

# Q2(R2) VALIDATION OF ANALYTICAL PROCEDURES

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For questions regarding this draft document, contact (CDER) [David.Keire@fda.hhs.gov](mailto:David.Keire@fda.hhs.gov).

## 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.



INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL  
REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

**ICH HARMONISED GUIDELINE**

**VALIDATION OF ANALYTICAL PROCEDURES  
Q2(R2)**

Draft version

Endorsed on 24 March 2022

*Currently under public consultation*

*At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.*

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**ICH HARMONISED GUIDELINE**  
**VALIDATION OF ANALYTICAL PROCEDURES**

**Q2(R2)**

**ICH Consensus Guideline**

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1 **1 INTRODUCTION**

2 This guideline presents a discussion of elements for consideration during the validation of  
3 *analytical procedures* included as part of registration applications submitted within the ICH  
4 member regulatory authorities. Q2(R2) provides guidance and recommendations on how to  
5 derive and evaluate the various *validation tests* for each analytical procedure. This guideline  
6 serves as a collection of terms, and their definitions. These terms and definitions are meant to  
7 bridge the differences that often exist between various compendia and documents of the ICH  
8 member regulatory agencies.

9 The objective of validation of an analytical procedure is to demonstrate that the analytical  
10 procedure is suitable for the intended purpose. A tabular summary of the characteristics  
11 applicable to common types of uses of analytical procedures is included (Table 1). Further  
12 general guidance is provided on how to perform *validation studies* for analytical procedures.

13 The document provides an indication of the data which should be presented in a regulatory  
14 submission. Analytical procedure validation data should be submitted in the corresponding  
15 sections of the application in the ICH M4Q THE COMMON TECHNICAL DOCUMENT FOR  
16 THE REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. All relevant data  
17 collected during validation (and any methodology used for calculating validation results)  
18 should be submitted to establish the suitability of the procedure for the intended use. Of note,  
19 suitable data derived from development studies (see ICH Q14) can be used in lieu of validation  
20 data. When an established *platform analytical procedure* is used for a new purpose, validation  
21 testing can be abbreviated, if scientifically justified.

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

25 Suitably characterized reference materials, with documented identity and purity or any other  
26 characteristics as necessary, should be used throughout the validation study. The degree of  
27 purity necessary for the reference material depends on the intended use.

28 In practice, the experimental work can be designed so that the appropriate validation tests can  
29 be performed to provide sound, overall knowledge of the performance of the analytical  
30 procedure, for instance: *specificity/selectivity*, *accuracy*, and *precision over the reportable*  
31 *range*.

32 As described in *ICH Q14*, the *system suitability test (SST)* is an integral part of analytical  
33 procedures and is generally established during development as a regular check of performance.  
34 *Robustness* typically should be evaluated as part of development prior to the execution of the  
35 analytical procedure validation study (ICH Q14).

36 **2 SCOPE**

37 This guideline applies to new or revised analytical procedures used for release and stability

38 testing of commercial drug substances and products (chemical and  
39 biological/biotechnological). The guideline can also be applied to other analytical procedures  
40 used as part of the control strategy (*ICH Q8-Q10*) following a risk-based approach. The  
41 scientific principles described in this guideline can be applied in a phase-appropriate manner  
42 during clinical development. This guideline may also be applicable to other types of products,  
43 with appropriate regulatory authority consultation as needed.

44 The guideline is directed to the most common purposes of analytical procedures, such as  
45 assay/potency, purity, impurity (quantitative or limit test), identity or other quantitative or  
46 qualitative measurements.

### 47 **3 ANALYTICAL PROCEDURE VALIDATION STUDY**

48 A validation study is designed to provide sufficient evidence that the analytical procedure meets  
49 its objectives. These objectives are described with a suitable set of *performance characteristics*  
50 and related *performance criteria*, which can vary depending on the intended use of the  
51 analytical procedure and the specific technology selected. The section “VALIDATION TESTS,  
52 METHODOLOGY AND EVALUATION” summarizes the typical methodology and validation  
53 tests that can be used (see flowchart in Annex 1). Specific non-binding examples for common  
54 techniques are given in Annex 2. Based on Annex 1 and the measured product attributes,  
55 typical performance characteristics and related validation tests are provided in Table 1.

56

57

58 **Table 1:** Typical performance characteristics and related validation tests for measured  
59 product attributes

Analytical Procedure Performance Characteristics to be demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY content/potency
		Quantitative	Limit	Other quantitative measurements (1)
Specificity (3) Specificity Test	+	+	+	+
Working Range Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+(5)	-	+(5)

60 - signifies that this test is not normally evaluated

61 + signifies that this test is normally evaluated

62 ( ) signifies that this test is normally not evaluated, but in some complex cases recommended

63 QL, DL: Quantitation Limit, Detection Limit

64 (1) other quantitative measurements can follow the scheme of impurity testing, if the working range is  
65 close to the detection or quantitation limits of the technology, otherwise following the assay scheme is  
66 recommended.

67 (2) some performance characteristics can be substituted with technology inherent justification or  
68 qualification in the case of certain analytical procedures for physicochemical properties.

69 (3) a combined approach can be used alternatively to evaluating accuracy and precision separately

70 (4) lack of specificity of one analytical procedure could be compensated by one or more other supporting  
71 analytical procedures.

72 (5) Reproducibility and intermediate precision can be performed as a single set of experiments.

