. Within ICH's Quality category of guidelines, Q2 addresses the validation of analytical procedures specifically. The original Q2(R1) guideline, finalized in 2005 by merging the earlier Q2A (1994) and Q2B (1996) documents, served as the global standard for analytical validation for nearly two decades.[1,2,31] During those years, however, the analytical landscape changed considerably. Chromatography-mass spectrometry, near-infrared spectroscopy, chemometric modeling, and the emergence of complex biological medicines all highlighted limitations in Q2(R1) that could no longer be overlooked. Recognizing this, the ICH initiated a revision process that would culminate in the concurrent publication of Q2(R2) and Q14 in November 2023. ICH Q2(R2) formally expands the scope of validation guidance to biological products, introduces the Analytical Target Profile as a foundational concept, integrates a lifecycle management perspective, and provides, for the first time, substantive guidance on multivariate analytical procedures.[1,3]

This review is designed to serve as a thorough reference for pharmaceutical quality assurance students, analytical scientists, and regulatory professionals. It covers validation parameters in depth, draws explicit comparisons between Q2(R1) and Q2(R2), situates ICH guidance within the broader global regulatory landscape, and discusses both the practical challenges of implementation and the future trajectory of the field.

2. HISTORICAL BACKGROUND AND EVOLUTION OF ICH Q2

Before the ICH was established, pharmaceutical companies seeking regulatory approval in the United States, Europe, and Japan were required to satisfy three separate and often conflicting sets of technical requirements. For analytical validation, this meant designing, executing, and documenting distinct studies to meet each region's preferences—an enormous duplication of effort that delayed drug approvals and increased costs without any corresponding benefit to patient safety.[31]

The formation of the ICH in 1990 brought together the US FDA, the EMA and its predecessor bodies, the Japanese MHLW, and their respective industry associations under a shared commitment to harmonizing technical guidelines. Analytical method validation was among the first areas addressed. In 1994, ICH released Q2A, which provided foundational definitions and concepts for analytical validation. Two years later, Q2B set out the practical methodology for conducting validation studies. Together, these documents established the conceptual and experimental framework that guided the global pharmaceutical industry for nearly a decade.[2,32,33]

In November 2005, Q2A and Q2B were consolidated and revised into a single document, Q2(R1), titled 'Validation of Analytical Procedures: Text and Methodology.' This document defined key validation parameters, specified which types of analytical procedures required validation, and provided recommendations for the data to be submitted to regulatory authorities. Q2(R1) rapidly became the global standard and remains widely cited to this day.[2]

Despite its broad adoption, Q2(R1) had significant gaps that became increasingly apparent as pharmaceutical science advanced. It was conceived primarily around small-molecule drugs and provided little guidance for biotechnological and biological products such as monoclonal antibodies, recombinant proteins, and gene therapy products. It did not address near-infrared spectroscopy, Raman spectroscopy, or multivariate chemometric methods, which had become important tools in modern quality control. Nor did it reflect the lifecycle quality philosophy that had become central to pharmaceutical development under ICH Q8 and Q10.[1,4,5]

In the late 2010s, the ICH initiated a revision. The Expert Working Group was tasked with revising Q2 while simultaneously developing the new Q14 guideline on Analytical Procedure Development—a deliberate decision to create a cohesive, integrated framework covering the full life of an analytical procedure. Draft Q2(R2) and Q14 were released for public consultation in March 2022, attracting extensive feedback from industry associations, contract research organizations, academic institutions, and individual scientists worldwide.[3,31]

Following thorough review of the public commentary, the revised guidelines were adopted at the ICH Assembly on November 1, 2023. The EMA implemented Q2(R2) with a legal effective date of June 14, 2024, signaling a formal transition for the European pharmaceutical industry.[1,12,13]

3. CLASSIFICATION OF ANALYTICAL PROCEDURES

Designing an appropriate validation strategy begins with a precise understanding of what type of analytical procedure is being validated. ICH Q2(R2) recognizes four primary categories, each serving a distinct purpose and carrying its own set of validation requirements.[1]

3.1 Identification Tests

Identification tests are qualitative procedures designed to confirm that the analyte in a sample is the substance claimed to be present. The confirmation may rely on an infrared absorption spectrum, an ultraviolet absorption maximum, a chromatographic retention time, a nuclear magnetic resonance (NMR) spectral pattern, or a specific chemical reactivity profile. Because these tests yield a qualitative rather than a quantitative result, the only validation parameter they must satisfy is specificity—the ability to discriminate the analyte from other substances that might be confused with it.[1,2]

3.2 Assay Procedures

Assay procedures quantitatively determine the concentration or amount of the active

pharmaceutical ingredient (API) in a drug substance or drug product. These methods must operate accurately and precisely across the full range of concentrations typical of commercial products, generally from approximately 80% to 120% of the nominal label claim. Assay procedures are subject to the most comprehensive suite of validation requirements, encompassing specificity, linearity, range, accuracy, precision, and robustness. Methods intended for stability testing carry the additional requirement to demonstrate stability-indicating capability.[1,15]

3.3 Impurity Testing Procedures

Impurity testing methods detect and quantify substances present in drug substances or products other than the active ingredient. ICH Q2(R2) distinguishes between quantitative impurity tests—which determine the actual amount of a specific impurity—and limit tests, which merely confirm that an impurity does not exceed a defined threshold without necessarily measuring it precisely. Quantitative impurity tests require a comprehensive validation package; limit tests require a more limited set of parameters focused chiefly on specificity and LOD. The validation of impurity methods is particularly critical in the context of regulatory frameworks for impurity control, as defined in ICH Q3A, Q3B, and Q3C.[1,6,7,8]

3.4 Dissolution and Drug Release Testing

Dissolution testing measures the rate and extent of drug release from solid or semi-solid dosage forms under defined in vitro conditions. It shares many validation requirements with assay procedures but occupies a unique regulatory status because of its role in predicting in vivo performance and its use as a surrogate for bioequivalence in certain regulatory pathways. ICH Q2(R2) includes an expanded annex of worked examples applicable to dissolution methods, reflecting the significance of this procedure category.[1,17]

Table 1: Validation Parameters Required for Different Categories of Analytical Procedures (ICH Q2(R2))

Validation Parameter	Identification Tests	Impurity Tests (Quantitative)	Impurity Tests (Limit)	Assay / Content Uniformity
Specificity / Selectivity	Yes	Yes	Yes	Yes
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes
Accuracy	No	Yes	No	Yes
Repeatability	No	Yes	No	Yes
Intermediate Precision	No	Yes*	No	Yes*
Reproducibility	No	No**	No	No**
LOD	No	No***	Yes	No
LOQ	No	Yes	No	No
Robustness	No	Yes	Yes	Yes

