

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

**ICH HARMONISED GUIDELINE**

**ANALYTICAL PROCEDURE DEVELOPMENT**  
**Q14**

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.*

**Q14**  
**Document History**

<b>Code</b>	<b>History</b>	<b>Date</b>
Q14	Endorsement by the Members of the ICH Assembly under <i>Step 2</i> and release for public consultation.	24 March 2022

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**ANALYTICAL PROCEDURE DEVELOPMENT**

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**ICH Consensus Guideline**

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1 **1. INTRODUCTION**

2 **1.1 Objective of the Guideline**

3 This guideline describes science and risk-based approaches for developing and maintaining *analytical*  
4 *procedures* suitable for the assessment of the quality of drug substances and drug products. The  
5 systematic approach suggested in *ICH Q8 Pharmaceutical Development* together with principles of  
6 *ICH Q9 Quality Risk Management* can also be applied to the development and lifecycle management  
7 of analytical procedures. When developing an analytical procedure, a minimal (also known as  
8 traditional) approach or elements of an enhanced approach can be applied.

9 Furthermore, the guideline describes considerations for the development of *multivariate analytical*  
10 *procedures* and for *real time release testing (RTRT)*.

11 This guideline is intended to complement *ICH Q2 Validation of Analytical Procedures*. Submitting  
12 knowledge and information related to development of analytical procedures to regulatory agencies  
13 may provide additional evidence to demonstrate that the analytical procedure is appropriate for its  
14 intended purpose.

15 Using the tools described in *ICH Q12 Technical and Regulatory Considerations for Pharmaceutical*  
16 *Product Lifecycle Management*, the guideline describes principles to support change management of  
17 analytical procedures based on risk management, comprehensive understanding of the analytical  
18 procedure and adherence to predefined criteria for *performance characteristics*. Knowledge gained  
19 from application of an enhanced approach to analytical procedure development can provide better  
20 assurance of the performance of the procedure, can serve as a basis for the *analytical procedure*  
21 *control strategy* and can provide an opportunity for more efficient regulatory approaches to related  
22 post approval changes.

23 The guideline also describes submission of analytical procedure development and related lifecycle  
24 information in the Common Technical Document (CTD) format (*ICH M4Q, The Common Technical*  
25 *Document for the Registration of Pharmaceuticals for Human Use: Quality – M4Q*).

26

27 **2. SCOPE**

28 This guideline applies to new or revised analytical procedures used for release and stability testing of  
29 commercial drug substances and products (chemical and biological/biotechnological). The guideline  
30 can also be applied to other analytical procedures used as part of the *control strategy (ICH Q10,*  
31 *Pharmaceutical Quality System)* following a risk-based approach. The scientific principles described  
32 in this guideline can be applied in a phase-appropriate manner during clinical development. This  
33 guideline may also be applicable to other types of products, with appropriate regulatory authority  
34 consultation as needed. Development of pharmacopoeial analytical procedures is out of scope.

35

36 **2.1 General Considerations for Analytical Procedure Development and Lifecycle Management**

37 The goal of development is to obtain an analytical procedure fit for its intended purpose: to measure  
38 an attribute or attributes of the analysed material with the needed *specificity/selectivity, accuracy*  
39 *and/or precision* over the *reportable range*.

40 In this section the minimal and enhanced approaches to analytical procedure development are  
41 described. While the minimal approach remains acceptable, some or all elements of the enhanced  
42 approach might be used to support development and lifecycle management of analytical procedures.

43 In certain cases, an established analytical procedure can be applied to multiple products with little or  
44 no modification of measurement conditions. For a new application of such *platform analytical*  
45 *procedures*, the subsequent development can be abbreviated, and certain *validation tests* can be  
46 omitted based on a science- and risk-based justification. Details of the performance characteristics  
47 considered for analytical procedure validation are described in *ICH Q2*.

48 In general, data gained during the development studies (e.g., robustness data from a design of  
49 experiments (DoE study)) can be used as validation data for the related analytical procedure  
50 performance characteristics and does not necessarily need to be repeated.

51

## 52 **2.2 Minimal versus Enhanced Approaches to Analytical Procedure Development**

53

### 54 ***Minimal Approach***

55 Analytical procedure development should include the following elements as appropriate:

- 56 • Identifying which attributes of the drug substance or drug product need to be tested by the  
57 analytical procedure.
- 58 • Selecting an appropriate analytical procedure technology and related instruments or suitable  
59 apparatus.
- 60 • Conducting appropriate development studies to evaluate analytical procedure performance  
61 characteristics such as specificity, accuracy and precision over the reportable range (including  
62 the *calibration model*, limits at lower and/or higher range ends) and *robustness*.
- 63 • Defining an appropriate analytical procedure description including the analytical procedure  
64 control strategy (e.g., parameter settings and system suitability).

65

### 66 ***Enhanced Approach***

67 The enhanced approach offers a systematic way of developing and refining knowledge of an  
68 analytical procedure. An enhanced approach should include one or more of the following elements in  
69 addition to those already described for the minimal approach:

- 70 • An evaluation of the sample properties and the expected variability of the sample based on  
71 manufacturing process understanding.
- 72 • Defining the *analytical target profile (ATP)*.
- 73 • Conducting risk assessment and evaluating prior knowledge to identify the *analytical*  
74 *procedure parameters* that can impact performance of the procedure.
- 75 • Conducting uni- or multi-variate experiments to explore ranges and interactions between  
76 identified analytical procedure parameters.
- 77 • Defining an analytical procedure control strategy based on enhanced procedure understanding  
78 including appropriate set-points and/or ranges for relevant analytical procedure parameters  
79 ensuring adherence to *performance criteria*.
- 80 • Defining a lifecycle change management plan with clear definitions and reporting categories  
81 of *established conditions (ECs)*, *proven acceptable ranges (PARs)* or *method operational*  
82 *design regions (MODRs)* as appropriate.

83

84 Applying elements of the enhanced approach to development can lead to more robust analytical  
85 procedures, better understanding of the impact of analytical procedure parameters and more flexibility  
86 for lifecycle management such as wider operating ranges, a more appropriate set of ECs and  
87 associated reporting categories for changes.

88



116 An ATP consists of a description of the intended purpose, appropriate details on the product attributes  
117 to be measured and relevant performance characteristics with associated *performance criteria*. The  
118 ATP includes the performance requirements for a single attribute or a set of quality attributes. The  
119 ATP drives the choice of analytical technology. Multiple available analytical techniques may meet  
120 the performance requirements. Consideration of the operating environment (e.g., at-line, in-line or  
121 off-line) should be included in the technology selection. Once a technology has been selected, the  
122 ATP serves as a foundation to derive the appropriate analytical procedure attributes and acceptance  
123 criteria for analytical procedure validation (*ICH Q2*). Formal documentation and submission of an  
124 ATP is optional but can facilitate regulatory communication irrespective of the chosen development  
125 approach.

126 The ATP facilitates the selection of the technology, the procedure design and development as well as  
127 the subsequent performance monitoring and continual improvement of the analytical procedure. The  
128 ATP is maintained over the lifecycle and can also be used as a basis for lifecycle management to  
129 ensure that the analytical procedure remains suitable for the intended use.

130 Illustrative examples of ATPs are provided in Annex A.

131

## 132 **4. KNOWLEDGE AND RISK MANAGEMENT IN ANALYTICAL PROCEDURE** 133 **DEVELOPMENT AND CONTINUAL IMPROVEMENT**

### 134 **4.1 Knowledge Management**

135 As with product and manufacturing process development (*ICH Q10*), *knowledge management* plays  
136 a critical role in analytical procedure development and during the subsequent lifecycle of the  
137 analytical procedure.

138 Prior knowledge is explicitly or implicitly used for informing decisions during analytical procedure  
139 development and lifecycle management. Prior knowledge can be internal knowledge from a  
140 company's proprietary development and analytical experience, external knowledge such as reference  
141 to scientific and technical publications or established scientific principles.

142 Prior product knowledge plays an important role in identifying the appropriate analytical technique.  
143 Knowledge of best practices and current state-of-the-art technologies as well as current regulatory  
144 expectations contributes to the selection of the most suitable technology for a given purpose. Existing  
145 platform analytical procedures (e.g., protein content determination by UV spectroscopy for a protein  
146 drug) can be leveraged to evaluate the attributes of a specific product without conducting additional  
147 procedure development.

148 As additional information is obtained, knowledge related to analytical procedures should be actively  
149 managed throughout the product lifecycle.

150

### 151 **4.2 Risk Management**

152 The use of quality risk management is encouraged to aid in the development of a robust analytical  
153 procedure to reduce risk of poor performance and reporting incorrect results. Risk assessment is  
154 typically performed early in analytical procedure development and is repeated as more information  
155 becomes available. Risk assessment can be formal or informal and can be supported by prior  
156 knowledge.

157 Risk assessment tools as described in ICH Q9 Annex 1 can be used to

- 158 • identify analytical procedure parameters (factors and operational steps) with potential impact  
159 on its performance, e.g., Annex A Figures 1 and 2 (Ishikawa diagrams).
- 160 • assess the potential impact of analytical procedure parameters on the analytical procedure  
161 performance.
- 162 • identify and prioritise analytical parameters to be investigated experimentally.  
163

164 Risk control principles can be used to establish an analytical procedure control strategy. To maintain  
165 a state of control for analytical procedure performance, *ongoing monitoring* is recommended as part  
166 of risk review.

167 Risk communication should be used to support continual improvement of the analytical procedure  
168 performance throughout its lifecycle. The outcome of quality risk management should be documented  
169 within the applicant's pharmaceutical quality system (PQS).

170

## 171 **5. EVALUATION OF ROBUSTNESS AND PARAMETER RANGES OF ANALYTICAL** 172 **PROCEDURES**

### 173 **5.1 Robustness**

174 The *robustness* of an analytical procedure is a measure of its capacity to meet the expected  
175 performance requirements during normal use. Robustness is tested by deliberate variations of  
176 analytical procedure parameters. Prior knowledge and risk assessment can inform the selection of  
177 parameters to investigate during the robustness study. Those parameters likely to influence procedure  
178 performance over the intended period of use should be studied.

179

180 For most procedures, robustness evaluation is conducted during development. If the evaluation of  
181 robustness was already conducted during development, it does not need to be repeated during  
182 validation as discussed in ICH Q2. Data from validation studies (e.g., intermediate precision) can be  
183 used to complement robustness evaluation. For some analytical procedures with inherent high  
184 parameter variability (e.g., those requiring biological reagents) wider ranges may need to be  
185 investigated during robustness studies. Robustness of multivariate procedures may require additional  
186 considerations (see chapter 8). The outcome of the evaluation of robustness should be reflected in the  
187 analytical procedure control strategy.  
188

### 189 **5.2 Analytical Procedure Parameter Ranges**

190 Experiments to investigate parameter ranges can provide additional knowledge about the analytical  
191 procedure performance. The respective analytical procedure attributes and associated criteria could  
192 be derived from the ATP. Univariate examination of a single parameter can establish proven  
193 acceptable ranges (PAR) for the analytical procedure.

194 In an enhanced approach, the ranges for the relevant parameters and their interactions can be  
195 investigated in multi-variate experiments (DoE). Risk assessment and prior knowledge should be  
196 used to identify parameters, attributes and appropriate associated ranges to be investigated  
197 experimentally. Categorical variables (e.g., different instruments) can also be considered as part of  
198 the experimental design.

199 The outcome of development studies including DoE can provide an understanding of the relationships  
200 between analytical procedure variables (inputs) and the responses of the analytical procedure  
201 (outputs). Based on the results, fixed set-points may be defined for some parameters. For others,  
202 PARs could be defined while still others could be included into an MODR. An MODR consists of

203 combined ranges for two or more variables within which the analytical procedure is shown to be fit  
204 for the intended use.

205 Parameter ranges (e.g., PAR or MODR) can be proposed by the applicant based on development data  
206 and are subject to regulatory approval. Moving within an established parameter range does not require  
207 regulatory notification.

208 For practical reasons and following a risk-based approach, it may not be necessary or possible to  
209 validate the entirety of a MODR. The part of a PAR or a MODR intended for routine use in the  
210 analytical procedure must be covered by validation data. Validation approaches for MODRs are  
211 described in Annex B including an example table to present the performance characteristics combined  
212 with the analytical procedure attribute acceptance criteria, parameter ranges, analytical procedure  
213 control strategy and validation strategy. Analytical procedure validation is required only for those  
214 performance characteristics not covered by data from analytical procedure development. An  
215 *analytical procedure validation strategy*, e.g., as part of the analytical procedure validation protocol,  
216 can define the necessary extent of additional validation.

217

## 218 6. ANALYTICAL PROCEDURE CONTROL STRATEGY

219 An analytical procedure control strategy should ensure that the analytical procedure performs as  
220 expected during routine use throughout its lifecycle and consists of a set of controls, derived from  
221 current understanding of the analytical procedure including development data, risk assessment and  
222 robustness. Prior knowledge could also be used to develop the analytical procedure control strategy.  
223 The analytical procedure control strategy should be defined before validation (ICH Q2) and should  
224 be confirmed after validation has been finalized.

225 The analytical procedure control strategy includes analytical procedure parameters needing control  
226 and the *system suitability test* (SST) which is part of the analytical procedure description. The  
227 analytical procedure description should include the steps necessary to perform each analytical test.  
228 This can include (but is not limited to) the sample, the reference materials and the reagents, sample  
229 and control preparations, use of the apparatus, generation of the calibration curve, use of the formulae  
230 for the calculation of the *reportable results* and other necessary steps. The level of detail should  
231 enable a skilled analyst to perform the analysis and interpret the results (such as the level of detail in  
232 a regional pharmacopoeia for a similar substance).