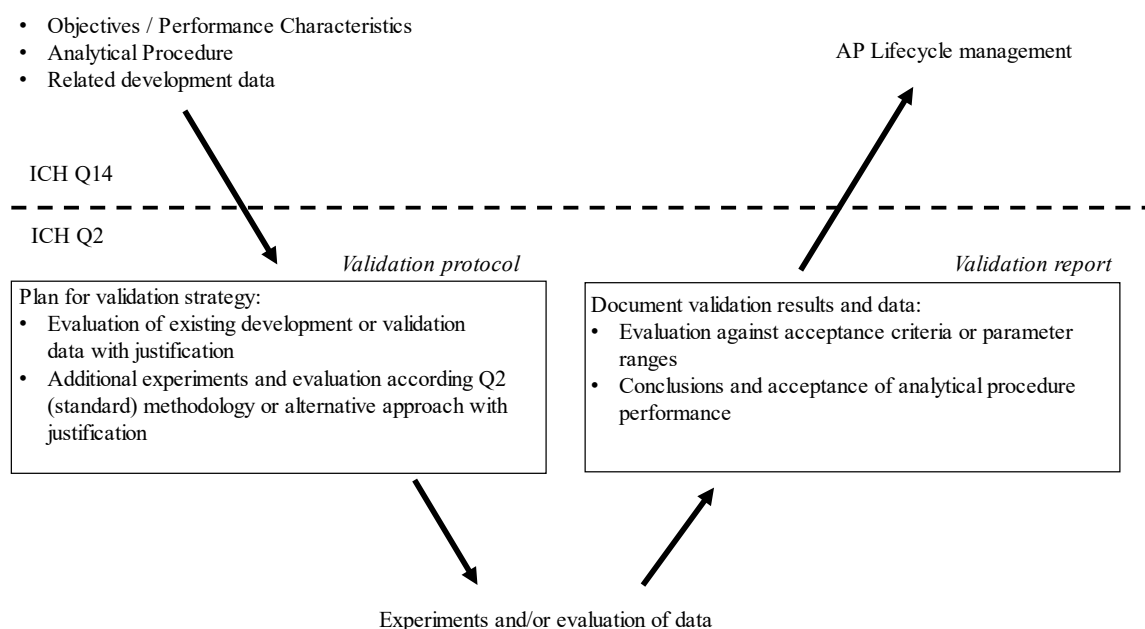
73

74 The objective of the analytical procedure, appropriate performance characteristics and  
 75 associated criteria and appropriate validation tests (including those excluded from the  
 76 validation protocol) should be documented and justified.

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

82 Figure 1 shows how knowledge can be generated during analytical procedure development as  
 83 described in ICH Q14 and aid the design of a validation study.

84 **Figure 1: Validation study design and evaluation**



85

86 **3.1 Validation during the lifecycle of an analytical procedure**

87

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

92 *Co-validation* can be used to demonstrate that the analytical procedure meets predefined  
 93 performance criteria by using data from multiple sites. When transferring analytical procedures  
 94 to a different laboratory, a subset of validation experiments is often performed.

95 *Cross-validation* is an approach which can be used to show that two or more analytical

96 procedures can be used for the same intended purpose. Cross-validation should demonstrate  
97 that the same predefined performance criteria are met for these procedures.

### 98 3.2 Reportable Range

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

104 The table below exemplifies recommended reportable ranges for some uses of analytical  
105 procedures; other ranges may be acceptable if justified. In some cases, *e.g.*, at low amounts,  
106 wider upper ranges may be more practical.

107 **Table 2: 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 drug substance or a finished (drug) product	80% of declared content or 80% of lower specification limit	120% of declared content or 120% of the upper specification limit
Potency	Lowest specification acceptance criterion -20%	Highest specification acceptance criterion +20%
Content uniformity	70% of declared content	130% of declared content
Dissolution testing	Q-45% (immediate release) of the dosage form strength first measurement timepoint or QL (modified release)	130% of declared content of the dosage form
Impurity testing	Reporting threshold	120% of specification limit
Purity testing (as area %)	80% of specification limit	100% of specification limit

### 108 3.3 Demonstration of stability indicating properties

109 If a procedure is a validated quantitative analytical procedure that can detect changes in relevant

110 quality attributes of a drug substance or drug product during storage, the procedure is  
111 considered a stability-indicating test. To demonstrate specificity/selectivity of a stability-  
112 indicating test, a combination of challenges should be performed with appropriate justification  
113 from development studies. These can include: the use of samples spiked with target analytes  
114 and all known interferences; samples that have been exposed to various physical and chemical  
115 stress conditions; and actual product samples that are either aged or have been stored at higher  
116 temperature and/or humidity.

117

### 118 **3.4 Considerations for multivariate analytical procedures**

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

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

125 Typically, calibration and validation are performed in two phases.

126 • In the first phase, model development consists of calibration and internal testing.  
127 Calibration data are used to create the calibration model. Test data are used for internal  
128 testing and optimisation of the model. The test data could be a separate set of data or  
129 part of the *calibration data set* used in a rotational manner. This internal test step is  
130 used to obtain an estimate of the model performance and to fine-tune an algorithm's  
131 parameters (*e.g.*, the number of *latent variables* for partial least squares (PLS)) to select  
132 the most suitable model within a given set of data and prerequisites.

133 • In the second phase, *model validation*, an independent validation data set with  
134 *independent samples* is used for validation of the model.

#### 135 **3.4.1 Reference analytical procedure(s)**

136 Samples used for the validation of quantitative or qualitative multivariate procedures should  
137 have values or categories assigned to each sample, typically obtained by a validated procedure  
138 or pharmacopeial reference procedure.

139 When a reference analytical procedure is used, its performance should match the expected  
140 performance of the multivariate analytical procedure. Analysis by the *reference procedure* and  
141 multivariate data collection should be performed on the same samples (whenever possible),  
142 within a reasonable period of time to assure sample and measurement stability. In some cases,  
143 a correlation or conversion may be needed to provide the same unit of measure. Any  
144 assumptions or calculations should be described.

**145 4 VALIDATION TESTS, METHODOLOGY AND EVALUATION**

146 In the following chapters, experimental methodologies to evaluate the performance of an  
147 analytical procedure are described. The methodology described is grouped by the main  
148 performance characteristic the analytical procedure was designed for. However, it is  
149 acknowledged that information about other performance characteristics may be derived from  
150 the same dataset. Other approaches may be used to demonstrate that the analytical procedure  
151 meets the objectives and related performance criteria, if justified.

**152 4.1 Specificity / Selectivity**

153 The *specificity* or *selectivity* of an analytical procedure can be demonstrated through absence  
154 of interference, comparison of results to an orthogonal procedure or may be inherently given  
155 by the underlying scientific principles of the analytical procedure. Some experiments can be  
156 combined with accuracy studies.

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

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

**162 4.1.1 Absence of interference**

163 Specificity/selectivity can be shown by demonstrating that the identification and/or  
164 quantitation of an analyte is not impacted by the presence of other substances (*e.g.*, impurities,  
165 degradation products, related substances, matrix, or other components present in the operating  
166 environment).

**167 4.1.2 Orthogonal procedure comparison**

168 Specificity/selectivity can be verified by demonstrating that the measured result of an analyte  
169 is comparable to the measured result of a second, well characterized analytical procedure (*e.g.*,  
170 an orthogonal procedure).

**171 4.1.3 Technology inherent justification**

172 In some cases where the specificity of the analytical technology can be ensured and predicted  
173 by technical parameters (*e.g.*, resolution of isotopes in mass spectrometry, chemical shifts of  
174 NMR signals), no experimental study may be required, if justified.

**175 4.1.4 Recommended Data****176 4.1.4.1 Identification**

177 For identification tests, a critical aspect is to demonstrate the capability to identify the analyte  
178 of interest based on unique aspects of its molecular structure and/or other specific properties.

