Q2(R2) Validation of Analytical Procedures

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

March 2024
ICH-Quality
Revision 2

Q2(R2) Validation of Analytical Procedures

Guidance for Industry

Additional copies are available from:

Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353; Email: druginfo@fda.hhs.gov
https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs

and/or

Office of Communication, Outreach, and Development
Center for Biologics Evaluation and Research
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 71, Room 3128
Silver Spring, MD 20993-0002
Phone: 800-835-4709 or 240-402-8010; Email: ocod@fda.hhs.gov
https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

March 2024
ICH-Quality
Revision 2

FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner. By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

ICH is a consensus-driven process that involves technical experts from regulatory authorities and industry parties in detailed technical and science-based harmonization work that results in the development of ICH guidelines. The commitment to consistent adoption of these consensus-based guidelines by regulators around the globe is critical to realizing the benefits of safe, effective, and high-quality medicines for patients as well as for industry. As a Founding Regulatory Member of ICH, the Food and Drug Administration (FDA) plays a major role in the development of each of the ICH guidelines, which FDA then adopts and issues as guidance to industry.

TABLE OF CONTENTS

Contains Nonbinding Recommendations

Q2(R2) Validation of Analytical Procedures Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION (1)[2]

A. Objective (1.1)

This guidance presents elements for consideration during the validation of *analytical procedures* included as part of registration applications. Analytical procedure validation forms a part of the analytical procedure life cycle, as described within the International Council for Harmonisation (ICH) guidance for industry *Q14 Analytical Procedure Development* (March 2024) (ICH Q14).[3] This ICH guidance for industry, *Q2(R2) Validation of Analytical Procedures* (ICH Q2), provides guidance on selection and evaluation of the various *validation tests* for analytical procedures. This guidance includes a collection of terms and their definitions, which are meant to bridge the differences that often exist between various compendia and documents of the ICH member regulatory authorities.

The objective of validation of an analytical procedure is to demonstrate that the analytical procedure is fit for the intended purpose. Further general guidance is provided on *validation studies* for analytical procedures.

B. Scope (1.2)

This guidance applies to analytical procedures used for release and stability testing of commercial drug substances and products, hereafter referred to as *products*. The guidance can also be applied to other analytical procedures used as part of the *control strategy* (ICH guidance for industry *Q10 Pharmaceutical Quality System* (April 2009)) following a risk-based approach. The scientific principles described in this guidance can be applied in a phase-appropriate manner to analytical procedures used during clinical development.

---

[1] This guidance was developed within the Expert Working Group (*Quality*) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Assembly at *Step 4* of the ICH process, November 2023. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions.

[2] The numbers in parentheses reflect the organizational breakdown of the document endorsed by the ICH Assembly at Step 4 of the ICH process, November 2023.

[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

The guidance is directed to common uses of analytical procedures, such as assay, potency, purity, impurity (quantitative or limit test), identity, or other quantitative or qualitative measurements.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. GENERAL CONSIDERATIONS FOR ANALYTICAL PROCEDURE VALIDATION (2)

This guidance indicates the data that should be presented in a regulatory submission. Analytical procedure validation data should be submitted in the corresponding sections of the application (ICH guideline *M4Q(R1) The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Quality*)[4]. Relevant data collected during validation (and any methodology used for calculating validation results) should be submitted to demonstrate the suitability of the procedure for the intended purpose. Suitable data derived from development studies (see ICH Q14) can be used as part of validation data. When an established *platform analytical procedure* is used for a new purpose, validation testing can be abbreviated, if scientifically justified.

Approaches other than those set forth in this guidance may be applicable and acceptable with appropriate science-based justification. The applicant is responsible for designing the validation studies and protocol most suitable for their product.

Reference materials, or other suitably characterized materials, with documented identity, purity, or any other characteristics as necessary should be used in the validation study.

In practice, the experimental work can be designed so that the appropriate *performance characteristics* are considered simultaneously to provide sound, overall knowledge of the performance of the analytical procedure, for instance: *specificity/selectivity*, *accuracy,* and *precision* over the *reportable range*.

As described in ICH Q14, the *system suitability test* is an integral part of analytical procedures and is generally established during development as a regular check of performance. *Robustness* is typically evaluated as part of development prior to the execution of the analytical procedure validation study (ICH Q14). Finally, the *analytical procedure validation strategy* is developed based on knowledge of the analytical procedure and the intended purpose. This includes the required analytical procedure performance to ensure the quality of the measured result (ICH Q14). If successfully executed, the analytical procedure validation strategy will demonstrate that the analytical procedure is fit for the intended purpose.

[4] Available at https://database.ich.org/sites/default/files/M4Q_R1_Guideline.pdf.

A. Analytical Procedure Validation Study (2.1)

A validation study is designed to provide sufficient evidence that the analytical procedure meets its objectives. These objectives are described with a suitable set of performance characteristics and related *performance criteria*, which can vary depending on the intended purpose of the analytical procedure and the specific technology selected. Section III (3), Validation Tests, Methodology, and Evaluation, summarizes the typical methodologies and validation tests that can be used (see also Figure 2 in Appendix A (6. Annex 1) on selection of validation tests). Specific nonbinding examples for common techniques are given in Appendix B (7. Annex 2). Table 1 (below) provides the measured quality attributes, typical performance characteristics and related validation tests, which are further illustrated in Appendix A (6. Annex 1).

The validation study should be documented. Prior to the validation study, a validation protocol should be generated. The protocol should contain information about the intended purpose of the analytical procedure, the performance characteristics to be validated, and the associated criteria. In cases where prior knowledge is used (e.g., from development or from previous studies), appropriate justification should be provided. The results of the validation study should be summarized in a validation report.

The experimental design of the validation study should reflect the number of replicates used in routine analysis to generate a *reportable result*. If justified, it may be acceptable to perform some validation tests using a different number of replicates or to adjust the number of replicates in the analytical procedure based on data generated during validation.