233 The SST depends on the type and intent of the analytical procedure and is typically conducted with  
234 one or more predefined materials (including use of positive or negative controls). The SST is designed  
235 to verify selected analytical procedure attributes. The acceptance criteria should be based on  
236 analytical procedure performance criteria. The components of the SST should be selected using risk  
237 assessment as well as knowledge and understanding from development data. The test is used to verify  
238 that the measurement system and the analytical operations associated with the analytical procedure  
239 are adequate during the intended time period of analysis and enable the detection of potential failures.  
240 Validity of the results of the analytical procedure depends on the outcome of the SST. In the enhanced  
241 approach, a well-designed set of SST parameters and criteria to ensure method performance could  
242 represent an important aspect of risk mitigation. For analytical procedures relying on multivariate  
243 models, data quality should be verified using appropriate software tools.

244 In addition to SST, *sample suitability assessment* may be required to ensure acceptable sample  
245 response. A sample and/or sample preparation is considered suitable if the measurement response of  
246 the sample satisfies pre-defined acceptance criteria for the analytical procedure attributes that have  
247 been developed for the validated analytical procedure (often used for biologics). In these cases,  
248 sample suitability is a prerequisite for the validity of the result along with a satisfactory outcome of

249 the SST. For analytical procedures relying on multivariate models, sample suitability assessment can  
250 be verified using appropriate software tools which check if the sample fits within the model space.  
251 This is commonly called data quality check.

252

253 Ongoing monitoring of selected analytical procedure outputs is recommended to look for any trends,  
254 in line with PQS expectations. Review of analytical procedure outputs facilitates the procedure  
255 lifecycle management and enables proactive intervention to avoid failures.

256

## 257 **6.1 Established Conditions for Analytical Procedures**

258 In line with ICH Q12, applicants may define established conditions (ECs) for an analytical procedure.  
259 ECs are proposed and justified by the applicant and approved by the regulatory authorities. ECs can  
260 be identified using tools highlighted in Chapter 4 including risk assessment, prior knowledge, and  
261 learnings from uni- and/or multi-variate experimentation. The nature and extent of ECs will depend  
262 on the development approach, the complexity of the analytical procedure and a demonstrated  
263 understanding of how parameters and other factors impact its performance.

264 With a minimal approach to development, the number of ECs may be extensive with fixed analytical  
265 procedure parameters and set points.

266 With an enhanced approach to development, there should be an increased understanding of the  
267 relationship between analytical procedure parameters and performance to facilitate identification of  
268 which factors require control and thus enable a more appropriate set of ECs. These can focus on  
269 performance characteristics (e.g., specificity, accuracy, precision).

270

271 ECs could consist of performance criteria (e.g., in the ATP or as part of SST), the analytical procedure  
272 principle (i.e., the physicochemical basis or specific technology), and set points and/or ranges for one  
273 or more parameters. Analytical procedure parameters which need to be controlled to ensure the  
274 performance of the procedure as well as those where the need for control cannot be reasonably  
275 excluded should be identified as ECs. If a parameter is controlled through performance characteristics  
276 and criteria, that parameter may not necessarily need to be defined as an EC or may be assigned a  
277 lower reporting category.

278 Use of the enhanced approach should not lead to providing a less detailed description of analytical  
279 procedures in a regulatory submission. A suitably detailed description of the analytical procedures in  
280 Module 3 of the CTD is expected to provide a clear understanding regardless of the approach used to  
281 identify ECs for analytical procedures. Description of analytical procedures can include supportive  
282 information as well as identified ECs.

283 Identification of reporting categories for ECs and the utilization of ECs in change management are  
284 described in the next chapter.

285

## 286 **7. LIFECYCLE MANAGEMENT AND POST-APPROVAL CHANGES OF ANALYTICAL** 287 **PROCEDURES**

288 Changes to analytical procedures can occur throughout the product lifecycle and could involve  
289 modification of existing procedures or a complete replacement including introduction of a new  
290 technology. Major changes in the performance characteristics or additional information on attributes  
291 could, in certain instances, lead to reevaluation of the ATP itself and/or a new procedure. Typically,  
292 process knowledge, analytical procedure knowledge and continual improvement are drivers for

293 change. If possible, changes should lead to improved analytical procedures in line with best practices  
294 and instrumentation. The tools and enablers discussed in ICH Q12 are applicable to analytical  
295 procedures, irrespective of the development approach and consist of:

- 296 • Existing risk-based categorisation of changes to analytical procedures (in applicable regional  
297 regulatory framework)
- 298 • ECs
- 299 • Post-Approval Change Management Protocols (PACMPs) which provide a detailed  
300 explanation of how future changes will be managed and provide the marketing authorization  
301 holder (MAH) with certainty about the acceptability of future changes and an associated  
302 reduced reporting category.
- 303 • The Product Lifecycle Change Management (PLCM) document which can facilitate  
304 regulatory communication about likely post-approval changes.
- 305 • The PQS (documentation of all changes including those not requiring regulatory submission,  
306 e.g., within a MODR or for parameters deemed not to have an impact on the method  
307 performance)
- 308 • The structured approach to frequent CMC changes (ICH Q12 Chapter 8).

309  
310 If a minimal approach to development is taken, then any changes should be reported according to  
311 existing regional reporting requirements. The use of different elements of the enhanced approach can  
312 facilitate management and regulatory communication of post-approval changes.

313 If appropriately justified and validated (see Chapter 5.2), a PAR or MODR allows flexibility within  
314 the approved range(s) to be managed within a company's PQS. Changes outside of the approved  
315 ranges or expansion of said ranges require regulatory reporting.

316 In cases where ECs are proposed, the risk associated with prospective changes should be assessed up  
317 front to define the appropriate reporting category. Factors to consider include the importance of the  
318 quality attribute being measured, the complexity of the technology and the extent of the change.  
319 Relevant risk reduction measures should be identified based on product and process knowledge as  
320 well as analytical procedure understanding and the proposed analytical procedure control strategy.  
321 Finally, the level of risk (high, medium or low) should be assigned.

322 In general, an understanding of the analytical procedure robustness and/or prior knowledge can be  
323 used to support risk mitigation associated with future changes. Submitting the outcomes of the risk  
324 assessments to regulatory agencies when ECs are identified can help justify reporting categories for  
325 future changes to analytical procedures.

326 Figure 2 summarizes how risk assessment and risk reduction measures can help identify appropriate  
327 reporting categories for ECs. Fixing performance criteria for performance characteristics identified  
328 as ECs, for example, in an ATP, can help mitigate risk associated with changes. This ensures that the  
329 analytical procedure remains fit for purpose subsequent to changes and thus forms the basis of a  
330 bridging strategy. Changes to parameters that are not ECs should be documented in the PQS but do  
331 not require regulatory reporting.

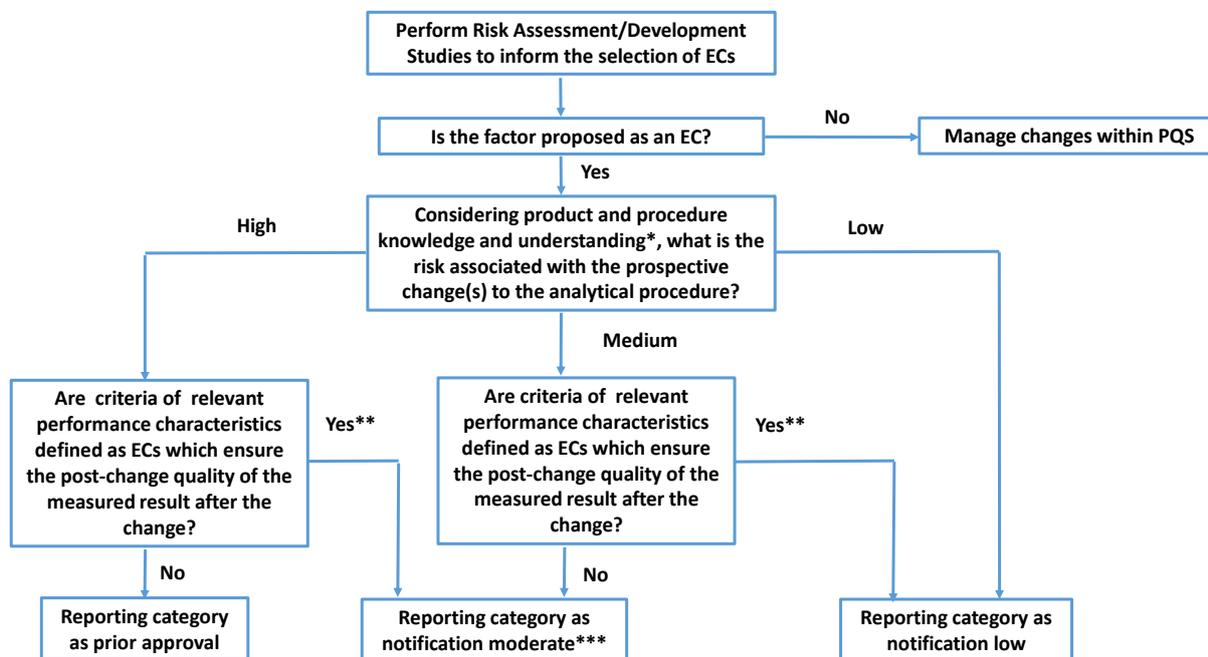
332 The ATP could also form the basis of a PACMP which would allow changes (e.g., a change between  
333 technologies) to be reported at a lower reporting category provided that the pre-defined requirements  
334 for a change are met.

335

336

337 **Figure 2: Risk-based approach to identification of ECs and reporting categories for associated**  
 338 **changes in the enhanced approach**

339



340

341 \* Including analytical procedure control strategy

342 \*\* Sufficient information or prior knowledge should be available to design appropriate  
 343 future bridging studies

344 \*\*\* In some cases, moderate risk changes proposed by the company may require prior  
 345 approval based on health authority feedback

346

347 In Annex A examples are given on how appropriate reporting categories can be proposed.

348 When implementing changes to analytical procedures, QRM can be used to evaluate the impact of  
 349 the changes and re-confirm that the originally agreed reporting category is still appropriate. The  
 350 outcome of this risk assessment informs the design and extent of the studies needed to support the  
 351 change including an appropriate bridging strategy to demonstrate that the revised or new procedure  
 352 is fit for purpose. The implementation of an already validated analytical procedure at a different  
 353 location, including the concepts of the analytical procedure transfer, should follow the same  
 354 verification and bridging strategies (Tables 1 and 2).

355



368 **Table 2: Examples of Analytical Procedure Change Evaluation**

<b>Risk Factor: Extent of change</b>	<b>Bridging strategy</b>	<b>Evidence of the suitability of a new procedure</b>
Change of analytical procedure principle (physicochemical/biochemical basis)	Full validation of new procedure And Comparative analysis of representative samples and standards. And/or Demonstration that the analytical procedure's ability to discriminate between acceptable and non-acceptable results remains comparable	Analytical procedure performance characteristics are evaluated and criteria are met after the change And Results are comparable after change or differences are acceptable and potential impact on specification evaluated
Change within same analytical procedure principle, for example:  1. Modification of procedures 2. Transfer of procedures to different locations/environments	Partial or full re-validation of the analytical procedure performance characteristics affected by the change And/or Comparative analysis of representative samples and standards	Analytical procedure attributes are evaluated and criteria are met after change And/or Results are comparable after change or differences are acceptable and potential impact on specification evaluated

369

370 To support the use of the tools described in this guideline, the company's PQS change management  
371 process should be effective and in line with recommendations described in ICH Q12. During the  
372 lifecycle the MAH should evaluate performance, perform trend analysis, assess knowledge gained  
373 and re-evaluate if the analytical procedure remains fit for purpose.

374

## 375 **8. DEVELOPMENT OF MULTIVARIATE ANALYTICAL PROCEDURES**

376 Multivariate analytical procedures are those where a result is determined through a multivariate  
377 calibration model utilizing more than one input variable. The considerations provided here are for  
378 models using *latent variables* that are mathematically related to directly measured variables. Other  
379 approaches, in machine learning, such as neural networks, or optimization techniques could use  
380 similar principles although the specific approach may vary and will not be discussed in detail.

381 Development of a robust multivariate analytical procedure includes scientifically justified sample  
382 selection and distribution over the range, sample size, model variable selection and data pre-  
383 processing.

### 384 **Sample and sample population**

385 Multivariate models link measured model variables with values obtained from a validated *reference*  
386 *procedure* or from *reference samples*. Therefore, samples in multivariate analysis consist of input  
387 measurements and their corresponding reference values, which are numeric values for quantitative  
388 measurements (e.g., assay) and classification categories for qualitative methods (e.g., identity). In  
389 some cases, one set of input measurements could be used for multiple models provided that more  
390 than one reference value exists. The reference values are determined using reference analytical  
391 procedure(s) or prepared reference samples with known values. Care should be taken to ensure that

392 uncertainty in the reference analytical procedure is sufficiently low in relation to the intended  
393 performance of the multivariate analytical procedure and that prepared reference samples are  
394 homogeneous. The approach to the reference procedure(s) or prepared reference samples should be  
395 explained and justified.  
396

397 The ranges of multivariate models are typically constructed by data from samples. Therefore, a  
398 careful strategy for sample selection is essential for obtaining the relevant information from the  
399 analytical data and contributes to the robustness of the resulting model. Based on the method and  
400 measurement principle, the sample population should encompass the sources of variability likely to  
401 occur during manufacture and analysis, such as raw material quality, manufacturing process  
402 variability, storage conditions, sample preparation and testing. Use of risk assessment tools can help  
403 to identify sources of variability with the potential to influence the measurements and resulting model  
404 outputs.

405 Obtaining samples with appropriate variability at commercial scale can be challenging. Therefore,  
406 development laboratory and pilot scale samples are often utilized to provide enough variability to  
407 improve accuracy and robustness of the model. Inclusion of commercial scale samples is  
408 recommended to capture variability related to specific equipment and/or processing conditions.  
409 Careful consideration should also be given to sample distribution in the calibration and *validation*  
410 *sets*, as this will influence the model predictive capability.