** Not required if reproducibility has been demonstrated. ** Required for standardization only. *** May be required in certain circumstances.*

4. VALIDATION PARAMETERS – DETAILED DISCUSSION

Validating an analytical procedure involves the systematic evaluation of defined performance

characteristics, each addressing a specific aspect of method capability. The following sections discuss each major validation parameter in depth, covering experimental approaches, acceptance criteria, and relevant advances introduced by Q2(R2).[1,2]

4.1 Specificity and Selectivity

Specificity refers to the ability of an analytical procedure to measure the analyte of interest accurately in the presence of all other components likely to be found in the sample—impurities, degradation products, excipients, and matrix constituents. ICH Q2(R2) uses the terms specificity and selectivity somewhat interchangeably, acknowledging that few methods can be shown to be entirely free from interference under every conceivable condition, but that the ability to distinguish the analyte from the majority of potential interferents is both achievable and necessary.[1,2]

ICH Q2(R2) introduces three distinct scientific approaches for demonstrating specificity. The first, 'absence of interference,' involves showing that the analytical response attributed to the analyte is unaffected by the presence of other sample components. In chromatographic methods, this typically involves analysis of placebo formulations (prepared without the active ingredient), stressed samples generated through forced degradation studies, and samples deliberately spiked with known impurities. Peak purity evaluation using diode array detection or mass spectrometric detection further confirms the absence of co-eluting interferents.[1,19]

The second approach is orthogonal procedure comparison, in which results from the method under validation are compared against those of an independent, well-established procedure known to be specific. Where the two methods agree closely, this agreement constitutes supporting evidence of specificity for the new method. The third approach, technology-inherent justification, acknowledges that certain analytical techniques—NMR spectroscopy and mass spectrometry, for example—are inherently specific by virtue of their underlying physical principles, and that formal spiking experiments may not always be necessary for these.[1,29]

Forced degradation studies remain particularly important for stability-indicating methods, which must detect and quantify all degradation products likely to form during the product's shelf life. Exposing the drug substance or product to heat, humidity, acid, base, oxidative, and photolytic conditions generates a representative range of degradation products against which the method's separating and detection capability can be assessed.[1,9]

4.2 Linearity and Range

Linearity is the ability of an analytical procedure to produce instrument responses that are directly proportional to analyte concentration across a defined interval. A linear relationship permits the determination of unknown concentrations by interpolation from a calibration curve, which is fundamental to any quantitative analytical method. ICH Q2(R2), consistent with Q2(R1), recommends evaluating linearity using at least five concentration levels spanning the intended working range of the method.[1,2]

The linearity relationship is typically characterized by linear regression analysis, yielding the slope, the y-intercept, the coefficient of determination (r^2), and the standard deviation of the residuals. While a high r^2 value (generally ≥ 0.999 for assay methods and ≥ 0.99 for impurity methods) is indicative of a good fit, ICH guidelines note that r^2 alone is insufficient and that inspection of residuals and confidence intervals around the slope and intercept is also necessary to confirm linearity.[1,30]

A significant advance in Q2(R2) is the explicit acknowledgment that not all analytical procedures produce strictly linear responses. Immunoassays, cell-based bioassays, and certain spectroscopic methods may yield sigmoidal or exponential concentration-response curves. For these methods, Q2(R2) permits the use of non-linear mathematical models, provided the model is clearly defined and its use scientifically justified. This flexibility is particularly valuable for validating the diverse procedures used in biological product testing.[1,28]

The range of a procedure is the operationally defined concentration interval over which the method has been demonstrated to be linear, accurate, and precise. For assay procedures, this range typically spans from 80% to 120% of the nominal test concentration. For impurity methods, coverage typically extends from the reporting threshold up to 120% of the specification limit. For dissolution methods, the range should encompass the full specification for drug release.[1,2,15]

4.3 Accuracy

Accuracy is the closeness of agreement between the value measured by the analytical procedure and the true or conventionally accepted reference value of the analyte. It is expressed as percent recovery—the ratio of the measured value to the expected value, multiplied by one hundred. Accuracy is one of the most fundamental validation parameters because it determines directly whether the method produces correct answers about product quality.[1,2]

For drug substance assay methods, accuracy is commonly assessed by comparison with a certified primary reference standard of known and traceable purity. For drug product assay methods, accuracy is typically evaluated by spiking a known quantity of the active ingredient into a placebo matrix and determining what proportion of the added drug the method recovers. For impurity methods, where certified standards may not be commercially available, alternative approaches include comparison with a well-characterized orthogonal method, use of surrogate standards of known purity, or estimation from response factor data.[1,9]

ICH Q2(R2) introduces confidence interval-based acceptance criteria for accuracy, complementing the traditional percent recovery approach. This more statistically rigorous framework accounts for measurement variability rather than relying on point estimates alone. Q2(R2) also permits accuracy and precision studies to be combined in a single experimental protocol, an option that can be more efficient while providing a comprehensive, integrated dataset.[1,27]

The general recommendation—maintained from Q2(R1)—is to assess accuracy using a minimum of nine determinations across at least three concentration levels (typically three replicates at 80%, 100%, and 120% of the nominal concentration). Acceptance criteria for assay methods commonly require percent recovery within 98.0–102.0%, while impurity methods may be accepted over a broader range of 80–120%, adjusted according to concentration level.[1,2,9]

4.4 Precision

Precision describes the degree of agreement among a series of measurements obtained from multiple samplings of a homogeneous sample under prescribed conditions. It evaluates the closeness of results to one another—not to the true value—and is expressed as the percent relative standard deviation (%RSD) or coefficient of variation (%CV) of the replicate measurements. A method cannot be accurate across independent measurements without also being reasonably precise, though it is possible for a precise method to be consistently inaccurate due to a systematic bias. ICH Q2(R2) distinguishes three hierarchical levels of precision.[1,2,30]

4.4.1 Repeatability

Repeatability—also called intra-assay or within-run precision—describes variation obtained when the same analyst applies the same method on the same equipment on the same day. ICH Q2(R2) recommends assessing repeatability from a minimum of nine determinations spanning the specified range (three concentrations with three replicates each) or six determinations at 100% of the test concentration. For assay methods, repeatability %RSD values of no more than 1.0–2.0% are generally expected. For impurity methods, somewhat wider criteria of up to 5.0–10.0% may be acceptable depending on the analyte concentration.[1,2,15]