179 The capability of an analytical procedure to identify an analyte can be confirmed by obtaining  
180 positive results comparable to a known reference material with samples containing the analyte,  
181 along with negative results from samples which do not contain the analyte. In addition, the  
182 identification test can be applied to materials structurally similar to or closely related to the  
183 analyte to confirm that an undesired positive *response* is not obtained. The choice of such  
184 potentially interfering materials should be based on scientific judgement with a consideration  
185 of any interference that could occur.

#### 186 4.1.4.2 Assay, purity- and impurity test(s)

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

189 Representative data (*e.g.*, chromatograms, electropherograms or spectra) should be used to  
190 demonstrate specificity and individual components should be appropriately labelled.

191 Suitable discrimination should be investigated at an appropriate level (*e.g.*, for critical  
192 separations in chromatography, specificity can be demonstrated by the resolution of the two  
193 components which elute closest to each other). Alternately, spectra of different components  
194 could be compared to assess the possibility of interference.

195 In case a single procedure is not considered sufficiently selective, an additional procedure  
196 should be used to ensure adequate specificity. For example, where a titration is used to assay a  
197 drug substance for release, the combination of the assay and a suitable test for impurities can  
198 be used.

199 The approach is similar for both assay and impurity tests:

#### 200 Impurities or related substances are available:

201 For assay, discrimination of the analyte in the presence of impurities and/or excipients should  
202 be demonstrated. Practically, this can be performed by spiking drug substance or drug product  
203 with appropriate levels of impurities and/or excipients and demonstrating that the assay result  
204 is unaffected by the presence of these materials (*e.g.*, by comparison with the assay result  
205 obtained on unspiked samples).

206 For an impurity test, discrimination can be established by spiking drug substance or drug  
207 product with appropriate levels of impurities and demonstrating the unbiased measurement of  
208 these impurities individually and/or from other components in the sample matrix.

#### 209 Impurities or related substances are not available:

210 If impurity, related substances or degradation product materials are unavailable, specificity can  
211 be demonstrated by comparing the test results of samples containing typical impurities, related  
212 substances or degradation products with a second well-characterized procedure (*e.g.*,  
213 pharmacopeial procedure or other validated orthogonal analytical procedure).



**214 4.2 Working Range**

215 Depending on the sample preparation (*e.g.*, dilutions) and the analytical procedure selected, the  
216 reportable range will lead to a specific working range. Typically, a corresponding set of sample  
217 concentrations or purity levels is presented to the analytical instrument and the respective signal  
218 responses are evaluated.

**219 4.2.1 Response****220 4.2.1.1 Linear Response**

221 A linear relationship between analyte concentration and response should be evaluated across  
222 the working range of the analytical procedure to confirm the suitability of the procedure for the  
223 intended use. The response can be demonstrated directly on the drug substance (*e.g.*, by dilution  
224 of a standard stock solution) or separate weighings of synthetic mixtures of the drug product  
225 components, using the proposed procedure.

226 Initially, linearity can be evaluated with a plot of signals as a function of analyte concentration  
227 or content. Test results should be evaluated by appropriate statistical methods (*e.g.*, by  
228 calculation of a regression line by the method of least squares).

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

235 For the establishment of linearity, a minimum of five concentrations appropriately distributed  
236 across the range is recommended; however, additional concentrations may be required for more  
237 complex models. Other approaches should be justified.

238 To obtain linearity, the measurements can be transformed, and a weighting factor applied to the  
239 regression analysis (*i.e.*, in case of populations of data points with different variability  
240 (heteroscedasticity), including log or square root).

241 Other approaches should be justified.

**242 4.2.1.2 Non-linear Response**

243 Some analytical procedures may show non-linear responses. In these cases, a model or function  
244 which can describe the relationship between response of the analytical procedure and the  
245 concentration is necessary. The suitability of the model should be assessed by means of non-  
246 linear regression analysis (*e.g.*, coefficient of determination).

247 For example, immunoassays or cell-based assays may show an S-shaped response. S-shaped  
248 test curves occur when the range of concentrations is wide enough that responses are

249 constrained by upper and lower asymptotes. Common models used in this case are four-  
250 parameter or five-parameter logistical functions, though other acceptable models exist.

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

#### 256 **4.2.1.3 Multivariate calibration**

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

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

#### 266 **4.2.2 Validation of lower range limits**

267 The lower range limits, *detection limit* (DL) and *quantitation limit* (QL), can be estimated using  
268 different approaches.

##### 269 **4.2.2.1 Based on signal-to-noise**

270 This approach can only be applied to analytical procedures which exhibit baseline noise.  
271 *Determination* of the signal-to-noise ratio is performed by comparing measured signals from  
272 samples with known low concentrations of analyte with those of blank samples. Signals in an  
273 appropriate baseline region can be used instead of blank samples. The DL or QL are the  
274 minimum concentrations at which the analyte can be reliably detected or quantified,  
275 respectively. A signal-to-noise ratio of 3:1 is generally considered acceptable for estimating the  
276 detection limit. For quantitation limit, a ratio of at least 10:1 is considered acceptable.

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

280

281 **4.2.2.2 Based on the Standard Deviation of a Linear Response and a Slope**

282 The detection limit (DL) can be expressed as:

283 
$$DL = \frac{3.3\sigma}{S}$$

284 while the quantitation limit (QL) can be expressed as:

285 
$$QL = \frac{10\sigma}{S}$$

286 where  $\sigma$  = the standard deviation of the response

287 S = the slope of the calibration curve

288 The slope S can be estimated from the regression line of the analyte. The estimate of  $\sigma$  can be  
289 carried out in a variety of ways, for example:290 Based on the Standard Deviation of the Blank291 Measurement of the magnitude of background response is performed by analysing an  
292 appropriate number of blank samples and calculating the standard deviation of the responses.293 Based on the Calibration Curve294 A specific calibration curve should be evaluated using samples containing an analyte in the  
295 range of the DL and QL. The residual standard deviation of a regression line (i.e., root mean  
296 square error/deviation) or the standard deviation of y-intercepts of the regression lines can be  
297 used as the standard deviation.298 Based on visual evaluation

299 Visual evaluation can be used for both non-instrumental and instrumental procedures.

300 The limit is determined by the analysis of samples with known concentrations and by  
301 establishing the minimum level at which the analyte can be reliably resolved and detected or  
302 quantified.303 **4.2.2.3 Based on Accuracy and Precision at lower range limits**304 Instead of using estimated values as described in the previous approaches, the QL can be  
305 directly validated by accuracy and precision measurements.306 **4.2.2.4 Recommended Data**307 The DL and the approach used for its determination should be presented. If the DL is  
308 determined based on visual evaluation or based on signal to noise ratio, the presentation of the  
309 relevant data is considered an acceptable justification.