411 The number of samples used to create a calibration model for quantitative analysis will depend on the  
412 complexity of the sample matrix and/or interference by the matrix in the analyte signal of interest  
413 (i.e., for more complex sample matrices, generally more samples are needed).

414 Sufficient samples should be available to allow for creation of independent calibration and validation  
415 sets of appropriate size and variability, i.e., samples in the validation set are not incorporated in  
416 calibration or *internal testing sets*. A validation sample set generated with samples from independent  
417 batches can be used to demonstrate model robustness.

#### 418 **Variable selection**

419 Variable selection is performed during model development. For example, wavelength range selection  
420 is frequently applied in spectroscopic applications to select a region of a spectrum that gives the best  
421 estimation of the selected chemical or physical property to be evaluated (modeled). Variable selection  
422 depends on the measurement principle, application and other factors, and should be justified.

#### 423 **Data transformation**

424 The selection of the *data transformation* method(s) can be driven by the type of data, instrument or  
425 sample, the intended use of the model and/or prior knowledge. Caution should be exercised when  
426 performing any transformation because artefacts can be introduced, or essential information lost. Any  
427 transformation of data should be documented and justified.

#### 428 **Robustness**

429 Model development should minimize the prediction error and provide a robust model that consistently  
430 assures the long-term performance of multivariate models. The robustness should be built into the  
431 model by including relevant sources of variability related to materials, process, environment,  
432 instrumentation or other factors. Sources of variability can be identified from prior knowledge and  
433 risk assessments and evaluated using statistical tools. Robustness depends on multiple factors, e.g.,  
434 composition of the calibration set, data transformation method, variable selection and the number of  
435 latent variables.

436 Optimization of the multivariate model is an important step in development and often requires a trade-  
437 off between accuracy and robustness. A critical factor is the number of latent variables to be used in  
438 the calibration model which ensures the model is optimized for its intended purpose. Selection of the

439 number of latent variables occurs during model development and is confirmed during internal testing.  
 440 Too many latent variables can result in model overfitting, potentially resulting in decreased  
 441 robustness and a need for more frequent model updates. Justification for the final number of latent  
 442 variables used should be provided. Diagnostic plots provided by software packages can be useful to  
 443 support the justification.

444 **Re-calibration and model maintenance**

445 Tracking the calibration model performance is an important part of ongoing monitoring for a  
 446 multivariate analytical procedure. Various statistical tools can be employed as diagnostics to ensure  
 447 that the model assumptions are upheld. For latent variable models, these diagnostic tools can include:

- 448 • examination of residuals to determine unmodeled features of the data (e.g., x-residuals or F-  
 449 probability)
- 450 • *outlier diagnostics* to determine if the data is within the bounds of the model construction  
 451 (e.g., Hotelling's T-squared or Mahalanobis distance)

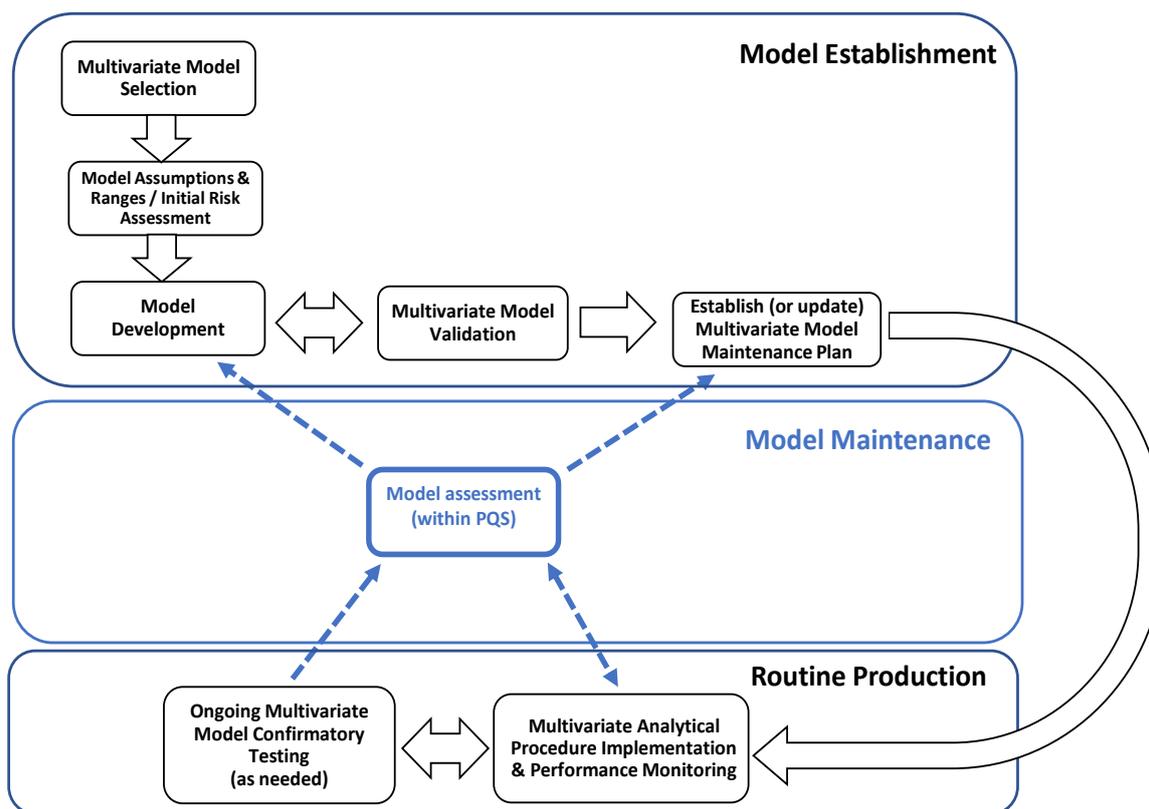
452 Software packages allow for the application of diagnostic tools for every model prediction.

453 Additionally, continued performance of the calibration model should be confirmed on a periodic and  
 454 event-driven basis by comparison of the model predictions with the reference samples or reference  
 455 method results. This confirmatory testing helps to ensure that the calibration model continues to  
 456 perform as expected. Examples of events that could trigger confirmatory testing include new known  
 457 process variability, unexpected process events or scheduled instrument maintenance.

458 Monitoring of the model can be used to trigger model rebuilding (recalibration) as a part of continual  
 459 improvement. In general, the same considerations hold as for the original model building and internal  
 460 testing. Based on the cause of the model update (e.g., a process shift), new data may need to be  
 461 included and old non-relevant data may be taken out.

462 Once the new calibration model is established, the updated analytical procedure can be validated  
 463 against the same performance criteria as the one included in the original model. Aspects that are not  
 464 expected to change from the model update may not need to be evaluated (e.g., specificity).

465

466 **Figure 3: Multivariate Model Lifecycle**

467

468 The multivariate model lifecycle is iterative and can be broken down into 3 major components: (1)  
 469 model establishment, (2) routine production and (3) *model maintenance*.

470 The choice of a multivariate model is based on the analytical procedure requirements and the  
 471 measurement technology selected. Prior to model development, the performance factors for the model  
 472 are defined, including the underlying model assumptions and desired ranges for model applicability.  
 473 An initial risk assessment can be valuable to understand potential sources of variability in the  
 474 materials and process that could affect the model performance and therefore should be considered  
 475 during the model calibration. Model development, including calibration and internal testing, follows  
 476 the considerations outlined in this chapter. Once the model is developed, it is validated using  
 477 independent data not previously used in the calibration set. The last step in model establishment is  
 478 development of a multivariate model maintenance plan, which includes the procedures and limits for  
 479 outlier diagnostics, and defines the frequency and circumstances for confirmatory testing, if needed.

480 Routine analysis of the multivariate analytical procedure typically includes monitoring the  
 481 appropriateness of every measurement using outlier diagnostics. Confirmatory testing against a  
 482 reference procedure is recommended on a pre-defined periodic or event driven basis (e.g., equipment  
 483 maintenance, new raw materials or process changes). Model assessment can be triggered by failure  
 484 of confirmatory testing or outlier diagnostics to meet the predefined criteria, or from data trending  
 485 indicating potential issues with the model, the process or the materials being measured (examples of  
 486 multivariate model lifecycle components are provided in Annex C).

487 Model assessment is performed within the PQS and utilizes knowledge management and risk  
 488 assessment. If an issue is identified, model development and revalidation may be needed, for example,  
 489 to add samples into the calibration set and remove those that are no longer relevant. In some cases,  
 490 the model may be performing appropriately, but additional experience may identify the need to  
 491 modify the limits of the model maintenance plan. In other cases, the issue identified could be related

492 to the measurement system (e.g., a misaligned sample interface) and no model update would be  
493 needed. The dashed arrows in the figure illustrates reintroduction into the lifecycle flow based on the  
494 potential outcomes of the model assessment.

495

## 496 **9. DEVELOPMENT OF ANALYTICAL PROCEDURES FOR REAL TIME RELEASE** 497 **TESTING: SPECIAL CONSIDERATIONS**

498 *Real Time Release Testing (RTRT)* is the ability to evaluate and ensure the quality of in-process and/or  
499 final product based on process data, which typically include a valid combination of measured material  
500 attributes and process controls (*ICH Q8*). RTRT measurements work in conjunction with all elements  
501 of the control strategy (e.g., process monitoring or in-process controls) to ensure product quality.  
502 RTRT can be applied to active substances, intermediates and finished products.

503 RTRT can be based on an appropriate combination of one or more process measurements and/or  
504 material attributes to provide a prediction of one or more product CQAs and needs to be specific for  
505 that CQA. The relationship between the RTRT approach and the product CQAs, as well as acceptance  
506 criteria, should be fully justified. As appropriate, an RTRT procedure should be validated as  
507 recommended in ICH Q2 and it should be demonstrated that the process measurements have  
508 appropriate specificity for the targeted product quality attribute.

509 Sampling and the sample interface are important considerations when designing any on-line or in-  
510 line test method, including those used for RTRT. The measurement point(s) should be chosen to be  
511 representative of the entire material being processed with the sample duration or amount appropriately  
512 chosen (e.g., relative to a unit dose). Additionally, the sample interface should remain consistent over  
513 the duration of manufacturing and should be robust to expected processing and environmental  
514 variations.

515 The RTRT approach should be included in the product specification along with a reference to the  
516 RTRT analytical procedure(s) and the related acceptance criteria, which are discussed in ICH Q6A  
517 and Q6B. Quantitative RTRT results should be expressed in the same units as those for traditional  
518 testing. The product specification will typically also include the analytical procedures to be used for  
519 off-line testing. If the dossier includes a registered alternate control strategy to RTRT (e.g., traditional  
520 end-product testing for when process analytics are unavailable), the related analytical procedures and  
521 when they would be applied should also be included in the submitted product specifications.

522

## 523 **10. SUBMISSION OF ANALYTICAL PROCEDURE RELATED INFORMATION**

### 524 **10.1 General Regulatory Considerations and Documentation**

525 The analytical procedure description(s) should be included in the ICH M4Q CTD section 3.2.S.4.2  
526 for drug substance or section 3.2.P.5.2 for drug product. Validation data and any supportive  
527 information needed to justify the analytical procedure control strategy should be included in the CTD  
528 section 3.2.S.4.3 for drug substance or section 3.2.P.5.3 for drug product. Other analytical procedures  
529 used as part of the control strategy can be included in relevant CTD sections (e.g., 3.2.S.2, 3.2.P.3  
530 and 3.2.P.4). The analytical procedure should describe the steps in sufficient detail for a skilled  
531 analyst to perform the analysis as elaborated in Chapter 6. Submission of validation data should  
532 follow the recommendations in ICH Q2. The criteria used in the validation study should be included  
533 in the submission. In some cases, depending on the intended use (e.g., dissolution testing) and/or the  
534 selected technique it may be appropriate to submit development data as justification.

535 Where ECs are proposed for analytical procedures as elaborated in Chapter 6, the ECs should be  
536 clearly differentiated from supportive information. Additional development and validation  
537 information can be included in sections 3.2.S.4.3 and 3.2.P.5.3 to justify ECs and their reporting  
538 categories. When other lifecycle management elements as described in ICH Q12 are included in the  
539 submission, the applicant should follow the principles described in ICH Q12 and Chapter 7 of this  
540 document.

## 541 **10.2 Documentation for the Enhanced Approach**

542 If an enhanced approach to development leads to the incorporation of enhanced elements into the  
543 analytical procedure control strategy, then these should be justified.

544 Performance characteristics and acceptance criteria (e.g., described in an ATP) and other elements of  
545 the enhanced approach (e.g., MODRs or PARs), should be described in the dossier sections for  
546 analytical procedure description (e.g., 3.2.S.4.2 and 3.2.P.5.2). If ECs are proposed, then these should  
547 also be included in the analytical procedure description, accompanied by supportive information. Use  
548 of the enhanced approach should not lead to providing a less detailed description of analytical  
549 procedures in a regulatory submission.

550 If ECs are proposed, risk-based categorization of changes and corresponding reporting categories  
551 should be included in the submission. Appropriate justification should be given for parameters that  
552 are ECs and those that are not ECs (see Chapter 6). For parameters that are not ECs and are typically  
553 not included in a minimal procedure description a justification is not expected.

554 Appropriate information from analytical procedure risk assessment and development studies to  
555 support the proposed lifecycle management strategy should be summarized and submitted in the  
556 regulatory submission sections for analytical procedure validation (e.g., 3.2.S.4.3 and 3.2.P.5.3).

## 557 **10.3 Documentation for Multivariate Analytical Procedures and RTRT**

558 Development information related to multivariate analytical procedures should be provided  
559 commensurate with the level of impact of the model (*Guide for ICH Q8/Q9/Q10 Implementation*).  
560 The process development section of the dossier (e.g., 3.2.S.2.6 or 3.2.P.2) should include the model  
561 development information for multivariate models used as part of manufacturing development studies  
562 or for in-process controls or tests. Supportive development information for RTRT multivariate models  
563 can be included in either the appropriate analytical procedure validation or process development  
564 section.