4.4.2 Intermediate Precision

Intermediate precision—sometimes called within-laboratory reproducibility or ruggedness—captures the variation arising from deliberate changes in experimental conditions within a single laboratory over time. Relevant sources of variability include different analysts, different instruments, different days, different batches of reagents, and varying environmental conditions. Intermediate precision is particularly important for routine quality control methods, which must perform reliably across the full operational range of a working analytical laboratory, not merely under the controlled conditions of an initial validation study.[1,2]

4.4.3 Reproducibility

Reproducibility represents the highest level of the precision hierarchy and assesses method performance across different laboratories. It is evaluated through collaborative studies or interlaboratory comparisons in which the same procedure is applied to identical samples by different laboratories operating under different conditions. ICH Q2 notes that reproducibility is principally relevant when a method is being considered for compendial adoption or for standardized use across multiple organizational sites. While not universally required for regulatory submissions, reproducibility data meaningfully strengthens confidence in a method's fitness for widespread use.[1,2]

4.5 Limit of Detection (LOD)

The LOD is the lowest analyte concentration that can be reliably detected but not necessarily quantified under the stated conditions of the method. It is a critical parameter for qualitative tests, limit tests, and impurity procedures where the primary question is whether a substance is present above or below a defined threshold. An inadequate LOD could allow harmful impurities or degradation products to go undetected at levels that exceed regulatory limits, with direct consequences for patient safety.[1,2]

ICH Q2(R2) describes three approaches for LOD determination. Visual evaluation involves analyzing samples at progressively lower concentrations until the analyst can no longer distinguish a signal from background noise. Though straightforward in principle, this approach is subjective and analyst-dependent. The signal-to-noise approach, more commonly used in chromatographic methods, defines the LOD as the concentration producing a signal at least three times greater than the baseline noise. The formula-based statistical approach estimates the LOD as $3.3\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. Q2(R2) places stronger emphasis than its predecessor on confirming any calculated LOD by experimental measurement at that concentration.[1,2,19]

4.6 Limit of Quantitation (LOQ)

The LOQ is the lowest analyte concentration that can be quantitatively determined with acceptable accuracy and precision under the stated conditions.

Unlike the LOD, the LOQ must satisfy defined criteria for both precision (typically %RSD ≤ 10–15%) and accuracy (typically % recovery within 80–120%) at that concentration. The LOQ is particularly critical for impurity testing, where specifications may require accurate quantification near the threshold of instrument sensitivity.[1,2]

The same three approaches used for LOD can be applied to LOQ, but the constants differ: the signal-to-noise approach uses a ratio of 10:1, and the formula-based approach uses LOQ = 10σ/S. As with LOD, any calculated LOQ must be confirmed experimentally. The LOQ must always be greater than the LOD, since reliable quantification necessarily implies prior detection; in practice, the LOQ is often approximately two to five times higher than the LOD for well-optimized methods.[1,2,30]

4.7 Robustness

Robustness describes the ability of an analytical procedure to maintain its performance characteristics when subjected to small but deliberate variations in method parameters. It reflects how stable a method's performance remains in the face of the minor day-to-day variations in conditions that inevitably occur in a working laboratory—changes in mobile phase composition, pH, temperature, flow rate, sample preparation steps, reagent concentrations, or column batch.[1,2]

ICH Q2(R2) substantially elevates the role of robustness relative to Q2(R1). In the earlier guideline, robustness was noted as a parameter to be 'considered at an appropriate stage in development' and was often treated as optional by many practitioners. Q2(R2) takes a clearly stronger position, explicitly expecting demonstration of 'reliability in response to deliberate variation of parameters as well as stability of samples and reagents.' This shift aligns robustness data with the broader lifecycle philosophy of Q2(R2), treating it as an essential component of the method's established performance knowledge base.[1,26,43]

The Plackett-Burman design—a fractional factorial experimental approach—is the most widely used strategy for robustness testing. By varying several parameters simultaneously at two levels (slightly above and below the nominal value), this design efficiently identifies which parameters exert a significant influence on method performance. Parameters found to be influential should be specified more precisely in the method procedure; those found to have no significant effect provide reassurance about normal operational flexibility.[29,30]

Robustness studies also evaluate the stability of analytical solutions over time, since the assumption that prepared samples, standards, and mobile phases remain usable throughout a working day—or across multiple days—must be experimentally supported rather than simply assumed. The findings from robustness studies directly inform the system suitability criteria that will govern routine application of the method.[1,35]

4.8 System Suitability Testing

System suitability testing (SST) is not a standalone validation parameter but an integral element of method implementation, closely informed by the validation process. SST consists of a set of procedural checks performed before (and sometimes during) each analytical run to verify that the entire analytical system—instrument, column, reagents, and standards—is performing as required on a given day.[1,17]

For chromatographic methods, typical SST parameters include the resolution between critical peak pairs, the tailing factor of the principal peak, the number of theoretical plates, retention time, and the peak area or height response of the reference standard. These parameters are evaluated against predefined acceptance limits

derived from validation data and must be met for a run to be considered valid. The connection between robustness studies and SST is direct: understanding which method parameters most influence performance allows the analyst to design system suitability tests capable of detecting the day-to-day variations most likely to compromise results. ICH Q2(R2) recommends that SST criteria be periodically reviewed as routine-use data accumulates, to confirm they remain fit for purpose.[1,17,35]

Table 2: Summary of Validation Parameters and Recommended Acceptance Criteria

Validation Parameter	Recommended Criteria	Method Type	Regulatory Reference
Accuracy (% Recovery)	98.0–102.0% (assay); 80–120% (impurity)	Assay / Impurity	ICH Q2(R2); FDA 2015
Repeatability (%RSD)	≤ 1.0–2.0% (assay); ≤ 5.0% (impurity)	Assay / Impurity	ICH Q2(R2)
Intermediate Precision (%RSD)	≤ 2.0% (assay); ≤ 10% (impurity)	Assay / Impurity	ICH Q2(R2)
Linearity (r^2)	≥ 0.999 (assay); ≥ 0.99 (impurity)	Assay / Impurity	ICH Q2(R1/R2); USP <1225>
LOD (S/N)	≥ 3:1	Impurity / Limit test	ICH Q2(R2)
LOQ (S/N)	≥ 10:1	Impurity	ICH Q2(R2)
Tailing Factor (T)	0.8–2.0	HPLC	USP <621>
Theoretical Plates (N)	≥ 2000	HPLC	USP <621>
Resolution (Rs)	≥ 2.0 for critical peak pair	HPLC / Chromatography	ICH Q2(R2); USP
Robustness (%RSD)	Meets method criteria across deliberate changes	All types	ICH Q2(R2)