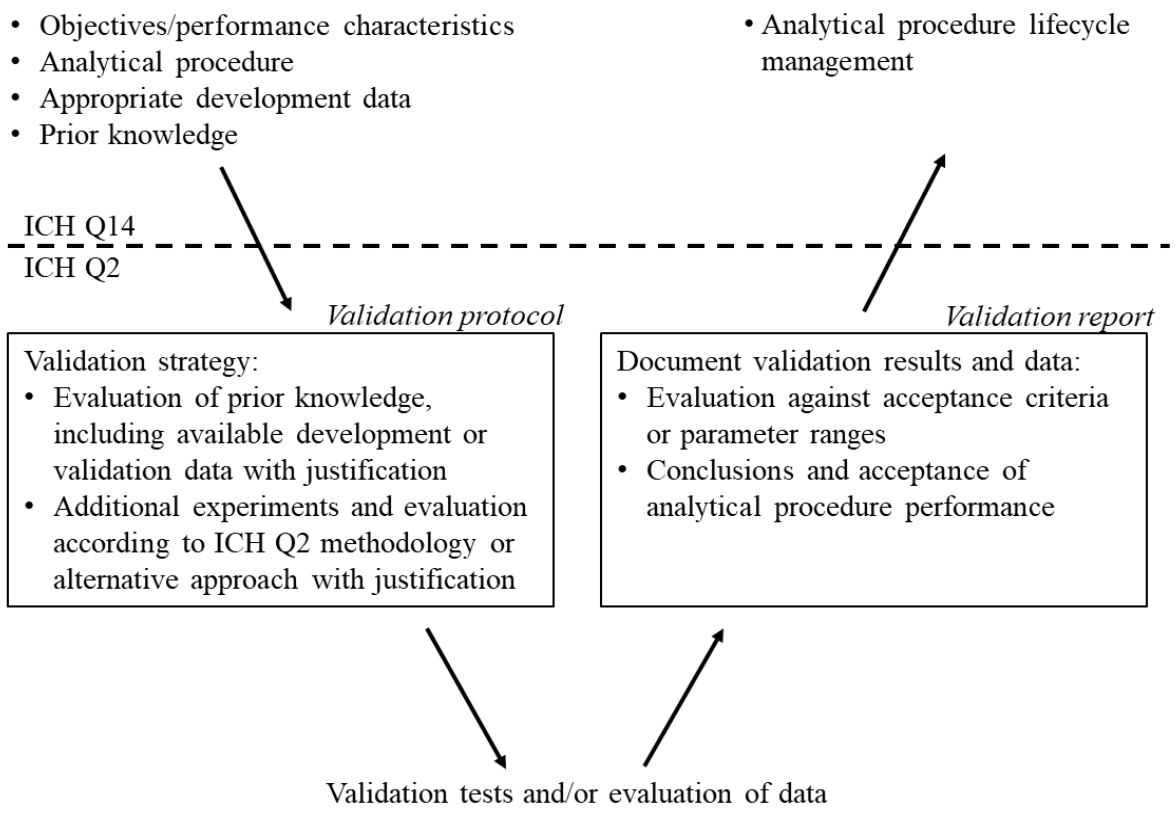
Figure 1 (below) shows the interrelationship between ICH Q2 and ICH Q14 and how knowledge generated during analytical procedure development as described in ICH Q14 aids the design of a validation study.

Table 1: Typical Performance Characteristics and Related Validation Tests for Measured Quality Attributes

Measured Quality Attribute / Analytical Procedure Performance Characteristics to be Demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other Quantitative Measurements (1)		ASSAY Content or Potency
		Quantitative Test	Limit Test	Other Quantitative Measurements (1)
Specificity (3) Specificity Test	+	+	+	+
Range Response (*Calibration Model)*	-	+	-	+
Lower Range Limit	-	QL†	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

- signifies that this test is not normally conducted.
+ signifies that this test is normally conducted.
† In some complex cases DL may also be evaluated.
QL = quantitation limit; DL = detection limit.
(1) Other quantitative measurements can follow the scheme for impurity, if the range limit is close to the DL/QL; other quantitative measurements can follow the scheme for assay (content or potency), if the range limit is not close to the DL/QL.
(2) Some performance characteristics can be substituted with technology-inherent justification in the case of certain analytical procedures for physicochemical properties.
(3) Lack of specificity of one analytical procedure should be compensated by one or more other supporting analytical procedures, unless appropriately justified.
(4) Alternatively, a combined approach can be used to evaluate accuracy and precision.
(5) Where reproducibility has been performed and intermediate precision can be derived from the reproducibility data set, an independent study for intermediate precision is not required.

Figure 1: Validation Study Design and Evaluation



ICH Q14 = International Council for Harmonisation (ICH) guidance for industry *Q14 Analytical Procedure Development* (March 2024).
ICH Q2 = ICH guidance for industry *Q2(R2) Validation of Analytical Procedures.*

B. Validation During the Life Cycle of an Analytical Procedure (2.2)

Changes may be required during the life cycle of a validated analytical procedure. In such cases, partial or full *revalidation* may be required. Science and risk-based principles can be used to justify whether or not a given performance characteristic needs revalidation. The extent of revalidation depends on the performance characteristics impacted by the change.

Transfer of a validated analytical procedure should be considered in the context of analytical life cycle changes in line with ICH Q14. When transferring analytical procedures to a different laboratory, a partial or full revalidation of the analytical procedure performance characteristics and/or comparative analysis of representative samples should be performed. Justification for not performing additional transfer experiments should be provided if appropriate.

Covalidation can be used to demonstrate that the analytical procedure meets predefined performance criteria by using data generated at multiple sites and could also satisfy the requirements of analytical procedure transfer at the participating sites.

C. Reportable Range (2.3)

The required reportable range is typically derived from the specification and depends on the intended use of the procedure. The reportable range is confirmed by demonstrating that the analytical procedure provides results with acceptable *response*, accuracy, and precision. The reportable range should be inclusive of the upper and lower specification or reporting limits, as applicable.

Table 2 (below) exemplifies recommended reportable ranges for common uses of analytical procedures; other ranges may be acceptable if justified. In some cases, (e.g., at low amounts) wider upper ranges may be more practical.

D. Demonstration of Stability-Indicating Properties (2.4)

A validated quantitative analytical procedure that can detect changes in relevant quality attributes of a product during storage is considered to be stability indicating. To demonstrate specificity/selectivity of a stability-indicating test, samples containing relevant degradation products should be included in the study. These can include samples spiked with target analytes and known interferences; samples that have been exposed to various physical and chemical stress conditions; and actual product samples that are either aged or have been stored under stressed conditions.

E. Considerations for Multivariate Analytical Procedures (2.5)

For *multivariate analytical procedures,* results are determined through a multivariate calibration model utilizing more than one input variable (e.g., a spectrum with many wavelength variables). The multivariate calibration model relates the input data to a value for the property of interest (i.e., the model output).

Successful validation of a multivariate procedure should consider calibration, *internal testing*, and validation.

Typically, development and validation are performed in two phases.

- In the first phase, model development consists of calibration and internal testing. Calibration data are used to create the calibration model. Test data are used for internal testing and optimization of the model. The test data could be a separate set of data or part of the *calibration set* used in a rotational manner. This internal test step is used to obtain an estimate of the model performance and to fine-tune an algorithm's parameters (e.g., the number of *latent variables* for partial least squares) to select the most suitable model within a given set of data. For more details, see ICH Q14.

- In the second phase, *model validation,* a *validation set* with *independent samples* is used for validation of the model. For identification libraries, validation involves analyzing samples (i.e., challenge samples) not represented in the library to demonstrate the discriminative ability of the library model.

Table 2: Examples of Reportable Ranges for Common Uses of Analytical Procedures

Use of Analytical Procedure	Low End of Reportable Range	High End of Reportable Range
Assay of a product (1)	80% of declared content or 80% of lower specification acceptance criterion	120% of declared content or 120% of the upper specification acceptance criterion
Potency	Lowest specification acceptance criterion -20%	Highest specification acceptance criterion +20%
Content uniformity	70% of declared content	130% of declared content
Dissolution: Immediate release • one point specification • multiple point specification Modified release	 Q -45% of the lowest strength Lower limit of reportable range (as justified by the specification) or QL, as appropriate. Lower limit of reportable range (as justified by the specification) or QL, as appropriate.	 130% of declared content of the highest strength
Impurity (1)	Reporting threshold	120% of specification acceptance criterion
Purity (as area %)	80% of lower specification acceptance criterion	Upper specification acceptance criterion or 100%

(1) Where assay and impurity are performed as a single test and only one standard is used, linearity should be demonstrated for both the reporting level of the impurities and up to 120% of the specification acceptance criterion for assay.
QL = quantitation limit.