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

313 Also, the QL and the approach used for its determination should be presented.

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

318 For impurity tests, the quantitation limit for the analytical procedure should be equal to or  
319 below the reporting threshold.

### 320 **4.3 Accuracy and Precision**

321 Accuracy and precision can be evaluated independently, each with a predefined acceptance  
322 criterion. Combining these performance characteristics is an alternative approach for  
323 evaluation of analytical procedure suitability described in this chapter.

#### 324 **4.3.1 Accuracy**

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

329 Accuracy is typically verified through one of the studies described below. In certain cases (*e.g.*,  
330 small molecule drug substance assay), accuracy can be inferred once precision, response within  
331 the working range and specificity have been established.

##### 332 **4.3.1.1 Reference material comparison**

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

##### 336 **4.3.1.2 Spiking Study**

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

##### 341 **4.3.1.3 Orthogonal Procedure comparison**

342 The results of the proposed analytical procedure are compared with those of a second well-  
343 characterized procedure that ideally applies a different measurement principle (independent

344 procedure, see 1.2.). The accuracy of this second procedure should be reported. Orthogonal  
345 procedures can be used with quantitative impurity measurements to verify primary  
346 measurement values in cases where obtaining samples of all relevant components needed to  
347 mimic the matrix for spike recovery studies is not possible.

#### 348 **4.3.1.4 Recommended Data**

349 Accuracy should be assessed using an appropriate number of determinations and concentration  
350 levels covering the reportable range (e.g., 3 concentrations/3 replicates each of the full  
351 analytical procedure).

352 Accuracy should be reported as the mean percent recovery by the assay of a known added  
353 amount of analyte in the sample or as the difference between the mean and the accepted true  
354 value together with the confidence intervals.

355 An appropriate confidence interval (e.g., 95%) for the mean percent recovery or the difference  
356 between the mean and accepted true value (as appropriate) should be compared to the  
357 acceptance criterion to evaluate analytical procedure bias. The appropriateness of the  
358 confidence interval should be justified.

359 For assay, the confidence intervals found should be compatible with the corresponding assay  
360 specification.

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

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

#### 369 **4.3.2 Precision**

370 Validation of tests for assay and for quantitative determination of impurities or purity includes  
371 an investigation of precision.

372 Precision should be investigated using homogeneous, authentic samples or artificially prepared  
373 samples (e.g., matrix mixtures spiked with relevant amounts of the analyte in question). If a  
374 homogeneous sample is not available, then artificially prepared samples or a sample solution  
375 can be used.

##### 376 **4.3.2.1 Repeatability**

377 *Repeatability* should be assessed using:

378 a) a minimum of 9 determinations covering the reportable range for the procedure (e.g.,  
379 3 concentrations/3 replicates each);

380 or

381 b) a minimum of 6 determinations at 100% of the test concentration.

#### 382 **4.3.2.2 Intermediate Precision**

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

#### 390 **4.3.2.3 Reproducibility**

391 *Reproducibility* is assessed by means of an inter-laboratory trial. Investigation of  
392 reproducibility is usually not required for regulatory submission but should be considered in  
393 cases of standardization of an analytical procedure, for instance, for inclusion of procedures in  
394 pharmacopoeias.

#### 395 **4.3.2.4 Recommended Data**

396 The standard deviation, relative standard deviation (coefficient of variation) and confidence  
397 interval should be reported for each type of precision investigated and be compatible with the  
398 specification limits.

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

#### 401 **4.3.3 Combined approaches for accuracy and precision**

402 An alternative to separate evaluation of accuracy and precision is to consider their total impact  
403 by assessing against a combined performance criterion. The approach should be reflective of  
404 the individual criteria that would have been established for accuracy and precision.

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

407 Combined accuracy and precision can be evaluated by use of a prediction interval (to assess  
408 the probability that the next reportable value falls within the acceptable range) or a tolerance  
409 interval (to assess the proportion of all future reportable values that will fall within the  
410 acceptable range). Other approaches may be acceptable if justified.

411 **4.3.3.1 Recommended Data**

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

416 **4.4 Robustness**

417 The evaluation of the analytical procedure's suitability within the intended operational  
418 environment should be considered during the development phase and depends on the type of  
419 procedure under study. Robustness testing should show the reliability of an analytical  
420 procedure with respect to deliberate variations in parameters. The robustness evaluation can be  
421 submitted as part of development data for an analytical procedure on a case-by-case basis or  
422 should be made available upon request.

423 For further details, see ICH Q14.

424

**425 5 GLOSSARY****426 ACCURACY**

427 The accuracy of an analytical procedure expresses the closeness of agreement between the  
428 value which is accepted either as a conventional true value or as an accepted reference value  
429 and the value measured. (ICH Q2)

**430 ANALYTICAL PROCEDURE**

431 The analytical procedure refers to the way of performing the analysis. The analytical procedure  
432 description should include in detail the steps necessary to perform each analytical test. (ICH  
433 Q2)

**434 ANALYTICAL PROCEDURE ATTRIBUTE**

435 A technology specific property that should be within an appropriate limit, range or distribution  
436 to ensure the desired quality of the measured result. For example, attributes for chromatography  
437 measurements may include peak symmetry factor and resolution. (ICH Q14)

**438 ANALYTICAL PROCEDURE CONTROL STRATEGY**

439 A planned set of controls derived from current analytical procedure understanding that ensures  
440 the analytical procedure performance and the quality of the measured result. (ICH Q14)

**441 ANALYTICAL PROCEDURE PARAMETER**

442 Any factor (including reagent quality) or analytical procedure operational step that can be  
443 varied continuously (e.g., flow rate) or specified at controllable, unique levels. (ICH Q14)

**444 ANALYTICAL PROCEDURE VALIDATION STRATEGY**

445 An analytical procedure validation strategy describes how to select the analytical procedure  
446 performance characteristics for validation. In the strategy, data gathered during development  
447 studies (e.g., using *MODR* or *PAR*) and system suitability tests (SSTs) can be applied to  
448 validation and an experimental scheme for future movements of parameters within an  
449 *MODR/PAR* can be predefined. (ICH Q14)

**450 ANALYTICAL TARGET PROFILE (ATP)**

451 A prospective summary of the performance characteristics describing the intended purpose and  
452 the anticipated performance criteria of an analytical measurement. (ICH Q14)

**453 CALIBRATION MODEL**

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

456



457 **CONTROL STRATEGY**

458 A planned set of controls, derived from current product and process understanding, that assures  
459 process performance and product quality. The controls can include parameters and attributes  
460 related to drug substance and drug product materials and components, facility and equipment  
461 operating conditions, in-process controls, finished product specifications, and the associated  
462 methods and frequency of monitoring and control. (ICH Q10)