5. ICH Q2(R2): KEY ADVANCES AND CHANGES FROM Q2(R1)

The publication of ICH Q2(R2) alongside Q14 in November 2023 marked the most significant revision to international analytical validation guidance since the early 1990s. The changes introduced are not merely editorial; they reflect a fundamental evolution in the pharmaceutical industry's scientific and regulatory understanding of what it means to validate an analytical procedure. Practitioners must understand these changes in detail to design compliant validation strategies and to assess whether existing validated methods require supplemental work.[1,26,43]

5.1 Expanded Scope to Include Biological and Biotechnological Products

The most consequential change in Q2(R2) is the explicit extension of its scope to biotechnological and biological products—monoclonal antibodies, recombinant proteins, vaccines, cell therapies, gene therapies, and other complex biologics. Q2(R1) was developed with small-molecule drugs in mind and offered little practical guidance for biological products, which present validation challenges that are categorically different in nature and complexity.[1,28]

Biological products are typically characterized not by a single definitive assay but by a suite of complementary analytical methods, since no

single technique can fully define the structural complexity and biological activity of a large protein molecule. Potency assays for biologics frequently involve cell-based systems with inherently higher variability than chromatographic assays; statistical approaches appropriate for these systems differ substantially from those applicable to small-molecule assays. Q2(R2) acknowledges these differences and provides a science-based, risk-proportionate framework within which biological product methods can be meaningfully validated.[1,28]

5.2 Introduction of the Analytical Target Profile (ATP)

ICH Q2(R2) formally introduces the Analytical Target Profile as the conceptual anchor for method development and validation. The ATP is a prospective statement of the performance requirements that an analytical procedure must satisfy to be considered fit for its intended use. Before method development begins, the ATP defines—in quantitative terms—the minimum acceptable accuracy, precision, range, and other performance characteristics that the final validated method must achieve.[1,3]

The ATP concept borrows directly from the Quality Target Product Profile framework used in ICH Q8 for drug product development, reflecting the Quality by Design philosophy that has become central to modern pharmaceutical science. By establishing measurable performance criteria at the outset, the ATP provides a clear benchmark against which method performance can be evaluated throughout development and validation, encourages more systematic and less trial-and-error-based method development, and reduces the risk of discovering late in the process that a critical performance requirement cannot be met.[3,4]

5.3 Lifecycle Management of Analytical Procedures

Among the most philosophically significant aspects of Q2(R2) is the adoption of a lifecycle perspective on analytical method management. Rather than treating validation as a one-time event that permanently confers fitness for use, Q2(R2) conceptualizes the life of an analytical procedure in three interconnected stages: development (including early understanding of method behavior), validation (formal demonstration of fitness for purpose), and continued performance verification (CPV—ongoing monitoring of method performance during routine use).[1,3,16]

The CPV concept parallels the continued process verification approach applied to manufacturing processes under ICH Q10 and FDA process validation guidance. In practice, CPV involves control charts, periodic review of system suitability data, and trending of key performance indicators to detect any deterioration in method performance over time before it causes a significant failure. Data generated during development—early robustness studies, forced degradation experiments, initial calibration—forms part of the method's integrated knowledge base under this framework rather than being relegated to background documentation.[5,16,45]

5.4 Enhanced Requirements for Robustness

As discussed in Section 4.7, ICH Q2(R2) unequivocally elevates robustness from a parameter to 'be considered' to one that is explicitly expected to be demonstrated and documented as part of the formal validation package. The guideline now treats robustness data as a component of the method's design space, describing the boundaries of operational conditions within which the method can be reliably applied. This treatment aligns robustness with the QbD concept of a proven acceptable range—a defined operational envelope within which method performance remains adequate.[1,26]

5.5 Explicit Guidance on Multivariate Analytical Procedures

ICH Q2(R2) is the first revision of the Q2 guideline to substantively address multivariate analytical procedures, reflecting the growing use of near-infrared (NIR) spectroscopy, Raman spectroscopy, and fluorescence spectroscopy in pharmaceutical quality control—particularly in process analytical technology applications. These methods differ fundamentally from univariate chromatographic procedures in their data structure and validation requirements, relying on mathematical models built from large spectral datasets rather than simple calibration curves.[1,3]

Q2(R2) describes a two-phase validation approach for multivariate methods: a model calibration phase, in which the mathematical relationship between spectral responses and the analyte property of interest is established using a representative training set; and a model validation phase, in which the predictive accuracy and precision of the model are evaluated against an independent test set not included in model construction. This two-phase approach aligns Q2(R2) guidance with established chemometric practice and extends the validation framework to encompass modern spectroscopic technologies used in process analytical technology and real-time release testing.[1,3,29]

Table 3: Comparative Overview of ICH Q2(R1) vs. ICH Q2(R2)

Aspect	ICH Q2(R1) – 2005	ICH Q2(R2) – 2023	Significance
Scope	Small molecules only	Includes biologics and complex molecules	Broader applicability
Lifecycle Approach	Not included	Three-stage lifecycle (ATP, validation, CPV)	Continuous performance monitoring
ATP	Not mentioned	Formally introduced	Foundation for risk-based method design
Multivariate Methods	Not addressed	Explicitly addressed (NIR, Raman, chemometrics)	Enables advanced analytical platforms
Robustness	Optional; development phase	Explicitly required; integrated into validation	Stronger reliability requirements
Specificity approaches	Limited discussion	Three defined approaches	Scientific flexibility
Statistical Tools	Basic regression, RSD	Confidence intervals, ANOVA, total error	More rigorous data interpretation
Companion Guidance	None	Harmonized with ICH Q14	Integrated development-validation continuum

6. ANALYTICAL TARGET PROFILE AND LIFECYCLE MANAGEMENT

6.1 Definition and Purpose of the ATP

The ATP is a prospective, quantitative summary of the performance characteristics that an analytical procedure must achieve to serve its intended measurement purpose. It is established before method development begins and is derived from an understanding of how measurement uncertainty and analytical errors will affect the quality decisions made on the basis of the method's results. A well-constructed ATP specifies the analyte and sample matrix, the measurement range

over which acceptable performance is required, the maximum acceptable total analytical error or measurement uncertainty, and any specific selectivity requirements. Crucially, the ATP does not specify the technology or procedure to be used—it defines the required outcome and leaves the scientist free to select and develop the most appropriate analytical approach.[1,3]

6.2 ATP-Driven Method Development

Once the ATP is in place, method development proceeds with the explicit goal of satisfying those pre-defined performance requirements. Multiple candidate analytical technologies or platforms may be evaluated and compared against ATP criteria during early development stages. This systematic approach encourages broader consideration of analytical options, potentially leading to more appropriate technology choices than the traditional practice of defaulting to HPLC for all pharmaceutical quantitative analyses.[3,34]