Samples used for the validation of quantitative or qualitative multivariate procedures require values or categories assigned to each sample, typically obtained by a *reference analytical procedure* (i.e., a validated or pharmacopoeial procedure).

When a reference analytical procedure is used, its performance should equal or exceed the expected performance of the multivariate analytical procedure. Analysis by the reference

analytical procedure and multivariate data collection should be performed on the same samples (whenever possible) within a reasonable period of time to assure sample and measurement stability. In some cases, a correlation or conversion may be needed to provide the same unit of measure. Any assumptions or calculations should be described.

III. VALIDATION TESTS, METHODOLOGY, AND EVALUATION (3)

In the following sections, experimental methodologies to evaluate the performance of an analytical procedure are described. These methodologies are grouped according to main performance characteristics dictated by the analytical procedure design. It is acknowledged that information about multiple performance characteristics may be derived from the same dataset. Different approaches may be used to demonstrate that the analytical procedure meets the objectives and related performance criteria, if justified.

A. Specificity/Selectivity (3.1)

1. General Considerations (3.1.1)

The specificity or selectivity of an analytical procedure can be demonstrated through absence of interference or comparison of results to an orthogonal procedure. In some cases, specificity/selectivity may be inherently given by the underlying scientific principles of the analytical procedure. Some experiments can be combined with accuracy studies.

Selectivity could be demonstrated when the analytical procedure is not specific. However, the test for an analyte to be identified or quantitated in the presence of potential interference should minimize that interference and demonstrate that the analytical procedure is fit for the intended purpose.

Where one analytical procedure does not provide sufficient discrimination, a combination of two or more procedures is recommended to achieve the necessary specificity/selectivity.

a. Absence of interference (3.1.1.1)

Specificity/selectivity can be shown by demonstrating that the identification and/or quantitation of an analyte is not impacted by the presence of other substances (e.g., impurities, degradation products, related substances, matrices, or other components likely to be present).

b. Orthogonal procedure comparison (3.1.1.2)

Specificity/selectivity can be verified by demonstrating that the measured result of an analyte is comparable to the measured result of a second, well-characterized analytical procedure that ideally applies a different measurement principle.

c. Technology-inherent justification (3.1.13)

In some cases where the specificity of the analytical technology can be ensured and predicted by technical parameters (e.g., resolution of isotopes in mass spectrometry, chemical shifts in

nuclear magnetic resonance spectroscopy), additional experimental studies may not be required, if justified.

2. Recommended Data (3.1.2)

a. Identification (3.1.2.1)

For identification tests, a critical aspect is to demonstrate the capability to identify the analyte of interest based on unique aspects of its molecular structure and/or other specific properties. The capability of an analytical procedure to identify an analyte can be confirmed by obtaining positive results comparable to a reference material using samples containing the analyte, along with negative results from samples, which do not contain the analyte. In addition, the identification test should be applied to materials structurally similar to or closely related to the analyte to confirm that a positive result is not obtained. The choice of such potentially interfering materials should be based on scientific judgement with a consideration of interferences that could occur.

b. Assay, purity, and impurity test(s) (3.1.2.2)

The specificity/selectivity of an analytical procedure should be demonstrated to fulfill the accuracy requirements for the content or potency of an analyte in the sample.

Representative data (e.g., chromatograms, electropherograms, spectra, biological response) should be used to demonstrate specificity and relevant components should be labeled, if appropriate.

For separation techniques, suitable discrimination should be investigated at an appropriate level (e.g., for critical separations in chromatography, specificity can be demonstrated by the resolution of the two components, which elute closest to each other). Alternatively, spectra of different components could be compared to assess the possibility of interference.

For nonseparation techniques (e.g., bioassay, enzyme-linked immunosorbent assay, quantitative polymerase chain reaction), specificity can be demonstrated through the use of reference materials or other suitably characterized materials to confirm the absence of interference in relation to the analyte. In cases where the analyte is a process-related impurity, specificity (noninterference) must also be confirmed against the product.

In case a single procedure is not considered specific or sufficiently selective, an additional procedure should be used to ensure adequate discrimination. For example, where a titration is used to assay a drug substance for release, the combination of the assay and a suitable test for impurities may be used.

<u>Impurities or related substances are available or can be intentionally created:</u>

For assay or potency, discrimination of the analyte in the presence of impurities and/or excipients should be demonstrated. Practically, this can be performed by spiking product with appropriate amounts of impurities and consequently demonstrating that the assay result is unaffected by the presence of these materials (e.g., by comparison with the assay result obtained on unmanipulated samples). Alternatively, samples containing appropriate amounts of impurities could be generated through deliberate stressing of product materials.

For a purity or impurity test, discrimination can be established by stressing or spiking product to achieve appropriate levels of impurities or related substances and demonstrating the absence of interference.

Impurities or related substances are not available:

If impurities, related substances, or degradation products cannot be prepared or isolated, specificity can be demonstrated by comparing the test results of samples containing typical impurities, related substances, or degradation products with an orthogonal procedure. The approach taken should be justified.

B. Range (3.2)

1. General Considerations (3.2.1)

The range of an analytical procedure is the interval between the lowest and the highest results in which the analytical procedure has a suitable level of response, accuracy, and precision. The range can be validated through the direct assessment of reportable results (to generate a reportable range) using an appropriate calibration model (i.e., linear, nonlinear, multivariate). In some cases, the reportable range can be determined using one or more appropriate *working ranges*, depending on the sample preparation (e.g., dilutions) and the analytical procedure selected.

Typically, a working range corresponds to the lowest and the highest sample concentrations or purity levels presented to the analytical instrument for which the analytical procedure provides reliable results. Mathematical calculations are typically required to generate reportable results. Reportable range and working range could be identical.

In cases where materials of sufficient purity (or containing sufficient amounts of impurities) to validate the full range (e.g., 100 percent purity) cannot be generated, extrapolation of the reportable range may be appropriate and should be justified.

2. Response (3.2.2)

a. Linear response (3.2.2.1)

A linear relationship between analyte concentration and response should be evaluated across the range of the analytical procedure to confirm the suitability of the procedure for the intended purpose. The response can be demonstrated directly on the product or suitable reference materials, separate weighings of analyte, or predefined mixtures of the components (e.g., by dilution of a solution of known content), using the proposed procedure.

Linearity can be evaluated with a plot of signals as a function of analyte concentration or content and should demonstrate the analytical procedure capability across a given range to obtain values that are proportional to the true (known or theoretical) sample values. Test results should be evaluated by an appropriate statistical method (e.g., by calculation of a regression line by the method of least squares).

Data derived from the regression line may help to provide mathematical estimates of the linearity. A plot of the data, the correlation coefficient or coefficient of determination, y-intercept, and slope of the regression line should be provided. An analysis of the deviation of the actual data points from the regression line is helpful for evaluating linearity (e.g., for a linear response, the impact of any nonrandom pattern in the residuals plot from the regression analysis should be assessed).

To assess linearity during validation, a minimum of five concentrations appropriately distributed across the range is recommended.

The measured data can be mathematically transformed if necessary (e.g., through the use of a log function).

Other approaches to the assessment of linearity should be justified.

b. Nonlinear response (3.2.2.2)

Some analytical procedures may show nonlinear responses. In these cases, a model or function which can describe the relationship between the activity/concentration present and the response of the analytical procedure is necessary. The suitability of the model should be assessed by means of nonlinear regression analysis (e.g., coefficient of determination).