463 **CO-VALIDATION**

464 Demonstration that the analytical procedure meets its predefined performance criteria when  
465 used at different laboratories for the same intended purpose. Co-validation can involve all (full  
466 revalidation) or a subset (partial revalidation) of performance characteristics potentially  
467 impacted by the change in laboratories. (ICH Q2)

468 **CRITICAL QUALITY ATTRIBUTE (CQA)**

469 A physical, chemical, biological or microbiological property or characteristic that should be  
470 within an appropriate limit, range, or distribution to ensure the desired product quality. (ICH  
471 Q8)

472 **CROSS-VALIDATION**

473 Demonstration that two or more analytical procedures meet the same predefined performance  
474 criteria and can therefore be used for the same intended purpose. (ICH Q2)

475 **DETECTION LIMIT**

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

478 **DETERMINATION**

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

481 **ESTABLISHED CONDITIONS (ECs)**

482 ECs are legally binding information considered necessary to assure product quality. As a  
483 consequence, any change to ECs necessitates a submission to the regulatory authority. (ICH  
484 Q12)

485 **INTERMEDIATE PRECISION**

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

489 **KNOWLEDGE MANAGEMENT**

490 A systematic approach to acquiring, analysing, storing and disseminating information related  
491 to products, manufacturing processes and components. (ICH Q10)

492 **METHOD OPERABLE DESIGN REGION (MODR)**

493 A combination of analytical procedure parameter ranges within which the analytical procedure  
494 performance criteria are fulfilled and the quality of the measured result is assured. (ICH Q14)

495 **ONGOING MONITORING**

496 The collection and evaluation of analytical procedure performance data to ensure the quality  
497 of measured results throughout the analytical procedure lifecycle. (ICH Q14)

498 **PERFORMANCE CHARACTERISTIC**

499 A technology independent description of a characteristic to ensure the quality of the measured  
500 result. Typically, accuracy, precision, specificity/selectivity and range may be considered. The  
501 term was previously called VALIDATION CHARACTERISTIC. (ICH Q2)

502 **PERFORMANCE CRITERION**

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

505 **PLATFORM ANALYTICAL PROCEDURE**

506 A platform analytical procedure can be defined as a multi-product method suitable to test  
507 quality attributes of different products without significant change to its operational conditions,  
508 system suitability and reporting structure. This type of method would apply to molecules that  
509 are sufficiently alike with respect to the attributes that the platform method is intended to  
510 measure. (ICH Q2)

511 **PRECISION**

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

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

518 **PROVEN ACCEPTABLE RANGE FOR ANALYTICAL PROCEDURES (PAR)**

519 A characterised range of an analytical procedure parameter for which operation within this  
520 range, while keeping other parameters constant, will result in an analytical measurement  
521 meeting relevant performance criteria. (ICH Q14)

522 **QUALITY RISK MANAGEMENT**

523 A systematic process for the assessment, control, communication and review of risks to the  
524 quality of the drug (medicinal) product across the product lifecycle. (ICH Q9)

525 **QUANTITATION LIMIT**

526 The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively  
527 determined with suitable precision and accuracy. The quantitation limit for an analytical  
528 procedure should not be more than the reporting threshold. The quantitation limit is a parameter  
529 used for quantitative assays for low levels of compounds in sample matrices, and, particularly,  
530 is used for the determination of impurities and/or degradation products. (ICH Q2)

531 **RANGE**

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

535 **REPORTABLE RANGE**

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

539 **WORKING RANGE**

540 The working range of an analytical procedure is the lowest and the highest concentration  
541 that the analytical procedure provides meaningful results. Working ranges may be  
542 different before sample preparation (sample working range) and when presented to the  
543 analytical instrument (instrument working range). (ICH Q2)

544 **REAL TIME RELEASE TESTING (RTRT)**

545 The ability to evaluate and ensure the quality of the in-process and/or final product based on  
546 process data, which typically include a valid combination of measured material attributes and  
547 process controls. (ICH Q8)

548 **REPEATABILITY**

549 Repeatability expresses the precision under the same operating conditions over a short interval  
550 of time. Repeatability is also termed intra-assay precision. (ICH Q2)

551 **REPORTABLE RESULT**

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

554 **REPRODUCIBILITY**

555 Reproducibility expresses the precision between laboratories (e.g., inter-laboratory studies,  
556 usually applied to standardization of methodology). (ICH Q2)

557 **RESPONSE**

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

561 **REVALIDATION**

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

565 **ROBUSTNESS**

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

569 **SAMPLE SUITABILITY ASSESSMENT**

570 A sample or sample preparation is considered suitable if the measurement response on the  
571 sample satisfies pre-defined acceptance criteria for the analytical procedure attributes that have  
572 been developed for the validated analytical procedure. Sample suitability is a pre-requisite for  
573 the validity of the result along with a satisfactory outcome of the system suitability test. Sample  
574 suitability generally consists of the assessment of the similarity of the response between a  
575 standard and the test sample and may include a requirement of no interfering signals arising  
576 from the sample matrix. (ICH Q14)

577 **SPECIFICITY/SELECTIVITY**

578 Specificity and selectivity are both terms to describe the extent to which other substances  
579 interfere with the determination of a substance according to a given analytical procedure. Such  
580 other substances might include impurities, degradation products, related substances, matrix or  
581 other components present in the operating environment. Specificity is typically used to describe  
582 the ultimate state, measuring unequivocally a desired analyte. Selectivity is a relative term to  
583 describe to which extent particular analytes in mixtures or matrices can be measured without  
584 interferences from other components of similar behaviour. (ICH Q2)

585 **SYSTEM SUITABILITY TEST (SST)**

586 These tests are developed and used to verify that the measurement system and the analytical  
587 operations associated with the analytical procedure are adequate for the intended analysis and  
588 increase the detectability of potential failures (ICH Q14)

589

590 **TOTAL ANALYTICAL ERROR**

591 Total analytical error (TAE) represents the overall error in a test result that is attributed to  
592 imprecision and inaccuracy. TAE is the combination of both, systematic error of the procedure  
593 and random measurement error. (ICH Q14)

594 **VALIDATION STUDY**

595 An evaluation of prior knowledge, data or deliberate experiments to determine the suitability  
596 of an analytical procedure for its intended purpose. (ICH Q2)

597 **VALIDATION TEST**

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

600 **MULTIVARIATE GLOSSARY**

601 **CALIBRATION DATA SET**

602 A set of data with matched known characteristics and measured analytical results, that spans  
603 the desired operational range. (ICH Q2)

604 **DATA TRANSFORMATION**

605 Mathematical operation on model input data to assume better correlation with the output data  
606 and simplify the model structure. (ICH Q14)