Under the integrated Q2(R2)/Q14 framework, the development phase encompasses not only parameter optimization but also extensive characterization of method behavior—including robustness experiments to identify critical parameters and their acceptable ranges. This thorough characterization reduces the risk of failure during formal validation and builds a comprehensive knowledge base that will support the method's management throughout its lifecycle.[3,34]

6.3 Continued Performance Verification

CPV is the third stage of the analytical procedure lifecycle under Q2(R2). It involves systematic collection and statistical analysis of performance data generated during routine use, using tools such as control charts, trend analysis, and periodic capability assessments to monitor key performance indicators over time. Data sources include system suitability test results, reference standard performance data, interlaboratory comparison results, and outcomes of out-of-specification investigations.[1,16,45]

By tracking these data continuously, an analytical laboratory can detect changes in instrument performance, reagent quality, or operator technique that might otherwise go unnoticed until a significant failure occurs. The CPV program thereby functions as an early warning system for method performance issues and supports a culture of continuous improvement in analytical practice—reflecting the spirit of ICH Q10 applied to the analytical function.[5,16]

7. METHOD TRANSFER AND REVALIDATION

Method transfer is the process through which a validated analytical procedure is documented and its performance confirmed at a receiving laboratory, demonstrating that the receiving site can generate results equivalent to those produced by the sending laboratory. It is a routine requirement whenever methods must be applied at multiple sites—for example, when transferring from a research and development laboratory to a quality control facility, or from an originator to a contract manufacturer.[1,9]

7.1 Approaches to Method Transfer

ICH Q2(R2) recognizes several transfer approaches. The most rigorous is comparative testing, in which both the sending and receiving laboratories independently analyze a common set of validation samples; statistical comparison of the results then confirms equivalence. This approach is required when methods are transferred to sites with substantially different personnel, equipment, and environmental conditions.[1]

Co-validation—in which both laboratories conduct defined subsets of the validation experiments jointly—allows the receiving laboratory to participate in establishing

performance characteristics from the outset and is an efficient alternative where feasible. A third approach applies when transferring compendial or otherwise standardized procedures that require only verification rather than full validation at the receiving site. ICH Q2(R2) also permits waiver of a formal transfer study if the receiving laboratory can demonstrate sufficient method knowledge and instrument qualification, though any waiver must be scientifically justified and fully documented.[1,9]

7.2 Revalidation

Revalidation is required when changes are made to a validated procedure or when the conditions under which it was originally validated change in ways that could affect its performance. The extent of revalidation depends on the nature and magnitude of the change. Common triggers include changes to the synthesis route of the drug substance (potentially introducing new impurities), changes to the drug product formulation (potentially altering matrix effects), changes to analytical equipment (such as switching to a different HPLC column chemistry or instrument platform), and revisions to regulatory specifications that require the method to operate across a different range or with different acceptance criteria.[1,41]

ICH Q2(R2) directs that science- and risk-based principles govern revalidation decisions: any change that could reasonably be expected to affect method performance requires assessment, and the relevant performance parameters must be re-demonstrated. A structured gap analysis—systematically identifying which Q2(R2) requirements are not fully met by an existing validation package and evaluating the associated risk—is the recommended approach for managing the transition of legacy methods.[1,26,41]

8. MULTIVARIATE ANALYTICAL PROCEDURES

The increasing adoption of process analytical technology in pharmaceutical manufacturing, together with growing interest in real-time release testing as an alternative to conventional end-product testing, has accelerated the use of multivariate spectroscopic methods in pharmaceutical quality control. Near-infrared spectroscopy, Raman spectroscopy, and hyperspectral imaging offer the advantages of rapid, non-destructive analysis and the potential for at-line, online, or in-line measurement during manufacturing operations. However, these methods present validation challenges that are not adequately addressed by the conventional univariate validation framework of Q2(R1).[1,3,29]

A multivariate NIR method for assessing API content in a tablet, for example, relies on a complex chemometric calibration model built from a training set of samples covering the full expected range of sample composition and physical properties. The model's performance is then evaluated on an independent test set not used in model building, and its predictive ability is characterized by statistics such as root mean squared error of prediction (RMSEP) and bias. These statistics serve functions analogous to accuracy and precision parameters for univariate methods but are computed and interpreted differently, and the validation approach must reflect those differences.[29,30]

Critical validation parameters for multivariate methods include predictive accuracy (RMSEP), predictive bias, prediction uncertainty, model stability, and robustness to variations in sample physical properties or instrument conditions. Q2(R2) also addresses when a multivariate model requires recalibration or revalidation—potential triggers include changes in raw material sources, manufacturing equipment, or the analytical instrument used to generate spectral data.[1,3]

9. REGULATORY FRAMEWORKS – COMPARATIVE OVERVIEW

Although ICH Q2(R2) represents the most widely recognized international standard for analytical method validation, pharmaceutical companies operating across global markets must be familiar with the requirements of multiple regulatory agencies and compendial organizations. The following overview addresses the major frameworks governing analytical method validation worldwide.[9,10,12,14,15]

9.1 US Food and Drug Administration (FDA)

The FDA implemented ICH Q2(R2) through its own final guidance document, 'Q2(R2) Validation of Analytical Procedures,' published in March 2024. The broader FDA guidance, 'Analytical Procedures and Methods Validation for Drugs and Biologics' (2015), remains in effect and provides additional context on how validation data should be presented in NDA and BLA submissions. The FDA places particular emphasis on pre-approval inspection readiness, data integrity, and scientifically justified validation decisions. A separate guidance for bioanalytical method validation (2018) governs the validation of methods used to measure drug concentrations in biological matrices for pharmacokinetic studies.[9,10,11]

9.2 European Medicines Agency (EMA)

The EMA adopted ICH Q2(R2) as a Step 5 guideline effective June 14, 2024. EMA validation requirements are incorporated into the Common Technical Document (CTD) module 3 requirements for marketing authorization applications in the European Union. The EMA requires that the validation range encompasses all specification limits, that the method's performance across the entire specification range is clearly demonstrated, and that impurity methods are validated to detect and quantify impurities at or below the threshold levels required by ICH Q3A and Q3B.[12,13,6,7]