For example, immunoassays or cell-based assays may show an S-shaped response. S-shaped test curves occur when the range of concentrations is wide enough that responses are constrained by upper and lower asymptotes. Common models used in this case are four- or five-parameter logistic functions, though other acceptable models exist.

For these analytical procedures, the evaluation of linearity is separate from consideration of the shape of the concentration-response curve. Thus, linearity of the concentration-response relationship is not required. Instead, analytical procedure performance should be evaluated across a given range to obtain values that are proportional to the true (known or theoretical) sample values.

c. Multivariate calibration (3.2.2.3)

Algorithms used for construction of multivariate calibration models can be linear or nonlinear, as long as the model is appropriate for establishing the relationship between the signal and the quality attribute of interest. The accuracy of a multivariate procedure is dependent on multiple factors, such as the distribution of calibration samples across the calibration range and the reference analytical procedure error.

In multivariate analysis, the measured data are commonly pretreated through derivatives or normalization.

Linearity assessment, apart from comparison of reference and predicted results, should include information on how the analytical procedure error (residuals) changes across the calibration range. Graphical plots can be used to assess the residuals of the model prediction across the working range.

3. Validation of Lower Range Limits (3.2.3)

If the quality attribute to be measured requires the range of an analytical procedure to be close to the lower range limits of the procedure, *detection limit* (DL) and *quantitation limit* (QL), can be estimated using the following approaches.

a. Based on visual evaluation (3.2.3.1)

Visual evaluation can be used for both noninstrumental and instrumental procedures.

The limit is determined by the analysis of samples with known concentrations and by establishing the minimum level at which the analyte can be reliably resolved and detected or quantitated.

b. Based on signal-to-noise (3.2.3.2)

This approach is relevant for analytical procedures, which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. Alternatively, signals in an appropriate baseline region can be used instead of blank samples. The DL or QL are the minimum concentrations at which the analyte can be reliably detected or quantitated, respectively. A signal-to-noise ratio of 3:1 is generally considered acceptable for estimating the DL. For QL, a ratio of at least 10:1 is considered acceptable.

The signal-to-noise ratio should be determined within a defined region and, if possible, situated equally around the place where the peak of interest would be found.

c. Based on the standard deviation of a linear response and a slope (3.2.3.3)

The DL can be expressed as:

$$DL = \frac{3.3\sigma}{S}$$

while the QL can be expressed as:

$$QL = \frac{10\sigma}{S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S can be estimated from the regression line of the analyte. The estimate of σ can be carried out in a variety of ways, for example:

<u>Based on the standard deviation of the blank:</u>

Measurement of the magnitude of background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of the responses.

Based on the calibration curve:

A specific calibration curve should be evaluated using samples containing an analyte in the range of the DL and QL. The residual standard deviation of a regression line (i.e., root mean square error/deviation) or the standard deviation of y-intercepts of the regression lines can be used as the standard deviation.

d. Based on accuracy and precision at lower range limits (3.2.3.4)

Instead of using estimated values as described in the previous approaches, the QL can be directly validated by accuracy and precision measurements.

e. Recommended data (3.2.3.5)

The DL and the approach used for its determination should be presented. If the DL is determined based on visual evaluation or based on signal-to-noise ratio, the presentation of the relevant data is considered an acceptable justification.

In cases where an estimated value for the DL is obtained by calculation or extrapolation, this estimate can subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the DL.

The QL and the approach used for its determination should also be presented.

If the QL was estimated, the limit should be subsequently validated by the analysis of a suitable number of samples known to be near or at the QL. In cases where the QL is well below (e.g., approximately 10 times lower than) the reporting limit, this confirmatory validation can be omitted with justification.

For impurity tests, the QL for the analytical procedure should be equal to or below the reporting threshold.

C. Accuracy and Precision (3.3)

Accuracy and precision can be evaluated independently, each with a predefined acceptance criterion. Alternatively, accuracy and precision can be evaluated in combination.

1. Accuracy (3.3.1)

Accuracy should be established across the reportable range of an analytical procedure and is typically demonstrated through comparison of the measured results with expected values. Accuracy should be demonstrated under regular test conditions of the analytical procedure (e.g., in the presence of sample matrix and using described sample preparation steps).

Accuracy is typically verified through one of the studies described below. In certain cases, accuracy can be inferred once precision, response within the range, and specificity have been established.

a. Reference material comparison (3.3.1.1)

The analytical procedure is applied to an analyte of known purity (e.g., a reference material, a well-characterized impurity, a related substance) and the measured *versus* theoretically expected results are evaluated.

b. Spiking study (3.3.1.2)

The analytical procedure is applied to a matrix of all components except the analyte where a known amount of the analyte of interest has been added. In cases where all the expected components are impossible to reproduce, the analyte can be added to or enriched in the test sample. The results from measurements on unspiked and spiked/enriched samples are evaluated.

c. Orthogonal procedure comparison (3.3.1.3)

The results of the proposed analytical procedure are compared with those of an orthogonal procedure. The accuracy of the orthogonal procedure should be reported. Orthogonal procedures can be used with quantitative impurity measurements to verify primary measurement values in cases where obtaining samples of all relevant components needed to mimic the matrix for spiking studies is not possible.

d. Recommended data (3.3.1.4)

Accuracy should be assessed using an appropriate number of *determinations* and concentration levels covering the reportable range (e.g., three concentrations/three replicates each of the full analytical procedure).

Accuracy should be reported as the mean percent recovery of a known added amount of analyte in the sample or as the difference between the mean and the accepted true value, together with an appropriate 100(1-α) percent confidence interval (or justified alternative statistical interval). The observed interval should be compatible with the corresponding accuracy acceptance criteria, unless otherwise justified.

For impurity tests, the approach for the determination of individual or total impurities should be described (e.g., weight/weight or area percent with respect to the major analyte).

For quantitative applications of multivariate analytical procedures, appropriate metrics (e.g., root mean square error of prediction (RMSEP)) should be used. If RMSEP is found to be comparable to acceptable root mean square error of calibration, then this indicates that the model is sufficiently accurate when tested with an independent test set. Qualitative applications such as classification, misclassification rate, or positive prediction rate can be used to characterize accuracy.

2. Precision (3.3.2)

Validation of tests for assay and for quantitative determination of impurity (purity) includes an investigation of precision.

Precision should be investigated using authentic homogeneous samples or, if unavailable, artificially prepared samples (e.g., spiked matrix mixtures or samples enriched with relevant amounts of the analyte in question).

a. Repeatability (3.3.2.1)

Repeatability should be assessed using:

a) A minimum of nine determinations covering the reportable range for the procedure (e.g., three concentrations/three replicates each)

or

b) A minimum of six determinations at 100 percent of the test concentration.

b. Intermediate precision (3.3.2.2)

The extent to which *intermediate precision* should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include different days, environmental conditions, analysts, and equipment, as relevant. Ideally, the variations tested should be based on and justified by using analytical procedure understanding from development and risk assessment (ICH Q14). Studying these effects individually is not necessary. The use of design of experiments studies is encouraged.

c. Reproducibility (3.3.2.3)

Reproducibility is assessed by means of an interlaboratory trial. Investigation of reproducibility is usually not required for regulatory submission but should be considered in cases of standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias and in cases where analytical procedures are conducted at multiple sites.

d. Recommended data (3.3.2.4)

The standard deviation, relative standard deviation (coefficient of variation), and an appropriate 100(1-α) percent confidence interval (or justified alternative statistical interval) should be reported. The observed interval should be compatible with the corresponding precision acceptance criteria, unless otherwise justified.