607 **INDEPENDENT SAMPLE**

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

611 **INTERNAL TESTING**

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

614 Internal testing serves as means to establish the optimal number of latent variables, estimate  
615 the standard error and detect potential outliers. Internal testing is preferably done by using  
616 samples not included in the calibration set. Alternatively, internal testing can be done using a  
617 subset of calibration samples, while temporarily excluding them from the model calculation.  
618 (ICH Q2)

619 **INTERNAL TEST SET**

620 A set of data obtained from samples that have physical and chemical characteristics that span  
621 a range of variabilities similar to the samples used to construct the calibration set. (ICH Q14)

622 **LATENT VARIABLES**

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

625 **MODEL VALIDATION**

626 The process of determining the suitability of a model by challenging it with independent test  
627 data and comparing the results against prespecified criteria. For quantitative models, validation  
628 involves confirming the calibration model's performance with an independent dataset. For  
629 identification libraries, validation involves analysing samples (a.k.a., challenge samples) not  
630 represented in the library to demonstrate the discriminative ability of the library model. (ICH  
631 Q2)

632 **MODEL MAINTENANCE**

633 Safeguards over the lifecycle of a multivariate model to ensure continued model performance,  
634 often including outlier diagnostics and resulting actions for model redevelopment or change in  
635 the maintenance plans. (ICH Q14)

636 **MULTIVARIATE ANALYTICAL PROCEDURE**

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

639 **OUTLIER DIAGNOSTIC**

640 Tests that can identify unusual or atypical data in a multivariate analytical procedure. (ICH  
641 Q14)

642 **REFERENCE PROCEDURE**

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

645 **REFERENCE SAMPLE**

646 A sample representative of the test sample with a known value for the property of interest, used  
647 for calibration. (ICH Q14)

648 **VALIDATION SET**

649 A set of data used to give an independent assessment of the performance of the calibration  
650 model, ideally over a similar operating range. (ICH Q14)

651

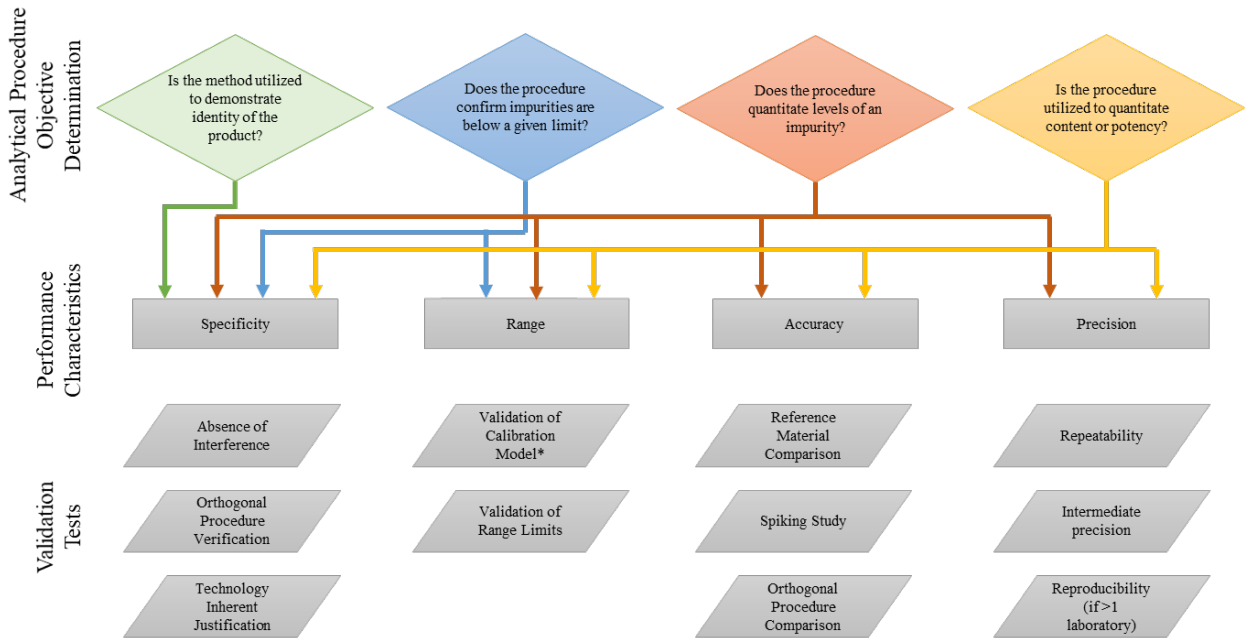
652 **6 References**

653 ICH Q14 Analytical Procedure Development

654

655 7 ANNEX 1 SELECTION OF VALIDATION TESTS

656 Figure 2: Selection of validation tests based on the objective of the analytical procedure  
 657



\* May not be needed for limit test

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659

660 **8 ANNEX 2 ILLUSTRATIVE EXAMPLES FOR ANALYTICAL TECHNIQUES**661 **Table 3: Examples for Quantitative separation techniques**

Technique	Separation techniques (HPLC, GC, CE) for impurities or assay	Separation techniques with Relative Area Quantitation, e.g., product-related substances such as charge variants
<b>Performance characteristic</b>	<b>Validation study methodology</b>	
Specificity / Selectivity	<p><u>Absence of relevant interference:</u> With DS, DP, buffer, or appropriate matrix, and between individual peaks of interest</p> <p>Spiking with known impurities / excipients</p> <p>or</p> <p>By comparison of impurity profiles by a secondary method</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples, if necessary.</p>	<p><u>Absence of relevant interference:</u> With DS, DP, buffer, or appropriate matrix, and between individual peaks of interest</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary.</p>
Precision	<p><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</p> <p><u>Intermediate precision:</u> Across e.g., days, environmental conditions, analysts, equipment</p>	
Accuracy	<p>For Assay: Comparison with suitably characterized material (e.g., standard)</p> <p>or</p> <p>Comparison with well-defined secondary procedure</p> <p>For impurities or related substances: Spiking/Recovery experiments with impurities</p> <p>Comparison of impurity profiles with well-defined secondary procedure</p>	<p>Comparison with well-defined secondary procedure and/or well-defined material (e.g., reference materials)</p> <p>and/or, accuracy can be inferred once precision, linearity and specificity have been established.</p> <p>and/or if needed, Spike/Recovery experiments with forced degradation samples and/or well-defined material</p>