9.3 World Health Organization (WHO)

WHO guidelines on analytical method validation are published primarily in the context of its prequalification programme, which assesses the quality of generic medicines intended for use in low- and middle-income countries. The WHO's guidance, published in Technical Report Series 1019 (2019), is broadly consistent with ICH Q2(R1) with some differences in emphasis and detail. WHO places particular importance on the validation of dissolution methods and impurity tests, reflecting their critical role in ensuring the quality and safety of essential medicines in resource-limited healthcare settings.[14]

9.4 United States Pharmacopeia (USP)

The USP publishes general chapters on analytical method validation that establish standards for compendial procedures and non-compendial methods alike. General Chapter <1225> addresses the validation of compendial procedures, while General Chapter <1226> covers verification of those procedures at user laboratories. In 2022, USP published General Chapter <1220>, 'The Analytical Procedure Lifecycle,' which formally adopts the lifecycle framework for analytical procedures, aligning USP guidance closely with the ICH Q2(R2) and Q14 approach and encompassing the same three stages: development, validation, and continued performance verification.[15,16,17,18]

Table 4: Major Regulatory Frameworks for Analytical Method Validation

Regulatory Body	Guideline / Document	Year	Key Focus	Region
ICH	Q2(R1)	2005	Foundational validation parameters for small molecules	Global

ICH	Q2(R2)	2023	Expanded scope; lifecycle; biologics; multivariate methods	Global
ICH	Q14	2023	Science- and risk-based method development; ATP	Global
US FDA	Analytical Procedures and Methods Validation	2015	NDA/BLA submissions; robustness; revalidation	USA
US FDA	Bioanalytical Method Validation	2018	PK/PD studies; matrix effects; QC samples	USA
EMA	ICH Q2(R2) Step 5 Implementation	2024	European adoption of ICH Q2(R2)	EU
WHO	TRS 1019	2019	Prequalification; developing countries; GMP alignment	Global
USP	<1225>, <1226>, <1220>, <621>	Ongoing	Compendial validation; lifecycle framework	USA / Int'l
CDSCO / India	Guidelines on Analytical Method Validation	Current	Regulatory submissions in India; aligned with ICH	India

10. PRACTICAL CHALLENGES IN ANALYTICAL METHOD VALIDATION

The theoretical framework of analytical method validation is clear and well-structured. Translating it into practice in a working pharmaceutical laboratory, however, presents a variety of challenges that analytical scientists and quality assurance professionals must navigate with both technical skill and regulatory awareness.[38]

10.1 Reference Standard Availability and Characterization

Accurate determination of validation parameters—particularly accuracy and linearity—depends fundamentally on the availability of reference standards of known purity. For established drug substances, certified primary reference standards are generally available from the USP, European Pharmacopoeia, or WHO. For impurities, degradation products, and novel chemical entities in early development, such standards may not exist commercially. In these situations, in-house reference materials must be characterized to sufficient purity using techniques such as quantitative NMR, mass balance calculations, or a combination of complementary analytical methods, to establish their suitability for use in the validation studies.[32,33]

10.2 Matrix Complexity in Drug Product Analysis

Drug product formulations contain numerous excipients—diluents, binders, lubricants, coating agents, preservatives—each of which may potentially interfere with the analytical response for the active ingredient or its impurities. Demonstrating specificity in a complex matrix requires careful experimental design: analysis of placebo samples, spiking studies at multiple concentration levels, and peak purity assessment using orthogonal detection techniques such as diode array or mass spectrometric detection. For particularly complex matrices such as creams, suppositories, or transdermal patches, sample preparation steps add further layers of complexity that must themselves be characterized and validated.[32,33]

10.3 Validation of Stability-Indicating Methods

Methods intended for stability testing carry the additional regulatory requirement to demonstrate stability-indicating capability. A stability-indicating method must detect and quantify all significant degradation products that might form during the product's shelf life, including those not identified in advance. The conventional approach is to subject the drug substance or product to forced degradation under a panel of conditions—acid hydrolysis, base hydrolysis, neutral hydrolysis, oxidation, thermal stress, and photolytic stress—to generate a representative population of degradation products. The selection of appropriate stress conditions and levels requires considerable scientific judgment, and the adequacy of the forced degradation study is a frequent focus of regulatory scrutiny.[1,9]

10.4 Statistical Challenges and Data Interpretation

ICH Q2(R2) introduces a greater emphasis on statistical rigor than Q2(R1), including the use of confidence intervals around accuracy and precision estimates, evaluation of regression model residuals, and application of statistical equivalence testing in method transfer studies. These requirements demand a higher level of statistical sophistication from both the validation scientist and the regulatory reviewer. The pharmaceutical industry has responded through training programs, adoption of validated statistical software, and guidance documents from industry organizations. Nevertheless, building adequate statistical competence across the analytical workforce remains an ongoing challenge.[26,27,38]

10.5 Legacy Methods and Retrofitting to Q2(R2)

Many pharmaceutical companies hold large portfolios of analytical methods validated under Q2(R1) that now face the question of how to demonstrate Q2(R2) compliance. Methods validated years ago may lack data on robustness, lifecycle performance monitoring, or multivariate model validation now expected by Q2(R2). A gap analysis approach—systematically identifying which Q2(R2) requirements are not met by the existing validation package and assessing the associated risk—is the recommended starting point. Where gaps are found to pose a material risk to data integrity or product quality assurance, supplemental validation studies should be conducted to address them.[26,41]

10.6 Biological Products and Novel Modalities

Validating analytical procedures for biological products remains among the most complex challenges in the field. Unlike chemically defined small-molecule drugs, biological products are heterogeneous populations of large molecules whose structural complexity and biological activity cannot be fully characterized by any single analytical method. The inherent biological variability of cell-based assays, matrix effects in complex biological fluids, and the sensitivity of certain biologics to analytical conditions all conspire to make achieving the precision and accuracy levels typical of small-molecule assays extremely difficult. A nuanced, fit-for-purpose approach—one that explicitly acknowledges the limitations inherent to biological measurement—is necessary.[1,28]

11. FUTURE PERSPECTIVES

11.1 Analytical Quality by Design

Analytical Quality by Design (AQbD) applies the principles of ICH Q8 to the development and validation of analytical procedures. Beginning with a well-defined ATP, AQbD proceeds through systematic design of experiments to characterize the analytical method design space and establishes an Analytical Control Strategy that keeps the method within its validated performance boundaries throughout its lifecycle. Methods

developed through AQbD tend to be better characterized and more robust than those developed through traditional approaches, and they offer the potential for regulatory flexibility in managing post-approval changes through pre-defined methodological knowledge spaces.[3,4,34]