Additionally, for multivariate analytical procedures, the routine metrics of RMSEP encompass accuracy and precision.

3. Combined Approaches for Accuracy and Precision (3.3.3)

An alternative to separate evaluation of accuracy and precision is to consider their total impact by assessing against a combined performance criterion.

Data generated during development may help determine the best approach and refine appropriate performance criteria to which combined accuracy and precision are compared.

Combined accuracy and precision can be evaluated by use of a prediction interval, a tolerance interval, or a confidence interval. Other approaches may be acceptable if justified.

a. Recommended data (3.3.3.1)

If a combined performance criterion is chosen, results should be reported as a combined value to provide appropriate overall knowledge of the suitability of the analytical procedure. If relevant to justify the suitability of the analytical procedure, the individual results for accuracy and precision should be provided as supplemental information. The approach used should be described.

D. Robustness (3.4)

The evaluation of the analytical procedure's suitability within the intended operational environment should be considered during the development phase and depends on the type of procedure under study. Robustness testing should show the reliability of an analytical procedure in response to deliberate variations in *analytical procedure parameters* as well as the stability of the sample preparations and reagents for the duration of the procedure, if appropriate. The robustness evaluation can be submitted as part of development data for an analytical procedure on a case-by-case basis or should be made available upon request.

For further details, see ICH Q14.

GLOSSARY (4)

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or as an accepted reference value and the value or set of values measured. (International Council for Harmonisation guidance for industry *Q2(R2) Validation of Analytical Procedures* (ICH Q2))[1]

Analytical procedure

The analytical procedure refers to the way of performing the analysis. The analytical procedure should describe in sufficient detail the steps necessary to perform each analytical test. (ICH Q2)

Analytical procedure parameter

Any analytical factor (including reagent quality) or analytical procedure operational condition that can be varied continuously (e.g., flow rate) or specified at controllable, unique levels. (ICH guidance for industry *Q14 Analytical Procedure Development* (March 2024) (ICH Q14))

Analytical procedure validation strategy

An analytical procedure validation strategy describes the selection of analytical procedure performance characteristics for validation. In the strategy, data gathered during development studies and system suitability tests can be applied to validation and an appropriate set of validation tests can be predefined. (ICH Q14)

Calibration model

A model based on analytical measurements of known samples that relates the input data to a value for the property of interest (i.e., the model output). (ICH Q2)

Control strategy

A planned set of controls, derived from current product and process understanding, that assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control. (ICH guidance for industry *Q10 Pharmaceutical Quality System* (April 2009))

Covalidation

Demonstration that the analytical procedure meets its predefined performance criteria when used at different laboratories for the same intended purpose. Covalidation can involve all (full

[1] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

revalidation) or a subset (partial revalidation) of performance characteristics potentially impacted by the change in laboratories. (ICH Q2)

Detection limit (DL)

The detection limit is the lowest amount of an analyte in a sample, which can be detected but not necessarily quantitated as an exact value. (ICH Q2)

Determination

The reported value(s) from single or replicate measurements of a single sample preparation as per the validation protocol. (ICH Q2)

Intermediate precision

Intermediate precision expresses intralaboratory variations. Factors to be considered should include potential sources of variability, for example, different days, different environmental conditions, different analysts, and different equipment. (ICH Q2)

Performance characteristic

A technology independent description of a characteristic that ensures the quality of the measured result. Typically, accuracy, precision, specificity/selectivity, and range may be considered. Previous ICH Q2 guidance versions referred to this as *validation characteristic*. (ICH Q2)

Performance criterion

An acceptance criterion describing a numerical range, limit, or desired state to ensure the quality of the measured result for a given performance characteristic. (ICH Q14)

Platform analytical procedure

An analytical procedure that is suitable to test quality attributes of different products without significant change to its operational conditions, system suitability, and reporting structure. This type of analytical procedure can be used to analyze molecules that are sufficiently alike with respect to the attributes that the platform analytical procedure is intended to measure. (ICH Q2)

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions. Precision can be considered at three levels: repeatability, intermediate precision, and reproducibility.

The precision of an analytical procedure is usually expressed as the variance, standard deviation, or coefficient of variation of a series of measurements. (ICH Q2)

Quantitation limit (QL)

The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter used for quantitative assays for low levels of compounds in sample matrices and, particularly, is used for the determination of impurities and/or degradation products. (ICH Q2)

Range

The range of an analytical procedure is the interval between the lowest and the highest results in which the analytical procedure has a suitable level of precision, accuracy, and response. (ICH Q2)

Reportable range

The reportable range of an analytical procedure includes all values from the lowest to the highest reportable result for which there is a suitable level of precision and accuracy. Typically, the reportable range is given in the same unit as the specification acceptance criterion. (ICH Q2)

Working range

A working range corresponds to the lowest and the highest levels of the quality attribute to be measured (e.g., content, purity) as presented to the analytical instrument and for which the analytical procedure provides reliable results. (ICH Q2)

Reference material

A suitably characterized material, sufficiently homogeneous and stable with regard to one or more defined attributes, which has been established to be fit for the intended purpose. Reference materials may include national/international reference standards, pharmacopoeial reference standards, or in-house primary/secondary reference materials. (ICH Q2)

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intraassay precision. (ICH Q2)

Reportable result

The result as generated by the analytical procedure after calculation or processing and applying the described sample replication. (ICH Q2)

Reproducibility

Reproducibility expresses the precision between laboratories (e.g., interlaboratory studies, usually applied to standardization of methodology). (ICH Q2)

Response

The response of an analytical procedure is its ability (within a given range) to obtain a signal which is effectively related to the concentration (amount) or activity of analyte in the sample by some known mathematical function. (ICH Q2)

Revalidation

Demonstration that an analytical procedure is still fit for the intended purpose after a change to the product, process, or the analytical procedure itself. Revalidation can involve all (full revalidation) or a subset (partial revalidation) of performance characteristics. (ICH Q2)

Robustness

The robustness of an analytical procedure is a measure of its capacity to meet the expected performance criteria during normal use. Robustness is tested by deliberate variations of analytical procedure parameters. (ICH Q14)

Specificity/Selectivity

Specificity and selectivity are both terms to describe the extent to which other substances interfere with the determination of an analyte according to a given analytical procedure. Specificity is typically used to describe the ultimate state, measuring unequivocally a desired analyte. Selectivity is a relative term to describe the extent to which particular analytes in mixtures or matrices can be measured without interferences from other components with similar behavior. (ICH Q2)

System suitability test (SST)

System suitability tests are developed and used to verify that the measurement system and the analytical operations associated with the analytical procedure are fit for the intended purpose and increase the detectability of unacceptable performance. (ICH Q14)

Validation study

An evaluation of prior knowledge, data, or deliberate experiments (i.e., validation tests) to determine the suitability of an analytical procedure for the intended purpose. (ICH Q2)

Validation test

Validation tests are deliberate experiments designed to authenticate the suitability of an analytical procedure for the intended purpose. (ICH Q2)

<u>Multivariate Glossary</u>

Calibration set

A set of data with matched known characteristics and measured analytical results. (ICH Q14)

Independent sample

Independent samples are samples not included in the calibration set of a multivariate model. Independent samples can come from the same batch from which calibration samples are selected. (ICH Q2)

Internal testing

Internal testing is a process of checking if unique samples processed by the model yield the correct predictions (qualitative or quantitative).