565 Validation information for multivariate analytical procedures used for release of drug product or drug  
566 substance, including RTRT, should be included in the validation information section of the dossier  
567 (e.g., 3.2.S.4.3 or 3.2.P.5.3). Additionally, these sections should include validation information on  
568 analytical procedures used as reference methods. The model development, calibration and validation  
569 information can be included directly in the CTD section or be in an appended document.

570 For multivariate models used as part of drug substance or drug product specifications, including  
571 RTRT approaches, the description of the validation approach and results should include:

- 572 • Description of the independent validation sample set
- 573 • The performance criteria to be met during validation of the multivariate model
- 574 • Evaluation of the *model validation* results against the performance criteria
- 575 • Discussion of the relationship between the model performance criteria and the attribute  
576 specification limits
- 577 • High level overview of the PQS elements for model monitoring and maintenance, such as  
578 diagnostic tools for determining the appropriateness of the sample data for the model and the  
579 approach taken when outliers are identified.

580

581 The description of the multivariate analytical procedure used for RTRT should be provided in the  
582 CTD section 3.2.S.4.2 for drug substance or section 3.2.P.5.2 for drug product and typically includes:

- 583 • The property or attribute of interest to be determined by the multivariate analytical  
584 procedure and the desired quantitative ranges or limits
- 585 • A description of the measurement principle and pertinent instrument operating parameters  
586 (e.g., sample presentation, sample interrogation time and measurement frequency)
- 587 • An overview of how the multivariate model calibration data are obtained (e.g., sample  
588 preparation approach, reference method)
- 589 • The type of multivariate model (e.g., principal component analysis)
- 590 • A description of reference analytical procedure or high-level description of prepared  
591 reference samples preparation
- 592 • Any calculations needed to adjust the model output into the reported value  
593

594 Additionally, section 3.2.S.4.2 for drug substance or section 3.2.P.5.2 for drug product should include  
595 description of any analytical procedures that are part of a registered alternate control strategy to  
596 RTRT.

597

598 **11. GLOSSARY**

599 **ACCURACY**

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

603 **ANALYTICAL PROCEDURE**

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

606 **ANALYTICAL PROCEDURE ATTRIBUTE**

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

610 **ANALYTICAL PROCEDURE CONTROL STRATEGY**

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

613 **ANALYTICAL PROCEDURE PARAMETER**

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

616 **ANALYTICAL PROCEDURE VALIDATION STRATEGY**

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

622 **ANALYTICAL TARGET PROFILE (ATP)**

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

625 **CALIBRATION MODEL**

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

628

629

630

631 **CONTROL STRATEGY**

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

637 **CO-VALIDATION**

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

642 **CRITICAL QUALITY ATTRIBUTE (CQA)**

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

645 **CROSS-VALIDATION**

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

648 **DETECTION LIMIT**

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

651 **DETERMINATION**

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

654 **ESTABLISHED CONDITIONS (ECs)**

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

657 **INTERMEDIATE PRECISION**

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

661 **KNOWLEDGE MANAGEMENT**

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

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

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

667 **ONGOING MONITORING**

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

670 **PERFORMANCE CHARACTERISTIC**

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

674 **PERFORMANCE CRITERION**

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

677 **PLATFORM ANALYTICAL PROCEDURE**

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

682 **PRECISION**

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

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

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

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

693 **QUALITY RISK MANAGEMENT**

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

696

697 **QUANTITATION LIMIT**

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

703 **RANGE**

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

707 **REPORTABLE RANGE**

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

711 **WORKING RANGE**

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

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

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

720 **REPEATABILITY**

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

723 **REPORTABLE RESULT**

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

726 **REPRODUCIBILITY**

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

729

730

731 **RESPONSE**

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

735 **REVALIDATION**

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

739 **ROBUSTNESS**

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

743 **SAMPLE SUITABILITY ASSESSMENT**

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

751 **SPECIFICITY/SELECTIVITY**

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

759 **SYSTEM SUITABILITY TEST (SST)**

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

763

764

765

766 **TOTAL ANALYTICAL ERROR**

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

770 **VALIDATION STUDY**

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

773 **VALIDATION TEST**

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

776

777 **MULTIVARIATE GLOSSARY**

778 **CALIBRATION DATA SET**

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

781 **DATA TRANSFORMATION**

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

784 **INDEPENDENT SAMPLE**

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

788 **INTERNAL TESTING**

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

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

795 **INTERNAL TEST SET**

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

798 **LATENT VARIABLES**

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

801 **MODEL VALIDATION**

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

807 **MODEL MAINTENANCE**

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

811 **MULTIVARIATE ANALYTICAL PROCEDURE**

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

814 **OUTLIER DIAGNOSTIC**

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

816 **REFERENCE PROCEDURE**

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

819 **REFERENCE SAMPLE**

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

822 **VALIDATION SET**

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

825

826 **12. References**

827 ICH Q2 Validation of Analytical Procedures

828 ICH Q8 Pharmaceutical Development

829 ICH Q9 Quality Risk Management

830 ICH Q10 Pharmaceutical Quality System

831 ICH Q12 Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle  
832 Management

833 ICH M4Q The Common Technical Document for the Registration of Pharmaceuticals for Human  
834 Use: Quality – M4Q

835

836 **13. ANNEX**

837 **13.1 Annex A – Analytical Procedure Lifecycle**

838 *The examples provided in this Annex are mock examples for illustrative purposes. They suggest how*  
839 *the concepts described in ICH Q14 could be applied and should not be used as a template or the sole*  
840 *basis for a regulatory submission.*

841 *The examples have been created to illustrate*

- 842 • *how analytical procedure performance characteristics derived from the product context and*  
843 *knowledge could be summarized in an ATP*
- 844 • *how performance characteristics described in the ATP could be applied to select a suitable*  
845 *analytical technology, guide the development of an analytical procedure and help define the*  
846 *analytical procedure control strategy*
- 847 • *how performance characteristics described in the ATP could aid the design of the validation*  
848 *study for the analytical procedure*
- 849 • *how to identify ECs for analytical procedures developed using the enhanced approach*
- 850 • *how QRM and the adherence to associated criteria for relevant performance characteristics*  
851 *and/or the subsequent execution of a bridging study can ensure the post-change quality of*  
852 *the measured result and help to justify the respective reporting categories for ECs and the*  
853 *post approval change management of analytical procedures*

854

855 *As described in chapter 4, QRM can be used to evaluate the impact of proposed changes for analytical*  
856 *procedures. The paragraph below describes examples of risk factors and risk reduction measures to*  
857 *identify the risk associated with the changes to an analytical procedure. The outcome of the risk*  
858 *assessment (risk level: high, medium or low) feeds into the design and extent of the studies needed to*  
859 *support the change*

860 ***Selected Risk (risk factors)***

- 861 • *Relevance of the test*
  - 862 • *Potential clinical impact of the measured attribute (efficacy, safety,*  
863 *pharmacokinetics and immunogenicity), e.g., controlling CQA vs non CQA*
  - 864 • *Extent of knowledge of the attribute*
  - 865 • *Attribute covered by other elements of the control system (testing or process control)*
- 866 • *Complexity of the technology*

- 867 • *Simple vs. complex technology*
- 868 • *Platform technologies*
- 869 • *Novel vs. established technology (e.g., in Pharmacopoeias)*
- 870 • *Several attributes reported as a sum (e.g., charge variants for large molecules)*
- 871 • *Biological assays, cell-based assays, immunochemical assays*
- 872 • *Multiaattribute assays*
- 873 • *Multivariate assays*
- 874 • *Extent of the change*
  - 875 • *Change of one or several parameters within MODR/PAR*
  - 876 • *Change of one or several parameters outside the already proven ranges*
  - 877 • *Change of the analytical procedure within existing analytical procedure*
  - 878 *performance characteristics*
  - 879 • *Change to analytical procedure performance characteristics (e.g., due to tightening*
  - 880 *a specification limit or a change to the intended purpose of the procedure to*
  - 881 *measure additional attributes)*

882

883 **Risk reduction**

884 *Risk reduction is defined in ICH Q9 as actions taken to lessen the probability of occurrence of harm*  
 885 *and the severity of that harm.*

886 *Different kinds of knowledge can lead to reduction of risk, for example:*

- 887 • *Product and Process knowledge*
  - 888 - *Knowledge about CQAs of the product/active substance and their acceptable ranges*
  - 889 - *Well justified AP performance criteria cover/link to CQAs and their acceptable*
  - 890 *range*
  - 891 - *Knowledge about CPPs of the manufacturing process including risk assessment of*
  - 892 *the process control capability over the CQA*
  - 893 - *Evidence to control the CQAs through the process parameter settings*
  - 894 - *Knowledge of the degradation pathways demonstrated by the analysis of relevant*
  - 895 *stressed samples*
  - 896 - *Other product knowledge (e.g., impurity profile, particle size and distribution)*
- 897 • *Analytical Procedure understanding and analytical procedure control strategy*
  - 898 - *Knowledge about analytical procedure parameters and their impact on measurement*
  - 899 *performance*
  - 900 - *Proven analytical procedure robustness, e.g., harmonized procedures (compendial*
  - 901 *tests)*
  - 902 - *Enhanced method understanding (e.g., DoE studies) supporting justification of*
  - 903 *acceptable ranges (e.g., PAR, MODR) to ensure quality of the result*
  - 904 - *Other knowledge from development of analytical procedure*
  - 905 - *System Suitability Test covers relevant analytical procedure attributes*
  - 906 - *Ongoing monitoring of method output*
  - 907 - *Clear link between signal and CQA to be measured (e.g., peak characterization*
  - 908 *available, specificity)*
- 909 • *Subsequent Bridging strategy for the actual change*
  - 910 - *Availability of well characterized reference material, relevant historical and or*
  - 911 *stressed samples to support method output assessment against performance*
  - 912 *requirements (demonstrated ability to control the CQA)*
  - 913 - *Comparison to output of previous method (understanding and acceptance of risk for*
  - 914 *potential differences)*

- 915 - Demonstrated understanding of risks associated with parameter changes and  
 916 potential interactions with other parameters (if applicable)  
 917 - Prior experience or literature with similar changes, analyte or technology  
 918 - Reference to previous filings or to platform analytical procedures (if appropriate).  
 919

920 **13.1.1 Measurement of Stereoisomers as Specific Process Related Impurities in a Small Molecule Drug**  
 921 **Substance (DS)**  
 922

923 **Introduction and Background**

924 “Sakuratinib Maleate” is a small molecule DS with multiple chiral centers. The chirality of the  
 925 molecule, its degradation pathway and the impurities are well characterized. From this knowledge  
 926 and the established manufacturing process controls the 5 Stereoisomers (Impurity A-E) were found  
 927 to be potentially present in the final product. Based on toxicological considerations, Impurity A-E  
 928 was specified at NMT 0.1%. One Stereoisomer F was found to be a process-related impurity but not  
 929 a degradation product. The stereoisomer was specified for release and re-test at NMT 0.5 % based on  
 930 toxicological data. Impurities G-J were other process-related impurities, of which process impurity J  
 931 was found to be also a degradation product of the DS. All specified impurities are isolated and  
 932 available as well characterized substances for procedure development and validation.

933 **Table 1: Analytical Target Profile:**

<b>Intended Purpose</b>		
Quantification of the stereoisomers A-F in Sakuratinib Maleate API for release testing.		
<b>Link to CQA (Chiral Purity)</b>		
The analytical procedures should allow for the individual quantification and determination of the total sum of the stereoisomers A-F to verify the CQA Chiral Purity $\geq 99.0\%$		
<b>Characteristics of the Reportable Results</b>		
<b>Characteristic</b>	<b>Acceptance Criteria</b>	<b>Rationale</b>
Performance Characteristics		
Accuracy	80-120% average recovery of spiked DS with Impurity A-E 90-110% average recovery of spiked DS with Impurity F	The values were derived from considerations of the significance of rounded values. At a specification level of 0.1%, 20% bias would lead to a variation of the analytical result of 0.02%, which was found acceptable for a release decision.
Precision	For impurity A-E Intermediate Precision RSD (n $\geq$ 6): Impurity A-E $\leq 15\%$ Impurity F $\leq 10\%$	In a similar fashion, values for precision were derived. The recovery criteria for accuracy were set with respect to the reported result and taking into consideration any correction or response factors.
Specificity	Analytical procedure should demonstrate to quantify with an acceptable bias of not more than 0.01% impurities A-F in presence of other likely process related substances or DS degradation products, which could be induced during chemical synthesis (Impurities G-J), and the salt forming agent.	Potential interference with quantification of specified impurities by other regular components in the sample
Reportable Range	Impurity A-E: at least 0.05-0.12% Impurity F: at least 0.05-0.6%	Reporting threshold to 120% of specification limit

934 **Initial Technology Selection**

935 Multiple analytical technologies for chiral separations were available: Chromatographic methods  
 936 such as gas chromatography (GC), liquid chromatography (HPLC), supercritical fluid  
 937 chromatography (SFC) and thin-layer chromatography (TLC) were established technologies using  
 938 different chiral separation principles. More recently, capillary zone electrophoresis (CZE) and  
 939 capillary electrochromatography (CEC) had been shown to be alternatives to chromatographic  
 940 methods. Besides meeting the desired performance characteristics, further practical criteria were  
 941 considered in the technology selection for development, based on general technical knowledge,  
 942 operational needs, availability of equipment and capabilities in the company at the time:

- 943 • Complexity and robustness of technology
- 944 • Time and costs of analysis
- 945 • Standardization of technology and availability of multiple instrument suppliers
- 946 • Existing expertise in the company

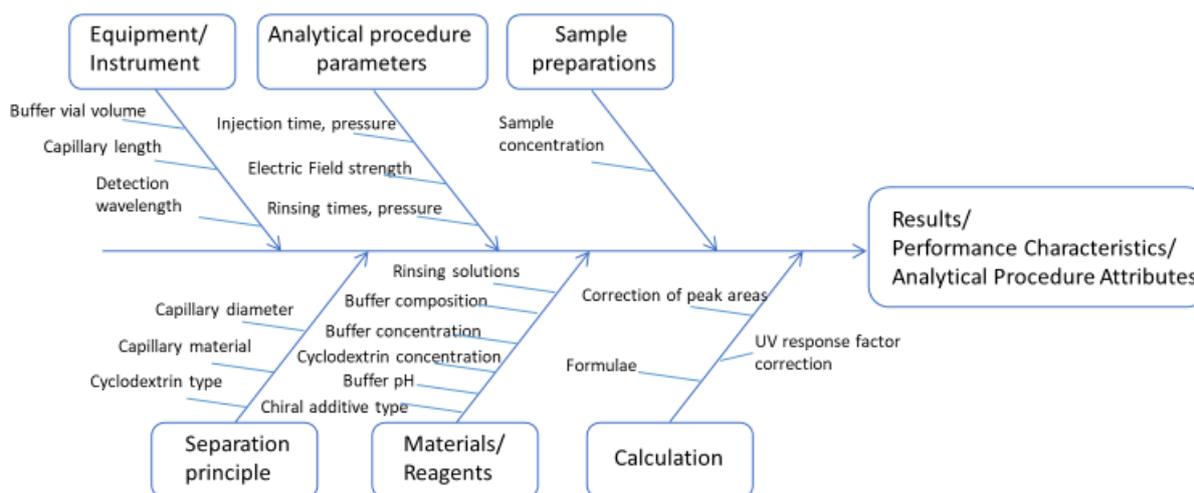
947  
 948 It was finally concluded to start method development with two technologies: Chiral HPLC and CZE.  
 949 As detection mode, UV detection was selected as it was known that the molecule had sufficient UV  
 950 absorption properties and standard for both separation techniques at the time.