ICH Q2(R2) Guideline

Technique	Separation techniques (HPLC, GC, CE) for impurities or assay	Separation techniques with Relative Area Quantitation, e.g., product-related substances such as charge variants
<b>Performance characteristic</b>	<b>Validation study methodology</b>	
Reportable Range	<p>Validation of calibration model across the range:</p> <p>Linearity: Dilution of the analytes of interest over the expected procedure range, at least 5 points</p> <p>Validation of lower range limits (for purity only): QL, DL through one selected methodology, e.g., signal-to-noise determination</p>	<p>Validation of calibration model across the range:</p> <p>Linearity: between measured (observed) relative result <i>versus</i> theoretically expected relative result across specification range(s); e.g., by spiking or degrading material</p> <p>Validation of lower range limits: QL (and DL) through selected methodology from Section 5.2 (e.g., signal-to-noise determination).</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p>Deliberate variation of parameters and stability of test conditions, e.g., Deliberate variations of test and sample preparation conditions, for example mobile phase, separation buffer, carrier gas composition and pH, columns, capillaries, temperature, extraction time, Stability of SST, test and reference solutions</p>	

662  
663

664 **Table 4: Example for Elemental Impurities by ICP-OES or ICP-MS as purity test**  
 665

Technique	Elemental Impurities by ICP-OES or ICP-MS as purity test
<b>Performance characteristic</b>	<b>Validation study methodology</b>
Specificity / Selectivity	<p><u>Spiking experiments</u> of elements into matrix and demonstration of sufficient non-interference and verification of accuracy/recovery:</p> <p>with the presence of components (<i>e.g.</i>, carrier gas, impurities, matrix)</p> <p>or <u>justification through technology/prior knowledge</u> (<i>e.g.</i>, specificity of technology for certain isotopes)</p>
Precision	<p><u>Repeatability:</u> Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering signals of interest</p> <p><u>Intermediate precision:</u> <i>e.g.</i>, across days, environmental conditions, analysts, equipment</p>
Accuracy	<p>Spiking/Recovery experiments with impurities</p> <p>or</p> <p>Comparison of impurity profiles with well-defined secondary procedure</p>
Reportable Range	<p><u>Validation of working range:</u></p> <p>Linearity: Dilution of the analytes of interest over the expected procedure range, at least 5 points, can be combined with multi-level accuracy experiment</p> <p><u>Validation of lower range (for impurities only):</u> QL, DL through one selected methodology</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p>Deliberate variation of parameters and stability of test conditions: Sample digestion technique and preparation, nebulizer and sheath flow settings, plasma settings</p>

666

667

668 **Table 5: Example for Dissolution with HPLC as product performance test for an**  
 669 **immediate release dosage form**

Technique	Dissolution with HPLC as product performance test for an immediate release dosage form	
Performance characteristic	<b>Demonstration of performance of dissolution step</b> <i>Typically demonstrated with development data</i>	<b>Validation testing methodology</b> <i>Typically demonstrated with final procedure</i>
Specificity/Selectivity	<u>Discriminatory power:</u> Demonstration of sufficiently different dissolution of acceptable versus non-acceptable batches	<u>Absence of interference</u> Demonstration of non-interference with excipients and dissolution media likely to impact the quantification of the main analyte
Precision	<u>Precision and intermediate precision:</u> Repeated dissolution experiments of a well-characterized product batch representative for the manufacturing process. <i>Note: The study will allow a combined assessment of product and analytical variations</i>	<u>Precision and Intermediate Precision:</u> 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 solubilized
Accuracy	(Not applicable for dissolution step)	<u>Spiking Study:</u> Add known amounts of the drug reference substance to the dissolution vessel containing excipient mixture in dissolution media and calculate recovery within defined working range.
Reportable Range	(Not applicable for dissolution step)	<u>Validation of calibration model across the range</u> <u>Linearity:</u> Demonstrate linearity from sample concentrations (as presented to quantitative measurement) in the range of Q-45% up to 120% of the content stated on the label, for immediate-release solid dosage forms.  <i>If lower concentration ranges are close to QL:</i> <u>Validation of lower range limits, see separation techniques</u>
Robustness (done as part of analytical procedure development as per Q14)	<u>Justification of the selection of the dissolution procedure parameters,</u> e.g., medium composition buffer or surfactant concentration, use of sinkers, pH, deaeration, volume, agitation rate, sampling time	<u>Deliberate variation of parameters of the quantitative procedure, see separation technique</u>

670 **Table 6: Example for Quantitative <sup>1</sup>H-NMR for the Assay of an API**

Technique	Quantitative <sup>1</sup> H-NMR (internal standard method) for the Assay of an API
<b>Performance characteristic</b>	<b>Validation testing methodology</b>
Specificity / Selectivity	<u>Absence of interference:</u> Identify a signal which is representative for the analyte and does not show interference with potential baseline artefacts, residual water or solvent signals, related structure impurities or other impurities, internal standards, non-target major component or potential isomers/forms.
Precision	<u>Repeatability:</u> Replicate measurements of at least 6 independent preparations at 100% level  <u>Intermediate Precision:</u> Not necessary to be conducted on target analyte (justified by technology principle, as typically verified through instrument calibration with a standard sample)
Accuracy	<u>Reference material comparison</u> verify with sample of known purity
Reportable Range	<u>Technology inherent justification:</u> Not necessary as the integral areas are directly proportional to the amount (mole) of reference standard and analyte.
Robustness (performed as part of analytical procedure development as per Q14)	<u>Deliberate variation of parameters, e.g.,</u> Temperature, Concentration, Field (shim), Tuning and Matching of the NMR probe  <u>Stability over the use period</u> of the test, e.g., solution stability

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672

673 **Table 7: Example for Biological Assays**

Technique	<b>Binding assay (e.g., ELISA, SPR) or Cell-based assay for determination of potency relative to a reference</b>
Performance characteristic	<b>Validation testing methodology</b>
Specificity / Selectivity	<p><u>Absence of interference:</u> Dose-response curve fulfils the response criteria demonstrating the similarity of the analyte and reference material, as well as non-interfering signal from the matrix, no dose-response from the cell line alone</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary.</p>
Precision	<p><u>Repeatability:</u> Repeated sample analysis on a single day or within a short interval of time covering the response range of the method (NLT 3 replicates at NLT 5 levels)</p> <p><u>Intermediate Precision:</u> Different analysts, Multiple independent preparations over multiple days at multiple potency levels through the method's range, inclusive of normal laboratory variation</p>
Accuracy	<p><u>Reference material comparison:</u> Assess recovery versus theoretical activity for multiple (NLT 3) independent preparations at multiple (NLT 5) levels through the method's range</p>
Reportable Range	<p><u>Validation of lower and higher range limits:</u> The lowest to highest relative potency levels that meet accuracy, precision, and response criteria, determined as NLT 5 mean potency levels</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p><u>Deliberate variation of parameters, e.g.,</u> Reagent lots (e.g., Capture/detection antibody, coating proteins, controls) Cell density, effector/target cell ratio, cell generation number Plate type Buffer components Incubation times Incubation conditions Instruments Reaction times Impact of sample degradation</p>