Internal testing serves as means to establish the optimal number of latent variables, estimate the standard error, and detect potential outliers. (ICH Q2)

Latent variables

Mathematically derived variables that are directly related to measured variables and are used in further processing. (ICH Q2)

Model validation

The process of determining the suitability of a model by challenging it with independent test data and comparing the results against predetermined performance criteria. (ICH Q2)

Multivariate analytical procedure

An analytical procedure where a result is determined through a multivariate calibration model utilizing more than one input variable. (ICH Q2)

Reference analytical procedure

A separate analytical procedure used to obtain the reference values of the calibration and validation samples for a multivariate analytical procedure. (ICH Q2)

Validation set

A set of data used to give an independent assessment of the performance of the calibration model. (ICH Q2)

REFERENCES (5)

International Council for Harmonisation guidance for industry *Q10 Pharmaceutical Quality System* (April 2009)[1]

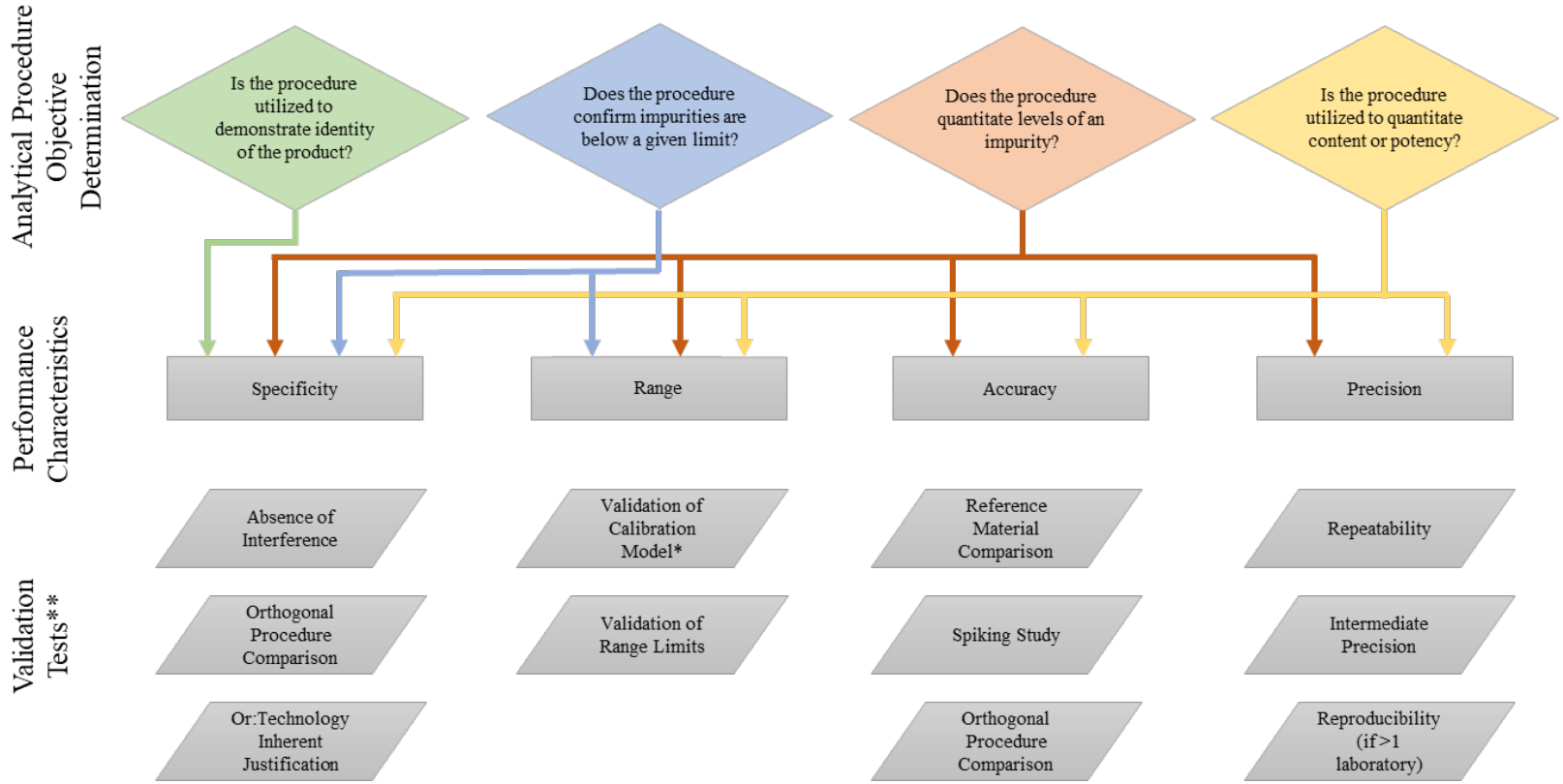
International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development* (March 2024)

International Council for Harmonisation guideline *M4Q(R1) The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Quality*[2]

[1] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.
[2] Available at https://database.ich.org/sites/default/files/M4Q_R1_Guideline.pdf.

APPENDIX A: SELECTION OF VALIDATION TESTS (6. ANNEX 1)

Figure 2: Examples of Relevant Validation Tests Based on the Objective of the Analytical Procedure



* May not be needed for limit tests

** Tests may be chosen from presented options. May not require all tests.

APPENDIX B: ILLUSTRATIVE EXAMPLES FOR ANALYTICAL TECHNIQUES (7. ANNEX 2)

The tables presented in this appendix are examples of approaches to analytical procedure validation for a selection of technologies. The technologies and approaches presented have been constructed to illustrate potential applications of the principles contained within this guidance and are not exhaustive. The examples are not intended to be mandatory, and alternative approaches (fulfilling the intent of the guidance) may also be acceptable.

Table 3: Examples for Quantitative Separation Techniques

Technique	**Separation Techniques (e.g., HPLC, GC, CE) for Impurities or Assay**	**Separation Techniques With Relative Area Quantitation (e.g., Product-Related Substances Such as Charge Variants)**
Performance Characteristic	**Validation Study Methodology**	
Specificity/ Selectivity	<u>Absence of relevant interference:</u> With product, buffer, or appropriate matrix, and between individual peaks of interest Spiking with known impurities/ excipients or By comparison of impurity profiles by an orthogonal analytical procedure Demonstration of stability-indicating properties through appropriate forced degradation samples, if necessary	<u>Absence of relevant interference:</u> With product, buffer, or appropriate matrix, and between individual peaks of interest Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary
Precision	<u>Repeatability:</u> Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering peak(s) of interest <u>Intermediate precision:</u> e.g., different days, environmental conditions, analysts, equipment	