951  
 952 **Analytical Procedure Development**

953  
 954 At initial development, a first screening was performed between HPLC and CZE technology. With  
 955 the technology and columns available at the time, only CZE could meet the expected performance for  
 956 specificity as described in the ATP, which served as primary endpoint for procedure development.  
 957 Therefore, the HPLC procedure development was discontinued at initial development.

958  
 959 A risk analysis for the developed CZE procedure was performed. Parameters, where impact on the  
 960 performance of the procedure could not reasonably excluded were identified. See Ishikawa diagram  
 961 below:

962  
 963 **Figure 1: Ishikawa-Diagram**



965  
 966 Analytical procedure parameters were investigated and their impact on the performance was  
 967 evaluated. The robustness of the CZE procedure was optimized and verified versus the performance  
 968 characteristics. Ultimately, the analytical procedure was optimized in the areas of sensitivity at QL,  
 969 repeatability of migration times and corrected peak areas, peak tailing of the API and stereoisomers,  
 970 and separation buffer depletion. Based on the development results, detailed instructions were given  
 971 in the analytical procedure description “Determination of the stereoisomers A-F in Sakuratinib

972 Maleate” and an SST was established on relative migration times resolution, LOQ, repeatability of  
 973 injection and the asymmetry of the DS peak as part of the analytical procedure control strategy.  
 974

974

975 **Table 2: Analytical Procedure Description**

976

Capillary:	Uncoated fused silica, 50 µm diameter, at least 70 cm length
Separation Buffer:	13.2 g/l solution of ammonium phosphate adjusted to pH 6.0 with phosphoric acid filtered and 100 mM β-cyclodextrin, both ends of capillary
Rinsing steps:	1M sodium hydroxide, water, 0.1M sodium hydroxide Rinsing time at 1 psi at least 2 minutes each step
Column temperature:	30°C
Injection:	Injection test solution (a) and the reference solution; injection for at least 3 s then CZE buffer injection for 2 s at about 0.5 psi
Separation field strength and polarity	217 V/cm, normal mode
Detection	UV 214 nm

977

### 978 **Method validation**

979

979 After the analytical procedure description was finalized, a technology specific validation study was  
 980 planned according to the recommendations in ICH Q2. In alignment with the performance  
 981 characteristics, a technology and procedure specific set of attributes and criteria were derived from  
 982 the performance characteristics:

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- The accuracy was measured by spiking three levels, 0.05, 0.1 and 0.12% for impurity A-E, 0.05, 0.5 and 0.6% for impurity F to the DS salt form at 100% level and the average recovery was calculated. The acceptance criteria for the average recovery of 80-120% and 90-110% respectively were met
- For precision (repeatability), 6 separate preparations of the 6 stereoisomers were made at specification limit. The RSD of 15% (Impurities A-E) respectively 10% (Impurity F) criteria for precision of the migration time corrected peak areas were met. Similarly, intermediate precision between operators, days and instruments were performed and evaluated in an ANOVA experiment.
- Specificity was demonstrated by spiking all 6 stereoisomers to the API salt form and impurities G-J, demonstrating sufficient baseline resolution (no detectable bias between peaks) between the individual analytes of interest and no interference with process related impurities. Additionally, blank injections of buffer and water were compared with a sample to demonstrate no interference with the analyte detection.
- To verify the reportable range, a linearity, QL and DL experiment was performed and compared to the technology specific acceptance criteria:
  - DL was confirmed to be above a signal to noise ratio of 3:1 for all stereoisomers
  - QL was confirmed by demonstrating the RSD of the corrected peak areas for the stereoisomers at the reporting threshold was NMT 10%
  - Linearity was found acceptable by demonstrating the correlation coefficient R was greater than 0.998 at 6 levels of stereoisomer concentrations ranging from 0.05-2.0% for all impurities and the drug substance. A wider range was chosen to allow the application of the procedure for a potential wider range and allow a more precise determination of relative UV response factors
  - Linearity slopes of the stereoisomers were compared to the linearity of drug substance to demonstrate a UV response factor of about 1.0 for each stereoisomer versus the drug substance

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After the performance of the validation study, the results were summarized in a validation report, which concluded that the analytical procedure would meet the acceptance criteria for the analytical procedure attributes. The related performance characteristics were met. The analytical procedure was concluded to be fit for the intended purpose.

**Description of Established Conditions (ECs), Reporting Categories, and Justifications**

Based on product and process understanding and considering the procedure development data and risk assessment (see introduction to this annex), the applicant proposed established conditions and reporting categories as part of the initial submission. Justification of reporting categories for changes included adherence to predefined acceptance criteria described in the ATP and additional performance controls (e.g., system suitability testing and control samples).

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*Note: The number of ECs and the associated reporting category listed in this table may depend on the extent of knowledge gained and information provided and is generated for this specific example only. The information provided in this example is not the entirety of the knowledge that is available and will be submitted to regulatory agencies and should not serve as general guidance. The extent of ECs, actual reporting categories, and data requirements may differ by region. Other parameters and conditions that are not identified as ECs in the table below may be required as EC for some cases depending on the region. The changes to other technologies may constitute different risks and may lead to different reporting categories. A PACMP may be required for some cases (e.g., a change between technologies) depending on region.*

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1034

**Table 3: Proposed established conditions and reporting categories applying principles of ICH Q12 in the enhanced approach**

Established Condition	Overall Risk Category	ICH Q12 Reporting Category	Justification/ rationale
Analytical Target Profile (ATP)	High	PA	If widening the ATP is necessary, it will be reported as PA.
Technology: Capillary Zone Electrophoresis with UV detection  Suitable chiral separation technique to meet performance characteristics defined in ATP	Low	NL	Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes. Changes to the method principle will be reported as NL. There is a strong understanding between product knowledge, intended purpose, and the analytical procedure performance established. In addition, well characterized analytical materials as well as a robust method development data set is available to allow a well-controlled bridging between technologies of similar separation capabilities (such as CZE to chiral HPLC).
Technology Specific Analytical Procedure Attributes	Low	NL	Accuracy and Precision (see ATP)  Specificity: Baseline Separation with R NLT 2.0 for Impurities A-F, DS, Salt forming agent and grouped impurities G-J. Impurities G-J do not need to be baseline separated amongst each other  Linearity: R NLT 0.990 with at least 5 points in the range between 0.05%-2.0% for  DL Impurities A-F: S/N NLT 3:1 below level 0.05%

ICH Q14 Guideline

Established Condition	Overall Risk Category	ICH Q12 Reporting Category	Justification/ rationale
<p>System Suitability Test and parameter-control relationship as part of the overall Analytical Procedure Control Strategy:</p> <p>SST 1: Verification of relative migration times of analytes as listed in the analytical procedure. Asymmetry factor of the DS <math>\leq</math> 1.5, Controlled factors:            Electric Field Strength            Rinsing agents &amp; times            Separation buffer concentration and pH            Effective Capillary Length            Capillary material            Chiral buffer additive type and concentration</p> <p>SST2: Resolution between critical peak pair: API Main Peak and Impurity D <math>\geq</math> 2.0, Controlled factors:            Chiral buffer additive type and concentration            Buffer composition            Buffer pH            Injection time/pressure (=volume)            Reference/Test solution concentration</p> <p>SST3: S/N at LOQ API at 0.05% <math>&gt;10 :1</math>, Controlled factors: Detection            Injection time and pressure            Sample and reference standard concentrations</p> <p>SST 4: Repeatability of injection of API at 0.5% level <math>\leq</math> 5%, Controlled factors:            Injection parameters            buffer filtration</p>	Low	NL	<p>QL Impurities A-F: S/N NLT 10:1 at level 0.05%</p> <p>SST was developed for the CZE procedure based on a risk analysis in alignment with the performance characteristics described in the ATP. The SST criteria are focused on critical performance characteristics during the regular application of the analytical procedure. Control relationships were established through prior knowledge (general principles of technique) or during method development. See further details with the parameters described below.</p> <p>A change in the SST should ensure similar or improved control of the associated factors listed in the left column.</p>
<p>Separation Principle:            Capillary: Material: uncoated fused silica capillary (diameter <math>\varnothing = 50 \mu\text{m}</math>) and <math>\beta</math>-cyclodextrin</p> <p>suitable instrumental and injection and buffer conditions to meet SST</p>	Low	NL	<p>The capillary material, diameter and the chiral agent are the main parameters, defining the separation mechanism and component migration order. Changing these parameters would likely result in the adaptation of the SST, and therefore the same reporting category in alignment with the SST is proposed. It was demonstrated that SST 1 and 2 provide controls for the parameters, therefore detectability is high, and the overall risk associated with changing these parameters was categorized as low.</p>

ICH Q14 Guideline

Established Condition	Overall Risk Category	ICH Q12 Reporting Category	Justification/ rationale
<b>The following conditions are not ECs in this example:</b>			
Buffer Conditions Chemicals (Pharmacopeial quality) Separation buffer (CZE): 13.2 g/l solution of ammonium phosphate adjusted to pH 6.0 with phosphoric acid filtered and 100 mM β-cyclodextrin	Low	-	During robustness studies, the variations of buffer pH +/- 0.5, ammonium phosphate concentration, and cyclodextrin concentration +/-10% were shown not having an impact on the performance of the analytical procedure. The relationship between the parameters and SST 1 and SST 2 was demonstrated during development. The data is provided as part of the Analytical Procedure Validation Report.
Instrumental conditions: Detection: 214 nm (UV) Electric Field Strength: 217 V/cm Temperature: 30 °C Separation: Separation buffer at both ends of the capillary Capillary effective length = at least 70 cm	Low	-	During robustness studies, typical variations in capillary temperature, and buffer concentrations and detection wavelength around +/-10% were shown not having an impact on the performance of the analytical procedure. The data is provided as part of the Analytical Procedure Validation Report. The relationship of electric field strength, voltage and capillary length is following scientific relationships as prior knowledge <sup>1</sup> During method development, SST 1-3 were demonstrated to be indicative for correct separation conditions. The data is provided as part of the Analytical Procedure Validation Report.
Capillary rinsing conditions: 1M sodium hydroxide, water, 0.1M sodium hydroxide Instrument parameters, Rinsing time at least 2 minutes each step at pressures greater than 1 PSI	Low	-	During method development, rinsing times were chosen to allow the capillary surface to be equilibrated with no impact on migration times within a wide range of rinsing (i.e., +/-0.5 minutes). Clear scientific relationships between pressure, capillary length and rinsing volume exist, allowing adjustments between various equipment <sup>1</sup> <small>Erreur ! Signet non défini.</small> . During method development, SST1 was demonstrated to be indicative for correct rinsing conditions. The data is provided as part of the Analytical Procedure Validation Report.
Sample Analysis Injection test solution (a) and the reference solution; injection for at least 3 s then CZE buffer injection for 2 s, about 0.5 psi pressure.	Low	-	Clear scientific relationships between pressure, capillary length and injection volume exist, allowing adjustments between various equipment <sup>1</sup> . During method development, SST1-3 were demonstrated to be indicative for correct injection conditions. The data is provided as part of the Analytical Procedure Validation Report.
API Reference Standard: Concentration of test solutions and reference standards: 1 mg/ml API in water	Low	-	The performance over the reportable working range has been demonstrated though the linearity experiments at validation. The lower concentration range control was established through SST3 based on clear scientific principles (Beer-Lambert law). The upper concentration limit is influenced by the ionic strength of the sample and a clear scientific relationship between ionic strength, field strength, Joule heating and resulting band broadening exists <sup>2</sup> . A control relationship was established with SST 1 and SST2.

<sup>1</sup> Harmonized pharmacopoeial chapters of Capillary Electrophoresis such as Phar. Eur. 2.2.47, USP <727>.

1036 Japanese Pharmacopoeia (general information capillary electrophoresis)  
1037 <sup>2</sup> M. I. Jimidar, Capillar Electrophoresis Methods for Pharmaceutical Analysis, Volume 9, 2008,  
1038 9-42 ISSN: 0149-6395  
1039

### 1040 **Change assessment and bridging strategy**

1041

1042 The assumption is that the information in the table above (ECs and reporting categories) has been  
1043 agreed upon up front with the regulatory agency.