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676 **Table 8: Example for quantitative PCR**

Technique	Quantitative PCR (quantitative analysis of impurities in drug substances or products)
Performance characteristic	Validation testing methodology
Specificity / Selectivity	<p><u>Orthogonal Procedure Comparison:</u> Test reaction specificity by electrophoresis gel, melting profile or DNA sequencing</p> <p><u>Absence of interference:</u></p> <ul style="list-style-type: none"> <li>- Positive template, no-reverse transcription control for RT-qPCR and no template control</li> <li>- Test probe target specificity against gene bank (nucleotide blast).</li> <li>- Evaluate the slope of standard curve for efficiency</li> </ul>
Precision	<p><u>Repeatability:</u> With n=6 replicates and calculation of inter-run variance: slopes, coefficient of variation (CV) and y-intercepts are compared using the criteria of 2 standard deviations for the set of curves, if justified.</p> <p><u>Intermediate precision</u> Comparison of measurements using the same procedure performed by another analyst on a different day.</p>
Accuracy	<p><u>Spiking Study:</u> 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</p>
Reportable Range	<p><u>Linearity:</u> Linear 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 linear dynamic range.</p> <p><u>Validation of lower working range limits based on the calibration Curve:</u></p> <p>DL defined by template spiking in samples or from standard curves DL is lowest point meeting the selected curve parameters, e.g., coefficient of determination (<math>R^2</math>), efficiency, 1st order polynomial fit and a standard deviation of the kurtosis distribution</p> <p>QL demonstrated through demonstrating sufficient recovery and acceptable coefficient of variations from the accuracy experiment</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p><u>Deliberate variation of parameters, e.g.,</u></p> <ul style="list-style-type: none"> <li>Equipment</li> <li>Master mix composition (concentrations of salts, dNTPs, adjuvants)</li> <li>Master mix lots</li> <li>Reaction volume</li> <li>Probe and primer concentrations</li> <li>Thermal cycling parameters</li> </ul>

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678 **Table 9: Example for particle size measurement**

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Technique	Particle size measurement (Dynamic light scattering; Laser diffraction measurement) as property test
Performance characteristic	Validation testing methodology
Specificity / Selectivity	<u>Absence of interference:</u>  If needed, evaluate blank and sample to determine the appropriateness of the equipment settings and sample preparation
Precision	<u>Repeatability:</u> test at least n=6 replicates at established analytical procedure parameters at target range.  <u>Intermediate precision:</u> analysis performed on different days, environmental conditions, analysts, equipment setup
Accuracy	<u>Technology inherent justification:</u> confirmed by an appropriate instrument qualification  Or  <u>Alternative option: Orthogonal Procedure comparison:</u> If needed, qualitative comparison using a different technique, like optical microscopy, to confirm results
Reportable Range	Technology specific justification, e.g., particle size range covered
Robustness (performed as part of analytical procedure development as per Q14)	<u>Deliberate variation of parameters, e.g.,</u> Evaluation of expected size ranges of the intended use of the analytical procedure. 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, if applicable Ultrasound percentage, if applicable.

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682 **Table 10: NIR**  
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Technique	NIR method validation example for core tablet assay
<b>Performance characteristic</b>	<b>Validation testing methodology</b>
Specificity / Selectivity	<p><u>Absence of interference:</u></p> <p>Comparison of API spectrum and the loadings plots of the model</p> <p>Rejection of outliers (e.g., excipient, analogues) not covered by the multivariate procedure</p>
Precision	<p><u>Repeatability:</u></p> <p>Repeated analysis with removal of sample from the holder between measurements.</p>
Accuracy	<p><u>Comparison with well-defined secondary procedure:</u></p> <p>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).</p>
Reportable Range	<p><u>Linearity:</u></p> <p>Demonstration of the linear relationship between predicted and reference values.</p> <p><u>Error (accuracy) across the range:</u></p> <p>Information on how the method error (accuracy) changes across the calibration range, e.g., by plotting the residuals of the model prediction vs. the actual data.</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p><u>Robustness</u></p> <p>Chemical and physical factors that can impact NIR spectrum and model prediction should be represented in data sets. Examples include various sources of API and excipients, water content, tablet hardness, and orientation in the holder.</p> <p><i>Note: NIR measurements are sensitive to changes in tablets composition and properties outside variation present in the calibration set.</i></p>

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686 Table 11: Example for Quantitative LC/MS

Technique	Quantitative LC/MS (quantitative analysis of impurities (e.g., genotoxic impurities) in drug substances or products)
Performance characteristic	Validation testing methodology
Specificity / Selectivity	<p><u>Technology inherent justification:</u> 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</p> <p><u>Absence of interference:</u> from other components in sample matrix.</p> <p><u>Orthogonal procedure comparison:</u> By comparison of impurity profiles determined by an alternative validated method</p>
Precision	<p><u>Repeatability</u> Measurement of at least three replicates at each of at least three spiking levels</p> <p><u>Intermediate precision</u> 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). Comparison of measurements of the same samples made in different laboratories</p>
Accuracy	<p><u>Spiking Study</u> Acceptable recovery of spiked impurity standards in sample matrix at multiple spiking levels</p> <p>Or:</p> <p><u>Comparison with well-defined secondary procedure:</u> Comparison of the measurement results to the 'true' values obtained from alternative validated procedures</p>
Reportable Range	<p><u>Validation of calibration model across the range:</u></p> <p><u>Linearity:</u> 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</p> <p><u>Validation of lower range limits:</u> DL: Use the measured signal to noise of the spiking level with coefficient of variation (CV) or calculated relative standard deviation (RSD or %RSD)</p>

ICH Q2(R2) Guideline

<b>Technique</b>	<b>Quantitative LC/MS (quantitative analysis of impurities (e.g., genotoxic impurities) in drug substances or products)</b>
<b>Performance characteristic</b>	<b>Validation testing methodology</b>
	<p>of responses (with 6 or more repeated injections) less than pre-defined acceptable value.</p> <p>QL: The lowest spiking level with acceptable accuracy and precision.</p> <p>The analytical procedure range extends from and inclusive of the LOQ to the highest spiking level with acceptable accuracy, precision, and linearity</p>
Robustness (performed as part of analytical procedure development as per Q14)	<p><u>Deliberate variation of parameters and stability of test conditions:</u> The following factors should be considered during assessment of analytical procedure performance: LC flow rate, LC injection volume, MS drying/ desolvation temperature, MS gas flow, mass accuracy and MS collision energy.</p>

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