Technique	**Separation Techniques (e.g., HPLC, GC, CE) for Impurities or Assay**	**Separation Techniques With Relative Area Quantitation (e.g., Product-Related Substances Such as Charge Variants)**
Performance Characteristic	**Validation Study Methodology**	
Accuracy	For Assay: Comparison with suitably characterized material (e.g., reference material) or Comparison with an orthogonal procedure For impurities or related substances: Spiking studies with impurities or Comparison of impurity profiles with an orthogonal procedure	Comparison with an orthogonal procedure and/or suitably characterized material (e.g., reference material) or Accuracy can be inferred once precision, linearity, and specificity have been established or Spiking studies with forced degradation samples and/or suitably characterized material
Reportable range	Validation of calibration model across the range: Linearity: Dilution of the analytes of interest over the expected procedure range, at least 5 points Validation of lower range limits (for purity only): QL, DL through a selected methodology (e.g., signal-to-noise determination)	Validation of calibration model across the range: Linearity: Between measured (observed) relative result *versus* theoretically expected relative result across specification range(s) (e.g., by spiking or degrading material) Validation of lower range limits: QL (and DL) through a selected methodology (e.g., signal-to-noise determination)

Technique	Separation Techniques (e.g., HPLC, GC, CE) for Impurities or Assay	Separation Techniques With Relative Area Quantitation (e.g., Product-Related Substances Such as Charge Variants)
Performance Characteristic	**Validation Study Methodology**	
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)*	Deliberate variation of relevant parameters, e.g., Sample preparation: extraction volume, extraction time, temperature, dilution Separation parameters: column/capillary lot, mobile phase/buffer composition and pH, column/capillary temperature, flow rate, detection wavelength Stability of sample and reference material preparations **Relative Response Factors** If the analyte has a different response from the reference material (e.g., a different specific UV absorbance), relative response factors should be calculated using the appropriate ratio of responses. This evaluation may be performed during validation or development, and should use the finalized analytical procedure conditions and be appropriately documented If the relative response factor is outside the range 0.8-1.2, then a correction factor should be applied. If an impurity/degradation product is overestimated, it may be acceptable not to use a correction factor	

HPLC = high-performance liquid chromatography; GC = gas chromatography; CE = capillary electrophoresis; QL = quantitation limit; DL = detection limit; UV = ultraviolet.

* We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

Table 4: Example for Elemental Impurities by ICP-OES or ICP-MS

Technique	Elemental Impurities by ICP-OES or ICP-MS
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Spiking of elements into matrix and demonstration of sufficient noninterference and confirmation of accuracy with the presence of components (e.g., carrier gas, impurities, matrix) or Justification through technology/prior knowledge (e.g., specificity of technology for certain isotopes)

Technique	**Elemental Impurities by ICP-OES or ICP-MS**
Performance Characteristic	**Validation Study Methodology**
Precision	Repeatability: Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering signals of interest Intermediate precision: e.g., different days, environmental conditions, analysts, equipment
Accuracy	Spiking studies with impurities or Comparison of impurity profiles with an orthogonal procedure
Reportable range	Validation of working range: Linearity: Dilution of the analytes of interest over the expected working range, at least 5 points, can be combined with multilevel accuracy experiment Validation of lower range: QL, DL through a selected methodology
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters and stability of test conditions, e.g., Sample digestion technique and preparation, nebulizer and sheath flow settings, plasma settings

ICP-OES = inductively coupled plasma optical emission spectroscopy; ICP-MS = inductively coupled plasma mass spectrometry; QL = quantitation limit; DL = detection limit.

Table 5: Example for Dissolution With HPLC as Product Performance Test for an Immediate Release Dosage Form

Technique	**Dissolution With HPLC as Product Performance Test for an Immediate Release Dosage Form**	
Performance Characteristic	**Demonstration of Performance of Dissolution Step** *Typically demonstrated with development data*	**Validation Testing Methodology** *Typically demonstrated with final procedure*
Specificity/ Selectivity	Discriminatory power: Demonstration of the discriminatory power to differentiate between batches manufactured with different critical process parameters and/or critical material attributes, which may have an impact on the bioavailability (performed as part of development of dissolution step)	Absence of interference: Demonstration of noninterference with excipients and dissolution media likely to impact the quantitation of the main analyte
Precision	Repeatability and intermediate precision: Understanding of variability by performing, e.g., vessel-to-vessel repeatability studies or intermediate precision studies (operators, equipment) *Note: The study provides a combined assessment of variability of product quality and product dissolution performance in addition to the variability of the quantitative procedure*	Repeatability and intermediate precision: Demonstration with a homogeneous sample from one dissolved tablet, e.g., several samples drawn from the same vessel, after analyte in sample has been fully dissolved
Accuracy	(Not applicable for dissolution step)	Spiking study: Add known amounts of the reference material to the dissolution vessel containing excipient mixture in dissolution media and calculate recovery within defined working range

Technique	**Dissolution With HPLC as Product Performance Test for an Immediate Release Dosage Form**	
Performance Characteristic	**Demonstration of Performance of Dissolution Step** *Typically demonstrated with development data*	**Validation Testing Methodology** *Typically demonstrated with final procedure*
Reportable range	(Not applicable for dissolution step)	Validation of calibration model across the range Linearity: Demonstrate linearity from sample concentrations (as presented to quantitative measurement) in the range of Q - 45% of the lowest strength up to 130% of the highest strength, for one point specification, and in the range of QL up to 130% of the highest strength, for multiple point specification *If lower concentration ranges are expected to be close to QL*: Validation of lower range limits, see separation techniques
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Justification of the selection of the dissolution procedure parameters, *e.g.,* medium buffer composition, surfactant concentration, use of sinkers, pH, deaeration, volume, agitation rate, sampling time	Deliberate variation of parameters of the quantitative procedure, see separation technique

HPLC = high-performance liquid chromatography; QL = quantitation limit.

Table 6: Example for Quantitative ^{1}H-NMR for the Assay of a Drug Substance

Technique	**Quantitative ^{1}H-NMR (Internal Standard Method) for the Assay of a Drug Substance**
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Absence of interference: Select a signal which is representative for the analyte and does not show interference with potential baseline artifacts, residual water or solvent signals, related structure impurities or other impurities, internal standards, nontarget major component, or potential isomers/forms
Precision	Repeatability: Replicate measurements of at least 6 independent preparations at 100% level Intermediate Precision: Not necessary to be conducted on target analyte (justified by technology principle, as typically verified through instrument calibration with a standard sample)
Accuracy	Reference material comparison: Confirm with sample of known purity
Reportable range	Validation tests are typically not necessary because the integral areas are usually directly proportional to the amount (mole) of reference material and analyte (technology-inherent justification)
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters, e.g., Temperature, concentration, field (shim), tuning and matching of the NMR probe, solution stability

^{1}H-NMR = hydrogen-1 nuclear magnetic resonance.