1044 For every change, the MAH will perform a structured risk assessment to evaluate potential impact on  
1045 the performance characteristics and the link to CQA (purity) as defined in the respective ATP. As a  
1046 potential outcome of the risk assessment, experimental bridging studies to demonstrate adherence to  
1047 the performance characteristics and associated criteria will be performed. These can include, if  
1048 necessary, partial or full (re-)validation of the analytical procedure performance characteristics  
1049 affected by the change and/or comparative analysis of representative samples and standards.

1050

1051 The MAH commits to not implement the modified analytical procedure using the predefined reporting  
1052 category if adherence to the performance characteristics and associated criteria defined in the ATP  
1053 cannot be demonstrated during the bridging studies. If the precondition of adherence to the ATP  
1054 cannot be met, a higher reporting category may apply.

1055

### 1056 **Change description and management**

1057 The following scenario illustrate examples of post- approval changes and illustrate the steps a MAH  
1058 would follow when actually implementing the change.

1059

### 1060 **Change #1: Change of buffer pH**

#### 1061 Background:

1062

1063 The company has monitored and trended the migration times of the stereoisomers during routine use  
1064 and found that the migration times could be reproduced in a more stable manner by shifting the buffer  
1065 pH from 6.0 to 6.5.

1066

#### 1067 Application of Enhanced Understanding

1068

1069 Elements of the enhanced approach (understanding the relationship between SST1 and procedure  
1070 performance, procedure control strategy) were used to define a control relationship between buffer  
1071 pH and SST1 and SST 2, as communicated in the submission.

1072

#### 1073 Risk assessment:

1074

1075 The intended change was a change of the analytical procedure parameter, and this was agreed to be  
1076 managed within the company's quality system following the adherence to commitments made (i.e.,  
1077 the parameter was not an EC).

1078

1079 *a) Risk of change to the patient, product, and manufacturing process (Relevance of the test):*

1080 The product is well established and characterized safe and efficacious. The current control strategy  
1081 of the product is considered as sufficient and will not be impacted by the change. As a result, the  
1082 specifications for the chiral impurities remain unchanged.

1083

1084 *b) Complexity of the technology:*

1085 CZE is a well-established technology and the relationship of buffer pH and ionic strength on the zeta  
1086 potential of the analytes and the capillary surface can be predicted through mathematical equations.

1087

1088 *c) Risk of change to the performance of the analytical procedure (Extent of the change)*

1089 The extent of the change is low as it is a minor adjustment of the buffer pH

1090

1091 Decision Tree Question #1: Considering product and procedure knowledge and understanding, what  
1092 is the risk associated with the proposed changes to the reported result?

1093 Answer: **Low**

1094

1095 Decision Tree Question #2: Are criteria of relevant performance characteristics defined in the  
1096 dossier which ensure the quality of the measured result after the change?

1097 Answer: **Yes**

1098

1099 Demonstration of analytical procedure performance after the change

1100

1101 As there is a clear control relationship established between buffer pH and SST1 and SST2,  
1102 demonstration of meeting the SST criteria is considered as appropriate along with meeting the  
1103 relevant performance characteristics and associated criteria in the ATP.

1104

1105 Conclusions

1106 Based on the initial risk assessment and the additional controls of SST 1 and SST 2 in place, the risk  
1107 of changing the buffer pH is considered to be very low.

1108

1109 Proposed Regulatory Reporting

1110 The original agreement with the regulator that this parameter is not an EC was confirmed as a result  
1111 of the steps that were performed to implement the actual change. Thus, no regulatory reporting is  
1112 needed. The company will document this change within the PQS.

1113

1114 **Change #2: from chiral CZE to chiral HPLC**

1115 Background

1116 As chiral column technology had advanced, the company could finally identify a suitable HPLC  
1117 column and conditions for the intended purpose. The company intends to implement the analytical  
1118 procedure for the control of stereoisomers of API for release of the final drug in an additional  
1119 manufacturing site. The company strategy is to use the current (CZE) and future (HPLC) analytical  
1120 procedures as alternative procedures. A well-established technology, chiral HPLC, is targeted in the  
1121 alternative development to allow the use of a more standardized technology platform for small  
1122 molecule drug substances. The intended change is not related to any quality issues of the product, or  
1123 the established CZE procedure and the company does not intend to modify the specifications for the  
1124 chiral impurities.

1125

1126 Application of Enhanced Understanding

1127 The anticipated change will neither impact the already established product understanding nor the  
1128 expected analytical procedure performance, as described in the ATP. Additionally, the fundamentals  
1129 of the analytical techniques are well understood as general methodology and described in  
1130 pharmacopoeias. Technology and analyte behaviour are predictable. The product, analytes, and  
1131 sample preparation are well characterized and understood. Elements of the enhanced approach, such  
1132 as the clear connectivity between SST and the analytical procedure performance as described in the  
1133 ATP and risk assessment were applied to make use of the control strategy. Similar enhanced  
1134 methodology used in the development of the CZE procedure will also be applied for the development  
1135 of the HPLC procedure.

1136

1137 Risk assessment:

1138 The intended change is a change in technology, and this was agreed as an EC with NL following the  
1139 adherence to commitments made.  
1140

1141 *a) Risk of change to the patient, product, and manufacturing process (Relevance of the test):*

1142 The product is well established and characterized safe and efficacious. The current analytical control  
1143 strategy of the product is considered as sufficient and will not be impacted by the change. As a result,  
1144 the specifications for the chiral impurities remain unchanged.  
1145

1146 *b) Complexity of the technology:*

1147 Only well-established separation technologies (HPLC and CZE) are in scope.  
1148

1149 *c) Risk of change to the performance of the analytical procedure (Extent of the change)*

1150 The performance of the analytical procedure for its intended purpose is described through accuracy,  
1151 precision, specificity, and result range. The intended change may have an impact on the analytical  
1152 procedure performance. Therefore, the company has used an analytical target profile as upfront  
1153 control element to minimize the risk of change.  
1154

1155 Decision Tree Question #1: Considering product and procedure knowledge and understanding, what  
1156 is the risk associated with the proposed changes to the reported result?

1157 Answer: Medium  
1158

1159 Decision Tree Question #2: Are criteria of relevant performance characteristics defined in the  
1160 dossier which ensure the quality of the measured result after the change?

1161 Answer: Yes  
1162

1163 Demonstration of Analytical Procedure performance after the change  
1164

1165 The procedure will be validated by establishing a technology specific validation protocol and  
1166 acceptance criteria. The analytical procedure will be validated in alignment with ICH Q2(R2) Annex  
1167 2, example separation technique. The acceptance criteria for validation will be derived from the ATP  
1168 and will result in matching or stricter technology specific tests and criteria. The company has a quality  
1169 system in place which ensures:

- 1170 • Appropriate analytical change control and risk evaluation
- 1171 • The ATP is translated into suitable validation tests and criteria once the technology is  
1172 selected
- 1173 • That only analytical procedures will be used and implemented, which fulfill the performance  
1174 criteria described in the ATP
- 1175 • Therefore, at any time, the appropriate analytical procedure performance will be guaranteed  
1176 before its implementation for regular use.  
1177

1178 Conclusions

1179 Based on the initial risk assessment and the additional controls in place, the risk of using an HPLC  
1180 method as alternative method to the CZE method is considered low. The original proposed reporting  
1181 category of NL was confirmed as a result of the additional assessment and development/validation  
1182 data.  
1183

1184 Proposed Regulatory Reporting

1185 The original EC with associated reporting category as agreed upon with the regulator per Table 3 was  
1186 confirmed as a result of the steps that were performed to implement the actual change, thus the change  
1187 will be submitted as notification low.  
1188

1189 ***13.1.2 Measurement of Potency for an anti-TNF-alpha Monoclonal Antibody***

1190

1191 **Introduction and Background**

1192 The example presented refers to the measurement of the relative potency of the drug, in this case an  
1193 anti-TNF-alpha monoclonal antibody, in drug substance and in drug product at release and for  
1194 stability testing.

1195

1196 In addition to performing measurements of product CQAs, testing of potency is a unique feature of  
1197 the release specification testing panel for biologics. Biological activity, measured by the potency,  
1198 describes the specific ability or capacity of a product to achieve a defined biological effect<sup>1</sup>. Often,  
1199 for complex molecules, the physicochemical information may be extensive but unable to confirm the  
1200 higher-order structure which, however, can be inferred from the biological activity<sup>1</sup>.

1201

1202 For the purpose of this example, it is assumed that the mode of action of the drug is the neutralisation  
1203 of the biological activity of soluble TNF-alpha by preventing TNF-alpha from binding to the TNF-  
1204 alpha receptor. Fc-effector functions are out of scope of the measurement described in the example.

1205 For the purpose of this example, it is assumed that the specification limits for the relative potency are  
1206 80% to 125% of the activity of the reference standard representative for the product.

1207

1208 During development, forced degradation studies highlighted some modifications in the structure of  
1209 the molecule as confirmed by physicochemical assays. The potency assay to be developed should be  
1210 able to detect a change and/or a shift in potency upon forced degradation.

1211

1212 The performance characteristics of the procedure used to generate the reportable result are accuracy,  
1213 precision, specificity and reportable range. The evaluation of the precision involves variation of the  
1214 key sources of variability of the analytical procedure such as analyst, days, key reagents (including  
1215 cell culture parameters, if appropriate), key equipment.

1216

1217

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<sup>1</sup> ICHQ6B – specifications: test procedures and acceptance criteria for biotechnological/biological products.

1218 **Table 4: Analytical target profile**

<b>Intended Purpose</b>		
Measurement of the relative potency of an anti-TNF-alpha monoclonal antibody in Drug Substance and in Drug Product at release and for stability testing.		
<b>Link to CQA (biological activity)</b>		
The mode of action of the drug is the neutralisation of the biological activity of soluble TNF-alpha by preventing TNF-alpha from binding to the TNF-alpha receptor. The assay should be able to measure the potency of the drug and detect if there are significant changes in biological activity upon forced degradation conditions.		
<b>Characteristics of the reportable result</b>		
<b>Characteristic</b>	<b>Acceptance criteria</b>	<b>Rationale</b>
<b>Performance characteristics</b>		
<b>Accuracy</b>	Relative accuracy <sup>1</sup> is assessed via a linearity experiment that covers the reportable range. No trend in relative bias is observed over the tested relative potency range.  The 95% Confidence Interval of the slope of the fitted regression line between theoretical and measured potency falls within a range of 0.8 to 1.25.  The upper and lower 90% confidence interval for the relative bias calculated at each potency level is not more than 20% <sup>2</sup> , considering the intended purpose of the measurement.	Parameters assessed based on compendial guidance e.g., USP<1033> <sup>3</sup>  Selected performance characteristic ensures that the intended method delivers the quality reportable result.
<b>Precision</b>	Upper 95% Confidence Interval for the average intermediate precision across levels across the reportable range (95% CI % geometric coefficient of variation <sup>4</sup> ) is not more than 20% <sup>4</sup> , considering the intended purpose of the measurement.	
<b>Total Analytical Error (TAE)<sup>3</sup></b> (alternative approach to individual assessment of accuracy and precision)	Different statistical measures can be used for evaluation of the capability of the method such as comparison of the TAE (combined accuracy and precision of the measurement) with the specification limit. <sup>5</sup>	During development the specification limit may be target limits while for commercial they will be the proposed specifications.
<b>Specificity</b>	Method is specific for the intended mechanism of action of the active ingredient.	Critical characteristic of a bioassay to ensure specificity towards the targeted biological activity.
	No interference from relevant process related impurities or matrix components.	For example, process related and matrix components do not significantly affect the characteristics of the dose response curve.
	Assay is stability indicating i.e., method capable of detecting a change in potency and/or a change in the shape of the dose response curve, confirmed using forced degraded samples (for example samples subjected to meaningful thermal, photostability, and oxidative stress).	To ensure that the product remains within specification over its shelf-life (e.g., retains the required safety and efficacy). <sup>5</sup>
<b>Reportable range</b>	The relative potency range is the range that meets accuracy and precision. It should include the specification range as a minimum (e.g., 80% to 120% of the specification range in this case corresponding to 64% to 150% for a specification of 80% to 125% relative potency)	Stated range for which the required accuracy and precision characteristics are demonstrated.

- 1219 <sup>1</sup> The relative accuracy of a relative potency assay is the relationship between measured relative potency and known relative  
 1220 potency. Definition from USP<1033> Biological Assay Validation, May 2017.  
 1221 <sup>2</sup> Individual values are just an example and can be different from product to product.  
 1222 <sup>3</sup> USP <1220> Analytical Procedure Life Cycle. USP-NF 2022 ISSUE 1; USP<1210> statistical tools for procedure validation  
 1223 and references therein; P. Jackson et al., Anal. Chem. 2019, 91, 4, 2577–2585  
 1224 <sup>4</sup> USP <1033> Biological Assay Validation, May 2017  
 1225 <sup>5</sup> The suitability of this approach will depend on the phase of development and/or prior knowledge on the process performance.

1226

1227 **Technology selection:**

1228 *General considerations*

1229 Based on the ATP above, there are several current technologies that may be a suitable choice for the  
 1230 measurement of the relative potency of an anti-TNF-alpha recombinant protein as illustrated in this  
 1231 example.

1232 It is common for the analytical technology for the measurement of potency to evolve during the  
 1233 product lifecycle for biologics, with ELISA-based technologies often being initially utilized prior to  
 1234 the subsequent development of a more technically challenging specific cell-based assay. The two  
 1235 methods rely on the binding of the active substance to the soluble TNF-alpha. While the signal of the  
 1236 ELISA is directly measuring the binding, the cell-based assay may target a later stage event, i.e., a  
 1237 downstream event in the signalling cascade.