11.2 Artificial Intelligence and Machine Learning

The application of AI and machine learning to analytical chemistry is an emerging field with far-reaching implications for method development and validation. Machine learning algorithms have been applied to chromatographic method development, spectral classification and quantitation, impurity prediction, and the identification of critical method parameters through virtual screening. As these tools mature and their reliability becomes better understood, they are likely to play an increasingly prominent role in next-generation analytical method development.[44]

Validating AI/ML-based analytical methods raises regulatory questions that current guidance documents are only beginning to address: how should machine learning models be validated, how should model drift over time be managed, and how should predictions from complex models be interpreted for regulatory purposes? The FDA and EMA are actively developing frameworks for AI/ML-based tools in pharmaceutical development and quality control, and this area of guidance is expected to evolve rapidly in coming years.[44]

11.3 Real-Time Release Testing and Continuous Manufacturing

Real-time release testing (RTRT) uses validated in-process measurements to confirm product quality without requiring conventional batch-level end-product testing. RTRT relies heavily on multivariate spectroscopic methods, process analytical technology, and statistically validated models linking in-process measurements to final product quality attributes. Validating methods for RTRT applications requires demonstrating not only the traditional performance characteristics of the measurement technology but also the predictive performance and robustness of the statistical models that translate analytical signals into quality statements about the product.[1,3]

11.4 Data Integrity and Digitalization

The pharmaceutical industry's ongoing digital transformation—electronic laboratory notebooks, laboratory information management systems (LIMS), cloud-based data storage, and remote analytical monitoring—is creating new opportunities and new responsibilities in the context of analytical validation. Digital systems offer powerful capabilities for data collection, trending, and analysis that can meaningfully strengthen lifecycle-based validation and CPV programs. At the same time, they introduce data integrity considerations related to access controls, audit trail management, electronic signatures, and computerized system validation. Regulatory agencies are devoting growing attention to data integrity in inspection programs, and analytical validation practices must be consistent with governance frameworks described in guidelines from the MHRA, PIC/S, and FDA.[37,44]

CONCLUSION

Analytical method validation stands as one of the most fundamental activities in pharmaceutical quality assurance. It is the scientific foundation upon which confidence in product quality is built, confirming that the procedures used to assess drug safety, efficacy, and quality will consistently deliver reliable, accurate, and reproducible results. The ICH Q2 guideline series has served as the global cornerstone for this activity for more than three decades, providing harmonized expectations

across the world's major pharmaceutical markets.[1,2]

The publication of ICH Q2(R2) in November 2023, together with the companion guideline Q14, marks a genuinely new era in pharmaceutical analytical validation. The revised guideline represents far more than an incremental update to Q2(R1). By formally introducing the Analytical Target Profile as the prospective anchor for method development, by adopting a lifecycle approach to method management, by extending validation guidance to biological products and multivariate analytical methods, and by making robustness and continued performance verification explicit and non-negotiable requirements, Q2(R2) charts a course for validation practice that is more scientifically rigorous, more operationally realistic, and more fully aligned with the goals of modern pharmaceutical quality systems.[1,3,26]

Implementing Q2(R2) will require concerted effort from both industry and regulatory agencies. Companies with portfolios of legacy methods must conduct gap analyses and prioritize supplemental validation work according to patient safety risk. Companies developing new methods must build lifecycle thinking into their validation strategies from the outset. Academic institutions must update curricula to reflect the changed landscape. Regulatory reviewers must develop expertise in the new statistical approaches and expanded validation frameworks that Q2(R2) introduces.

Despite these challenges, the underlying purpose remains constant and compelling. A pharmaceutical quality system anchored in thoroughly developed, carefully validated, and continuously monitored analytical procedures will be more capable of detecting product quality failures, more responsive to manufacturing process changes, and more reliable in serving the patient safety mission that is the ultimate purpose of pharmaceutical regulation. The principles and practices described in ICH Q2(R2) provide the roadmap for achieving that vision.[1,5]

REFERENCES

1. International Council for Harmonisation (ICH). Q2(R2) Validation of Analytical Procedures. Step 4 Guideline. Geneva: ICH; November 2023. Available from: https://database.ich.org/sites/default/files/ICH_Q2(R2)_Guideline_2023_1130.pdf
2. International Council for Harmonisation (ICH). Q2(R1) Validation of Analytical Procedures: Text and Methodology. Step 4 Guideline. Geneva: ICH; November 2005. Available from: https://database.ich.org/sites/default/files/Q2(R1)%20Guideline.pdf
3. International Council for Harmonisation (ICH). Q14 Analytical Procedure Development. Step 4 Guideline. Geneva: ICH; November 2023.
4. International Council for Harmonisation (ICH). Q8(R2) Pharmaceutical Development. Step 4 Guideline. Geneva: ICH; August 2009.
5. International Council for Harmonisation (ICH). Q10 Pharmaceutical Quality System. Step 4 Guideline. Geneva: ICH; June 2008.
6. International Council for Harmonisation (ICH). Q3A(R2) Impurities in New Drug Substances. Step 4 Guideline. Geneva: ICH; June 2006.
7. International Council for Harmonisation (ICH). Q3B(R2) Impurities in New Drug Products. Step 4 Guideline. Geneva: ICH; June 2006.
8. International Council for Harmonisation (ICH). Q3C(R8) Impurities: Guideline for Residual Solvents. Step 4 Guideline. Geneva: ICH; April 2021.
9. US Food and Drug Administration (FDA). Analytical Procedures and Methods Validation for Drugs and Biologics: Guidance

for Industry. Rockville: FDA; July 2015. Available from: https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf

10. US Food and Drug Administration (FDA). Q2(R2) Validation of Analytical Procedures: Guidance for Industry. Rockville: FDA; March 2024. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2r2-validation-analytical-procedures
11. US Food and Drug Administration (FDA). Bioanalytical Method Validation: Guidance for Industry. Rockville: FDA; May 2018. Available from: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf
12. European Medicines Agency (EMA). ICH Q2(R2) Guideline on Validation of Analytical Procedures. Step 5 – Revision 2. EMA/CHMP/ICH/82072/2006. Amsterdam: EMA; 2024 [effective 14 June 2024]. Available from: https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline
13. European Medicines Agency (EMA). ICH Q14 Analytical Procedure Development: Scientific Guideline. Amsterdam: EMA; 2024 [effective 14 June 2024]. Available from: https://www.ema.europa.eu/en/ich-q14-analytical-procedure-development-scientific-guideline
14. World Health Organization (WHO). Annex 4: WHO Guidelines on the Validation of Analytical Procedures Used for the Examination of Pharmaceutical Materials. Technical Report Series No. 1019. Geneva: WHO; 2019.
15. United States Pharmacopeia (USP). General Chapter <1225> Validation of Compendial Procedures. Rockville: USP; 2023.
16. United States Pharmacopeia (USP). General Chapter <1220> The Analytical Procedure Lifecycle. Rockville: USP; 2022.
17. United States Pharmacopeia (USP). General Chapter <621> Chromatography. Rockville: USP; 2023.
18. United States Pharmacopeia (USP). General Chapter <1010> Analytical Data Interpretation and Treatment. Rockville: USP; 2021.
19. Mehmood T, Hanif S, Azhar F, Ali I, Alafnan A, Hussain T, et al. HPLC method validation for the estimation of lignocaine HCl, ketoprofen, and hydrocortisone: Greenness analysis using AGREE score. Int J Mol Sci. 2023;24(1):440. doi: 10.3390/ijms24010440.
20. Pawar AY, Surana SJ. Analytical method validation: Principles and applications as per ICH guidelines. J Drug Deliv Ther. 2019;9(2-s):610–617. doi: 10.22270/jddt.v9i2-s.2499.
21. Sharma N, Gupta S, Kumari A. Recent advances in analytical method validation as per ICH Q2(R2): A comparative review with ICH Q2(R1). Int J Pharm Sci Res. 2024;15(3):892–906. doi: 10.13040/IJPSR.0975-8232.15(3).892-06.
22. Bhatt NM, Bhatt N. Analytical method development and validation: An overview. Int J Pharm Qual Assur. 2019;10(3):01–08.
23. Rao GV, Bansal MK. Implementation of Analytical Target Profile in HPLC method development under ICH Q2(R2). J Pharm Sci Technol. 2023;77(5):215–221.
24. Kumar A, Sequeira JA. Lifecycle management of capillary electrophoresis methods in biopharmaceutical analysis: Regulatory insights. Electrophoresis. 2022;43(7–8):822–835. doi: 10.1002/elps.202100296.

25. Vant'Hull B, Mao-Jones J. Real-world experiences implementing analytical lifecycle strategies: Lessons from industry. Pharm Eng. 2022;42(6):20–26.
26. Lim HK, Chan LC, Lim SC. Navigating ICH Q2(R2) compliance in analytical method validation: A gap analysis toolkit to streamline risk assessment and change management. J Pharm Sci. 2025;114(4):1051–1061. doi: 10.1016/j.xphs.2025.02.012.
27. Wuelfing WP, Reed RA, Bhatt D, Hammond S, Saxena A. ICH Q2(R2): Validation of analytical procedures. In: Ermer J, Limberger M, editors. Method Validation in Pharmaceutical Analysis. 2nd ed. Weinheim: Wiley-VCH; 2022. p. 319–346.
28. Parenteral Drug Association (PDA). Technical Report No. 57-2: Analytical Method Development and Qualification for Biotechnology Products. Bethesda: PDA; 2015.
29. Takacsova M, Chrisman R, Sahu N. Analytical Quality by Design-compliant development of a cyclodextrin-modified micellar electrokinetic chromatography method for the determination of trimecaine and its impurities. Pharmaceutics. 2023;15(6):1687. doi: 10.3390/pharmaceutics15061687. PMCID: PMC10302722.
30. Miller JN, Miller JC. Statistics and Chemometrics for Analytical Chemistry. 6th ed. Harlow: Pearson Education; 2010.
31. Federal Register. Q2(R2) Validation of Analytical Procedures and Q14 Analytical Procedure Development: International Council for Harmonisation; draft guidances for industry; availability. Fed Regist. 2022;87(167):52887–52889. Available from: https://www.federalregister.gov/documents/2022/08/29/2022-18516
32. Swartz ME, Krull IS. Handbook of Analytical Validation. Boca Raton: CRC Press; 2012.
33. Ermer J, Miller JH McB, editors. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. Weinheim: Wiley-VCH; 2005.
34. Shankar G, Devarakonda S. Advancing analytical method validation: Lifecycle and risk-based approaches under ICH Q2(R2). J Pharm Innov. 2025;20(1):15. doi: 10.1007/s12247-025-09930-1.
35. Borman P, Chatfield M, Nethercote P, Thompson D, Truman K. The application of quality by design to analytical methods. Pharm Technol. 2007;31(10):142–152.
36. Tonhi E, Collins KE, Jardim IC, Collins CH. Stationary phases for high-performance liquid chromatography based on organically modified silicas. J Chromatogr A. 2002;948(1–2):109–119. doi: 10.1016/S0021-9673(01)01536-6.
37. Bhattacharya S. The curse of digitalization: Data integrity challenges in pharmaceutical analytics. J Pharm Sci. 2022;111(3):595–600. doi: 10.1016/j.xphs.2021.10.017.
38. Menon A, Pillai RS. Bridging the skills gap in lifecycle-based method validation: An industry perspective. J Pharm Educ Res. 2022;13(3):110–116.
39. Hanna-Brown M, Borman PJ, Bale S, Szucs R, Monks K, Tan A, et al. Highlights and impact of ICH Q2(R2)/Q14 on analytical method development and validation. J Pharm Biomed Anal. 2024;242:116051. doi: 10.1016/j.jpba.2024.116051.
40. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on

Harmonization. J Chromatogr A. 2003;987(1–2):57–66. doi: 10.1016/S0021-9673(02)01536-4.

41. Patel DD, Shah KV. Challenges in retrofitting legacy analytical methods to meet ICH Q2(R2) requirements: A practical perspective. Indian J Pharm Sci. 2023;85(4):120–128. doi: 10.36468/pharmaceutical-sciences.1151.
42. Tan A, Hussain S, Musuku A, Massé R. Bioanalytical method validation: New FDA guidance vs. EMA guideline—better or worse? J Pharm Biomed Anal. 2019;165:442–448. doi: 10.1016/j.jpba.2018.12.018.
43. Peraman R, Bhadraya K, Reddy YP. Analytical quality by design: A tool for regulatory flexibility and robust analytics. Int J Anal Chem. 2015;2015:868727. doi: 10.1155/2015/868727.
44. Morais CLM, Lima KMG. Application of machine learning methods for the analysis of pharmaceutical data. Chemom Intell Lab Syst. 2021;215:104342. doi: 10.1016/j.chemolab.2021.104342.
45. Huber L. Validation and Qualification in Analytical Laboratories. 2nd ed. New York: Informa Healthcare; 2007.

HOW TO CITE: Ankita Mohite, Amit Kasabe, Analytical Method Validation A Comprehensive Review Of Principles, Parameters, Regulatory Frameworks, And Evolving Practices, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 2410-2430, https://doi.org/10.5281/zenodo.20610664