Table 7: Example for Biological Assays

Technique	**Binding Assay (e.g., ELISA, SPR) or Cell-Based Assay for Determination of Potency Relative to a Reference**
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Absence of interference: Dose-response curve fulfills the response criteria demonstrating the similarity of the analyte and reference material, as well as noninterfering signal from the matrix (for binding assay), or no dose response from the cell line alone (for cell-based assay) Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary
Precision	Repeatability: Repeated sample analysis on a single day or within a short interval of time covering the reportable range of the analytical procedure (at least 3 replicates over at least 5 levels) Intermediate Precision: Different analysts, multiple independent preparations over multiple days at multiple potency levels through the analytical procedure's reportable range, inclusive of normal laboratory variation
Accuracy	Reference material comparison: Assess recovery *versus* theoretical activity for multiple (at least 3) independent preparations at multiple (at least 5) levels through the analytical procedure's reportable range
Reportable range	Validation of range, including lower and higher range limits: The lowest to highest relative potency levels that meet accuracy, precision, and response criteria, determined over at least 5 potency levels
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters, e.g., Plate type, buffer components, incubation times, incubation conditions, instruments, reaction times, reagent lots including controls For binding assay procedures: coating proteins, capture/detection antibody For cell-based assay procedures: cell density, effector/target cell ratio, cell generation number

ELISA = enzyme-linked immunosorbent assay; SPR = surface plasmon resonance.

Table 8: Example for Quantitative PCR

Technique	**Quantitative PCR (quantitative analysis of impurities in drug substances or products)**
Performance Characteristic	**Validation Study Methodology**
Specificity/Selectivity	Orthogonal Procedure Comparison: Test reaction specificity by gel electrophoresis, melting profile, or DNA sequencing Absence of interference: Positive template, no-reverse transcription control for RT-qPCR and no template control. Test primer and probe target specificity against gene bank with sequence similarity search program (e.g., nucleotide BLAST). Evaluate the slope of standard curve for efficiency
Precision	Repeatability: Independent preparations of 5 positive control levels evenly distributed along the standard curve and assayed in triplicate within a single assay assessment. The results can be compared using (CV) Intermediate precision: At least 3 replicates per run at each positive control level in at least 6 runs over 2 or more days
Accuracy	Spiking Study: Test (e.g., n=6) replicates at 3 to 5 template spike levels from the standard curve concentrations Efficiency/consistency of RNA/DNA extraction method should be accounted for
Reportable range	Linearity: Working range should cover at least 5 to 6 log to the base 10 concentration values. Correlation coefficients or standard deviations should be calculated through the entire dynamic range Validation of lower working range limits based on the calibration curve: DL defined by template spiking in samples or from standard curves. DL is lowest point meeting the response curve parameters QL demonstrated through showing sufficient recovery and acceptable CVs from the accuracy experiment

Technique	**Quantitative PCR (quantitative analysis of impurities in drug substances or products)**
Performance Characteristic	**Validation Study Methodology**
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters, e.g., Equipment, master mix composition (concentrations of salts, dNTPs, adjuvants), master mix lots, reaction volume, probe and primer concentrations, thermal cycling parameters

qPCR = quantitative polymerase chain reaction; RT-qPCR = reverse transcription qPCR; CV = coefficient of variation; DL = detection limit; QL = quantitation limit; dNTPs = deoxynucleotide triphosphate.

Table 9: Example for Particle Size Measurement

Technique	**Particle Size Measurement (Dynamic Light Scattering; Laser Diffraction Measurement) as a Property Test**
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Absence of interference: Evaluate blank and sample to determine the appropriateness of the equipment settings and sample preparation
Precision	Repeatability: Test at least 6 replicates using established analytical procedure parameters at target range Intermediate precision: Analysis performed on different days, environmental conditions, analysts, equipment setup
Accuracy	Technology-inherent justification: Confirmed by an appropriate instrument qualification or Orthogonal procedure comparison: Qualitative comparison using a different technique, like optical microscopy, to confirm results
Reportable range	Technology specific justification, e.g., particle size range covered
Robustness and other considerations	Deliberate variation of parameters, e.g., Evaluation of expected size ranges for the intended use of the analytical procedure

(performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Dispersion stability for liquid dispersions (stability over potential analysis time, stir rate, dispersion energy equilibration or stir time before measurement) Dispersion stability for dry dispersions (sample amount, measurement time, air pressure and feed rate) Obscuration range (establish optimum percentage of laser obscuration) Ultrasound time/percentage for sample, if applicable

Table 10: Example for NIR Analytical Procedure

Technique	**NIR Analytical Procedure for Core Tablet Assay**
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Absence of interference: Comparison of drug substance spectrum and the loading plots of the model Rejection of outliers (e.g., excipient, analogues) not covered by the multivariate procedure
Precision	Repeatability: Repeated analysis with removal of sample from the holder between measurements
Accuracy	Comparison with an orthogonal procedure: Demonstration across the range through comparison of the predicted and reference values using an appropriate number of determinations and concentration levels (e.g., 5 concentrations, 3 replicates) Accuracy is typically reported as the standard error of prediction (SEP or RMSEP)
Reportable range	Response: Demonstration of the relationship between predicted and reference values Error (accuracy) across the range: Information on how the analytical procedure error (accuracy) changes across the calibration range, e.g., by plotting the residuals of the model prediction *versus* the actual data
Robustness and other considerations (performed as part of analytical	Deliberate variation of parameters, e.g., Chemical and physical factors that can impact NIR spectrum and model prediction should be represented in data sets. Examples include various sources of drug substance and excipients, water content, tablet hardness, and orientation in the holder

Technique	NIR Analytical Procedure for Core Tablet Assay
Performance Characteristic	**Validation Study Methodology**
procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	*Note: NIR measurements are sensitive to changes in tablet composition and properties outside variation present in the calibration set*

NIR = near infrared; SEP = square error of prediction; RMSEP = root mean square error of prediction.

Table 11: Example for Quantitative LC/MS

Technique	Quantitative LC/MS Analysis of Trace Impurities in Product
Performance Characteristic	**Validation Study Methodology**
Specificity/ Selectivity	Technology-inherent justification: Inferred through use of specific and selective MS detection (e.g., MRM transition with specified quantitative to qualitative ion ratio, accurate m/z value) in combination with retention time, consider potential for isotopes or Absence of interference from other components in sample matrix or Comparison of impurity profiles determined by an orthogonal analytical procedure
Precision	Repeatability Measurement of a minimum of 3 replicates at each of at least 3 spiking levels or a minimum of 6 replicates at 100% Intermediate precision Comparison of measurements of the same samples performed in the same laboratory but under varying conditions (e.g., different LC/MS systems, different analysts, different days)
Accuracy	Spiking study: Acceptable recovery of spiked impurity standards in sample matrix at multiple spiking levels or Comparison of the results to the *true* values obtained from an orthogonal procedure

Technique	**Quantitative LC/MS Analysis of Trace Impurities in Product**
Performance Characteristic	**Validation Study Methodology**
Reportable range	Validation of calibration model across the range: Linearity: Experimental demonstration of the linear relationship between analyte concentrations and peak responses (or the ratio of peak response if an internal standard was used) with reference materials at 5 or more concentration levels Validation of lower range limits: DL: Use the CV of responses at the spiking level (with 6 or more repeated injections) as a measure of signal-to-noise. The CV obtained must be less than or equal to a predefined acceptable value QL: The lowest spiking level with acceptable accuracy and precision The range extends from and is inclusive of the QL to the highest spiking level with acceptable accuracy, precision, and response
Robustness and other considerations (performed as part of analytical procedure development as per International Council for Harmonisation guidance for industry *Q14 Analytical Procedure Development*)	Deliberate variation of parameters, e.g., LC flow rate, LC injection volume, MS drying/desolvation temperature, MS gas flow, mass accuracy, MS collision energy, stability of test conditions

LC/MS = liquid chromatography/mass spectrometry; MRM = multiple reaction monitoring; CV = coefficient of variation.