1238 Cell-based bioassays can follow several assay methodologies. In the case of anti-TNF-alpha drugs,  
 1239 this includes neutralisation assays, where the assay measures the extent of soluble TNF-alpha-  
 1240 induced cytotoxicity and apoptosis in the presence of the drug. In addition, other formats such as  
 1241 reporter gene assay can be used.

1242 The ATP as described above can also be used in a risk assessment if the technology platform is  
 1243 changed.

1244 *Cell proliferation assay as a specific example*

1245 In this example, the format of the cell-based assay chosen to measure the relative potency of the anti-  
 1246 TNF-alpha recombinant protein is a neutralisation - cell proliferation assay. It is presumed in this  
 1247 example that the Fc-effector functions are not involved.

1248 The potency will be determined by comparison of dilutions of the sample to be tested with dilutions  
 1249 of the like for like reference standard using a suitable cell-based assay based on the inhibitory action  
 1250 of the drug on the biological activity of soluble TNF-alpha with a suitable readout for assessing the  
 1251 inhibitory effect. The cell proliferation assay was chosen. This assay has the capability to monitor the  
 1252 inhibition induced by the TNF-alpha on the proliferation of a responsive cell line (e.g., murine  
 1253 fibrosarcoma WEHI-164). The assay compares the dose response of a test sample with a designated  
 1254 standard to provide a quantitative measurement of relative potency. The cells are incubated with  
 1255 varying dilutions of test sample and reference standard in presence of TNF-alpha. The cell growth is  
 1256 assessed by a staining method using a tetrazolium salt which is converted by cellular dehydrogenases  
 1257 to a colored formazan product. The amount of released formazan is measured using a  
 1258 spectrophotometer at 450 nm and 650 nm. The spectrophotometric response is directly proportional  
 1259 to the number of living cells.

1260 The throughput of the cell proliferation technology was limited to a small number of samples per day.  
 1261 The test is performed on several 96-well plates and on multiple days. The number of plates run to  
 1262 generate a valid reportable result will be established during the development of the analytical  
 1263 procedure. The equipment required to run this method are commonly used in bioassay laboratories.  
 1264 There are no specific operational nor safety concerns in applying them for bioassay trained analysts.

1265 **Analytical Procedure Development**

1266 The development of the analytical procedure described has been performed based on extensive  
1267 knowledge of the molecule and relative potency assays.

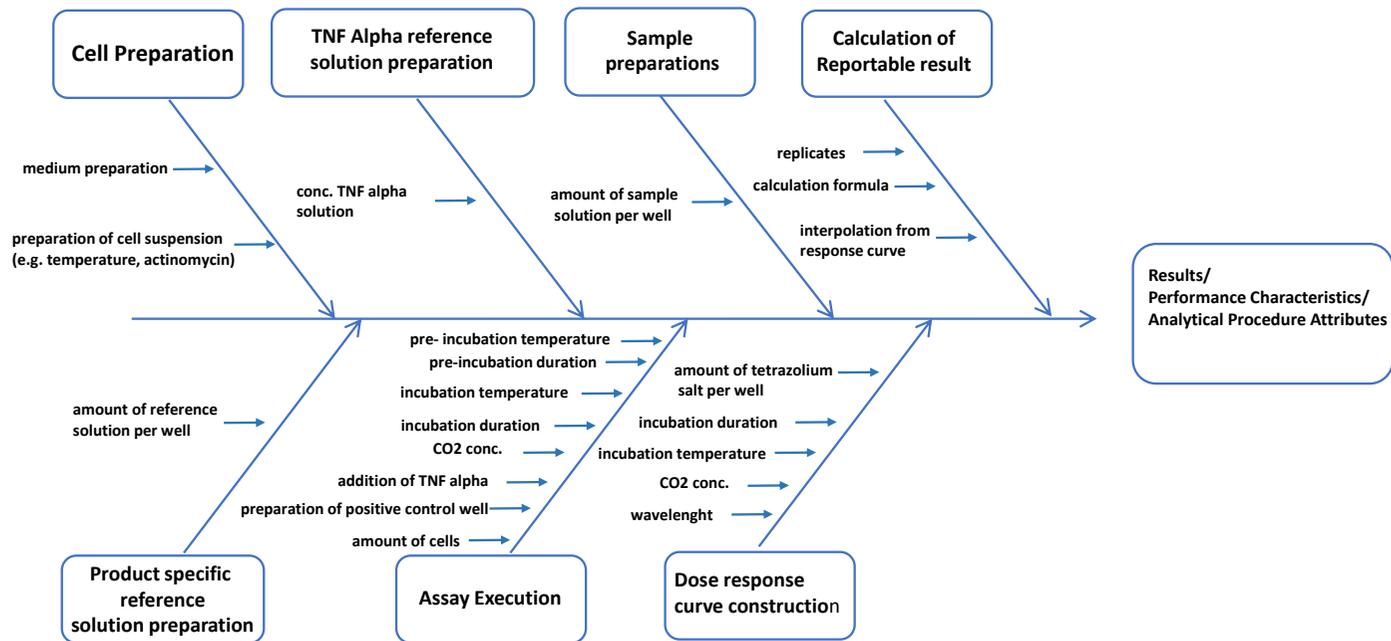
1268 The following points are considered in the establishment of the potency assay:

- 1269 - Purpose and context of the assay defined in the ATP:
- 1270 ○ The applicant has extensive knowledge about relevant factors that could impact the CQA  
1271 (relative potency of the drug) based on CQA assessment and process characterization and  
1272 has established the link between the mode of action (MOA) and the clinical performance.  
1273 Based on these data, the appropriate cell line and antigen binding conditions for the  
1274 potency assay have been selected.
  - 1275 ○ The molecule is characterized with other functional and/or physicochemical assays that  
1276 contribute to understanding of the molecule and binding properties (e.g., Fc effector  
1277 function). The other characterization assays are also continuously used in the lifecycle of  
1278 the drug.
  - 1279 ○ Performance characteristics for the analytical procedure are defined (e.g., *via* the TAE) to  
1280 support the specification acceptance criteria.
  - 1281 ○ Relative potency will be calculated for samples as compared to signal from a well-  
1282 characterized material (e.g., a reference standard) generated in the same analysis.
- 1283 - Extensive Knowledge was gained from development studies and prior knowledge on:
- 1284 ○ The **cell line** and its **performance** (viability, cultivation conditions, cell density, cell  
1285 line stability (e.g., minimum and maximum number of passages) are well understood.  
1286 Robustness of the cell cultivation conditions ensuring suitable cell metabolism was  
1287 confirmed during the development of the analytical procedure.
  - 1288 ○ Criteria for confluence and cell viability have been defined during development to  
1289 ensure the required cell metabolism and leading to an appropriate signal amplitude  
1290 and dose response curve.
  - 1291 ○ Extensive studies have been done to identify the appropriate **TNF alpha solution**  
1292 (antigen) leading to a **spectrophotometrically measurable sigmoidal dose response**  
1293 **curve** in the presence of the reference samples or test samples, with lower and upper  
1294 asymptotes corresponding to negative and positive controls, respectively.
  - 1295 ○ The assay conditions have been studied and the parameters which influence the assay  
1296 performance have been identified
  - 1297 ○ Serial dilution levels were developed to optimize the dose-response curve, e.g., to  
1298 ensure minimally three points in the linear segment of the dose-response curve and  
1299 two in each asymptote.
  - 1300 ○ The relative potency of the reference standard used in the procedure was qualified,  
1301 and criteria around its performance were established to ensure run-to-run variability  
1302 remains within suitable limits.
- 1303

1304 QRM principles were used to guide the design of development studies. Features considered during  
1305 risk assessment are shown in Figure 2.

1306

Figure 2: Ishikawa diagram



PQS requirements (e.g equipment qualification, operator training), human factors, material variability, environmental controls are considered during assessment of the individual steps, as appropriate

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1311**Table 5: Summary of development data and risk assessment**

Unit Operation	Procedure Parameter	Defined Target or Range	Investigated Range	Rationale	Risk*
Cell preparation	Cell Density (cells/mL)	1x10 <sup>6</sup> cells/mL	50 to 150 % of target value	To ensure appropriate sensitivity of the assay	medium
	Actinomycin D (µg/mL)	2 µg/mL	1-3 µg/mL	Actinomycin D is used in the assay to enhance cell susceptibility to TNF and will ensure proper sensitivity of the assay.	medium
	Cell viability	Minimum 80%	70-100%	To ensure appropriate sensitivity of the assay	medium
TNF Alpha reference standard solution preparation	Concentration of the TNF Alpha reference solution	Targeted working concentration	50 to 150% of targeted working concentration	To ensure appropriate potency determination of the anti-TNF drug	low
Reference Standard/Control Sample	Dilution factor	Target	Target	To ensure appropriate potency determination of the anti-TNF drug	low
Assay execution	Amount of cells added (µL)	50 µL	25 µL to 75 µL	Volume of cell suspension needed to ensure appropriate response of the test	low
	Pre-Incubation duration (h)	1 h	0.5 to 1.5 h	Combination of incubation conditions to allow generation of an appropriate dose response curve	low
	Pre-Incubation temperature (°C)	37°C	35-38°C	Combination of incubation conditions to allow generation of an appropriate dose response curve	low
	CO <sub>2</sub> concentration (%)	5%	3-7%	Combination of incubation conditions to allow generation of an appropriate dose response curve	low
	Incubation duration (h)	20 to 24 h	16 to 30 h	Combination of incubation conditions to allow generation of an appropriate dose response curve. For manipulation convenience, between 20 and 24 h has been	low

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				selected as target	
	Incubation temperature	37°C	35-38°C	Combination of incubation conditions to allow generation of an appropriate dose response curve	low
	CO <sub>2</sub> concentration (%)	5%	3-7%	Combination of incubation conditions to allow generation of an appropriate dose response curve	low
Dose response curve	Amount of tetrazolium salt added (µL of reconstituted solution)	10 µL	5 µl-15 µL	Salt needed to perform the colorimetric reaction and the formation of formazan	low
	Incubation duration	3 to 4 h	2 to 5 h	Duration of the incubation to ensure optimum formation of formazan. Combination of duration and temperature of incubation	low
	Incubation temperature	20°C	15-25°C	Temperature of the incubation to ensure optimum formation of formazan. Combination of duration and temperature of incubation	low

1312

1313

\* Risk refers to the impact on the reportable results (considering established controls (e.g., SST are fulfilled)

1314 **Analytical procedure description<sup>2</sup>**

1315 Equipment:

- 1316 - 96-well plates
- 1317 - Tissue culture flasks
- 1318 - CO<sub>2</sub> incubator
- 1319 - Biosafety cabinet
- 1320 - Plate reader

1321

1322 Solutions & reagents:

- 1323 - WEHI-164 cells (ATCC)
- 1324 - TNF-alpha solution:
  - 1325 ○ Dissolve the contents of a vial of TNF-alpha according to the supplier's
  - 1326 instructions. Further dilute with assay medium to obtain a suitable working
  - 1327 concentration. The cellular response to TNF-alpha varies and a suitable TNF-
  - 1328 alpha concentration (e.g., ED<sub>80</sub>) is determined using a TNF-alpha dose response
  - 1329 curve.
- 1330 - Assay medium composed of RPMI 1640, L-glutamine, heat-inactivated fetal bovine
- 1331 serum (10% v/v) and a penicillin/streptomycin solution (1% v/v)
- 1332 - Actinomycin D
- 1333 - Tetrazolium salt WST-8 (5-(2,4-disulfophenyl)-3-(2-methoxy-4-nitrophenyl)-2-(4-
- 1334 nitrophenyl)-2*H*-tetrazol-3-ium sodium)
- 1335 - Reference standard

1336

1337 Procedure:

1338 The number of assay plates and days for each sample will depend on the control strategy  
1339 defined for the method.

- 1340 - Reference solution and test solution:
  - 1341 ○ Dilute with assay medium to the appropriate concentration. Analyse in duplicate.
- 1342 - Plate preparation:
  - 1343 ○ Add 150 µL of assay medium to the wells designated for 'cell only control' and
  - 1344 for blanks on a 96-well microplate.
  - 1345 ○ Add 100 µL of assay medium and 50 µL of TNF-alpha working solution to the
  - 1346 wells designated for 'cell + TNF-alpha control'.
  - 1347 ○ Add 100 µL of assay medium to the sample wells and 200 µL of the test or
  - 1348 reference solutions.
  - 1349 ○ Further prepare a series of 2-fold dilutions.
  - 1350 ○ Then add 50 µL of TNF-alpha working solution.
  - 1351 ○ Incubate at 36.0-38.0°C for 1h in an incubator using 5±2% CO<sub>2</sub>.
- 1352 - Cell preparation
  - 1353 ○ Prepare a suspension of WEHI-164 cells containing 1x10<sup>6</sup> cells per milliliter,
  - 1354 using assay medium containing 2 µg/mL of actinomycin D.

1355

1356 <sup>2</sup> Contains binding information (ECs) and non-binding information  
1357

- 1358 - Plating cells
- 1359     o Add 50 µL of the cell suspension to each well maintaining the cells in a uniform
- 1360     suspension during addition.
- 1361     o Incubate at 36.0-38.0°C for 20-24 h in an incubator using 5±2% CO<sub>2</sub>.
- 1362 - Addition of tetrazolium salt and absorbance measurement
- 1363     o Remove 100 µL of medium from each well.
- 1364     o Add 10 µL of reconstituted WST-8 mixture to each well and reincubate for 3-4 h.
- 1365     o Measure the absorbance using a microplate reader at 450 nm and 650 nm.
- 1366     o Estimate the quantity of formazan produced by subtracting the reading at 650 nm
- 1367     from the reading at 450 nm.

1368  
1369 Calculations:

- 1370 - Calculate the potency of the preparation to be examined using the four-parameter
- 1371 logistic curve model.
- 1372 - The reportable result is calculated in accordance with the defined number of replicates
- 1373 which is determined during development. Replication strategy may include averaging of
- 1374 the results of multiple plates, typically 3. Individual results within the range of the assay
- 1375 and having passed the sample suitability assessment are used for the calculation of the
- 1376 reportable result.

1377  
1378 Analytical procedure control strategy

1379 The analytical procedure control strategy for relative potency determination using the cell

1380 proliferation assay (performed as described in the example above) can include the following

1381 elements:

1382 System Suitability Test

- 1383 - The dose-response curve obtained for the reference standard curve corresponds to a
- 1384 sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and
- 1385 ‘cell + TNF-alpha control’, respectively.
- 1386 - The dose-response curve obtained for the test sample corresponds to a sigmoid curve
- 1387 with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell treated with
- 1388 TNF-alpha control’, respectively.
- 1389 - The coefficient of determination calculated for each standard curve ( $r^2$ ) is not less than
- 1390 e.g., 0.97.
- 1391 - Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum e.g.,
- 1392 3.0.

1393 Sample suitability assessment:

1394 E.g., Assessment of similarity/ parallelism:

- 1395 - The upper asymptote ratio (A std/A test): e.g., 0.8-1.2
- 1396 - The lower asymptote ratio (D std/D test): e.g., 0.8-1.2
- 1397 - The Hill slope ratio (B std/B test): e.g., 0.8-1.2
- 1398 - The upper to lower asymptote ratio ((D-A) std/(D-A) test): e.g., 0.8-1.2

1399

1400 **Analytical procedure validation according to ICH Q2:**

- 1401 - Validation protocol including predefined acceptance criteria for cell-based assay
- 1402 ○ Performance characteristics as defined in the ATP:
- 1403     ▪ Accuracy
- 1404         Established by using various starting dilutions to generate different dose
- 1405         response curves
- 1406         • Acceptance criteria:
- 1407             ○ Relative accuracy is assessed via a linearity experiment that
- 1408             covers the reportable range. No trend in relative bias is
- 1409             observed over the tested relative potency range.
- 1410             ○ The 95% Confidence Interval of the slope of the fitted
- 1411             regression line between theoretical and measured potency falls
- 1412             within a range of 0.8 to 1.25.
- 1413             ○ The upper and lower 90% confidence interval for the relative
- 1414             bias calculated at each potency level is not more than 20%,
- 1415             considering the intended purpose of the measurement.
- 1416     ▪ Precision
- 1417         • Acceptance criterion:
- 1418             Upper 95% confidence interval for the average intermediate precision
- 1419             across the reportable range (95% CI % geometric coefficient of
- 1420             variation) is not more than 20% considering the intended purpose of
- 1421             the measurement.
- 1422     ▪ Specificity
- 1423         • Acceptance criteria:
- 1424             ○ The method is specific for the intended mechanism of action
- 1425             of the active ingredient, i.e., no dose response curve is
- 1426             obtained (failure of one or more of the assay acceptance
- 1427             criteria) when other biological products are tested using the
- 1428             same method parameters.
- 1429             ○ No interference from relevant process related impurities or
- 1430             matrix components, i.e., process related impurities and matrix
- 1431             components do not significantly affect the characteristics of
- 1432             the dose-response curve.
- 1433             ○ The assay is stability indicating, i.e., the method is capable of
- 1434             detecting a change in potency and/or a change in the shape of
- 1435             the dose-response curve, confirmed using forced degraded
- 1436             samples (for example samples subjected to meaningful
- 1437             thermal, photostability, or oxidative stress).
- 1438     ▪ Reportable range
- 1439         • Acceptance criterion:
- 1440             The relative potency range is the range that meets accuracy and
- 1441             precision. The reportable range should include the specification range
- 1442             as a minimum (e.g., 80% to 120% of the specification range). In this
- 1443             case, the reportable range corresponds to 64% to 150% relative
- 1444             potency.
- 1445
- 1446

- 1447 ○ Technology-dependent analytical procedure attributes:
- 1448     ▪ Linearity of the results
- 1449         The relative accuracy is the relationship between measured relative potency
- 1450         and known relative potency.
- 1451         • Acceptance criteria:
- 1452             ○ The upper and lower 90% confidence relative accuracy is
- 1453             assessed via a linearity experiment that covers the reportable
- 1454             range. No trend in relative bias is observed over the tested
- 1455             relative potency range.
- 1456             ○ The 95% confidence interval of the slope of the fitted
- 1457             regression line between theoretical and measured potency falls
- 1458             within a range of 0.8 to 1.25.
- 1459     ▪ Working range of the analytical procedure, i.e., upper to lower levels for
- 1460         which a suitable response curve is achieved.
- 1461         Individual potency results are used to generate the reportable result according
- 1462         to the replication strategy defined in the development.
- 1463         • acceptance criteria:
- 1464             ○ The final reportable result is within the specifications. The
- 1465             individual results agree to a defined RSD, 20%, and are
- 1466             covered by the validation range.
- 1467             ○ The validated range of the method is wide enough to
- 1468             encompass the individual result.

1469 - Execution of the validation

1470 The results were summarized in a validation report, which concluded that the analytical procedure  
1471 would meet the acceptance criteria for the analytical procedure attributes. Implicitly, the  
1472 performance characteristics were met and, in summary, the analytical procedure was suitable for  
1473 the intended purpose.  
1474

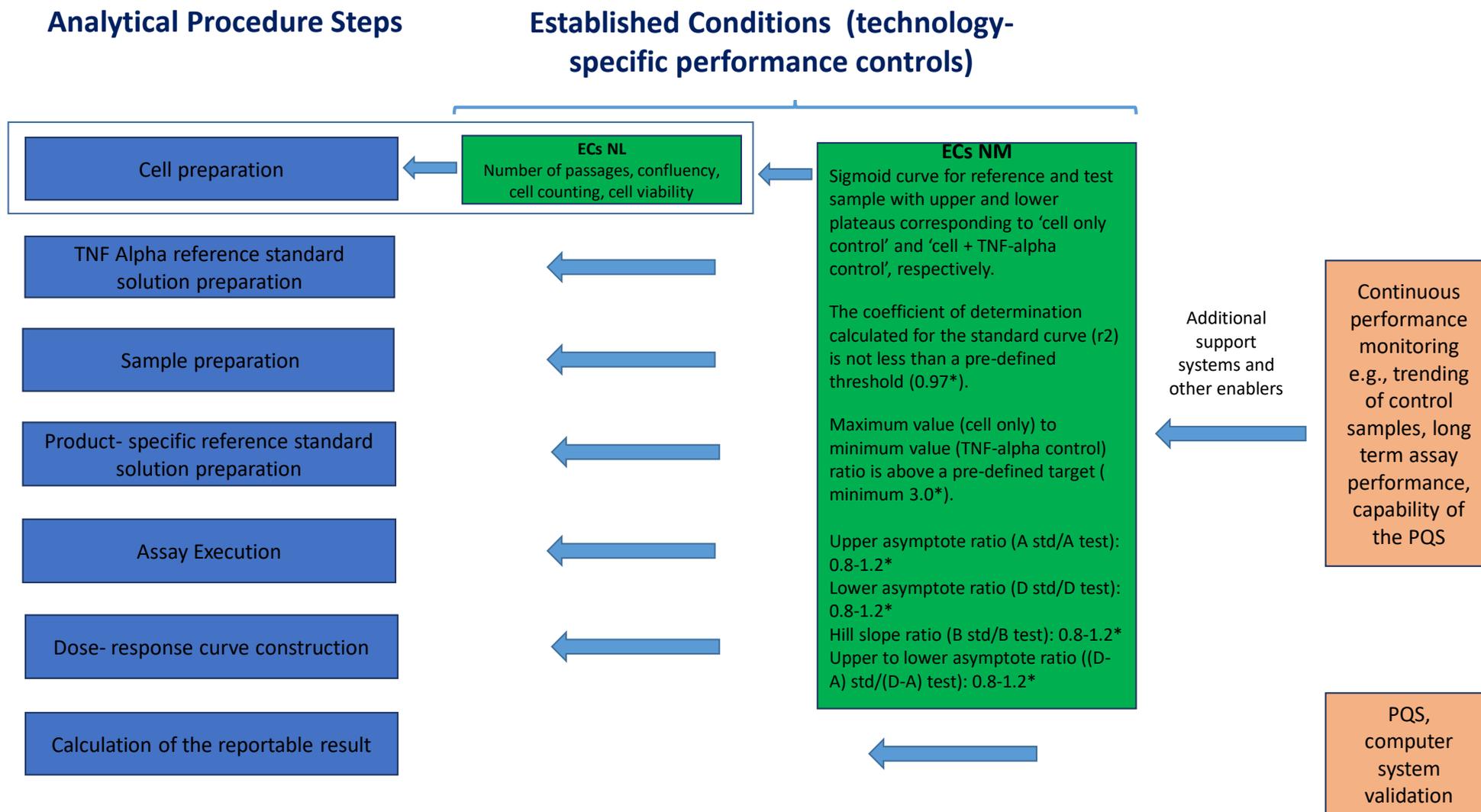
1475  
1476 **Description of Established Conditions, Reporting Categories, and Justifications**

1477 Based on product and process understanding, and considering the procedure development data, the  
1478 Applicant proposed Established Conditions and reporting categories, as part of the initial submission.  
1479 Justification of reporting categories for changes includes adherence to predefined acceptance criteria  
1480 described in the Analytical Target Profile and additional performance controls (e.g., system suitability  
1481 testing and control samples).

1482 Figure 3 illustrates which analytical procedure steps are relevant for the performance controls defined  
1483 as established conditions together with the additional continuous performance monitoring enablers.  
1484 Table 6 describes the ECs, their reporting categories and justification.

1485 *Note: The number of ECs, associated reporting category listed in this table may depend on the extent*  
1486 *of knowledge gained and information provided. The information provided in this example is not the*  
1487 *entirety of the knowledge that is available and will be submitted to regulatory agencies. The extent of*  
1488 *ECs, actual reporting categories, and data requirements may differ by region. Other parameters and*  
1489 *conditions that are not identified as ECs in the table below may be required as EC for some cases*  
1490 *depending on the region. The changes to other method principles may constitute different risks and*  
1491 *may lead to different reporting categories. PACMP may be required for some cases (e.g., a change*  
1492 *between technologies) depending on region.*

1493 **Figure 3: – Illustration of the performance control strategy of the analytical procedure**



\* Individual values are just an example and can be different from product to product

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**Table 6:** Proposed established conditions and reporting categories applying principles of ICH Q12 in the enhanced approach

Established conditions	ICH Q12 Reporting Category	Justification/rationale
Performance characteristics as reported in the ATP	PA	Relevant performance characteristics to control the CQA
Technology (principle) Cell Based Assay	PA or NM <sup>1</sup>	Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes
<b>Analytical procedure parameter</b>		
Related to the control strategy elements (SST, sample suitability assessment)		
The dose-response curve obtained for the reference standard curve corresponds to a sigmoid curve with upper and lower plateaus corresponding to 'cell only control' and 'cell + TNF-alpha control', respectively	NM	The long-term performance of the analytical procedure is ensured by the adherence to ATP and by successful execution of the bridging strategy and PQS.
The dose-response curve obtained for the test sample corresponds to a sigmoid curve with upper and lower plateaus corresponding to 'cell only control' and 'cell + TNF-alpha control', respectively.	NM	
Coefficient of determination calculated for each standard/sample curve ( $r^2$ ); $r^2$ is not less than 0.97 <sup>2</sup>	NM	
Maximum value (cell only) to minimum value (TNF-alpha control) ratio. Minimum ratio 3.0 <sup>2</sup>	NM	

## ICH Q14 Guideline

Established conditions	ICH Q12 Reporting Category	Justification/rationale
Assessment of similarity/ parallelism: e.g., The upper asymptote ratio (A std/A test): 0.8-1.2 <sup>2</sup> The lower asymptote ratio (D std/D test): 0.8-1.2 <sup>2</sup> The Hill slope ratio (B std/B test): 0.8-1.2 <sup>2</sup> The upper to lower asymptote ratio ((D-A) std/(D-A) test): 0.8-1.2 <sup>2</sup>	NM	
Cell Preparation		
Cell line; WEHI-164 cells (ATCC)	NM	Based on the understanding of the mode of action (link to CQA) the suitability of the responsive cell line will be confirmed by responding to the TNF-alpha (survival of the cell in presence of the drug and cell death without drug).  Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes.  Revised system suitability test should ensure the suitability of the cell line and its performance (number of passages, confluency, cell counting, cell viability, signal amplitude, shape of the response curve)
Preparation of cells: sub culturing	NL	Sufficient cell performance to detect changes in the quality of the drug is ensured by: System suitability of the method covers the suitability of the cell preparation (number of passages, confluency, cell counting, cell viability, signal amplitude, shape of the response curve). Changes in cell metabolism that impact performance of the method and link to CQA will be detected. Changes that lead to insufficient cell performance will not be implemented as they could have an impact on the defined performance characteristics and would require prior approval.
Medium composition: RPMI 1640, L-glutamine, heat-inactivated fetal bovine serum, and a suitable antibiotic	NL	Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes.

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Established conditions	ICH Q12 Reporting Category	Justification/rationale
Preparation of a suspension of WEHI-164 cells containing $1 \times 10^6$ cells per milliliter, using assay medium containing $2 \mu\text{g/mL}$ of actinomycin D.	NL	
<b>TNF-alpha reference standard solution preparation</b>		
<p>Concentration of the TNF-alpha solution: Dilute with assay medium to obtain a suitable working concentration (e.g., ED80) as determined using a TNF-alpha dose response curve and meeting the control strategy elements.</p> <p>Shape of the TNF-alpha dose response curve:</p>	NL	<p>The effect of the drug on the TNF-alpha, which is the basis of the mode of action of the drug, is demonstrated by:</p> <p>Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes.</p> <p>1/ The dose-response curve obtained for the reference standard curve corresponds to a sigmoid curve with upper and lower plateaus corresponding to 'cell only control' and 'cell + TNF-alpha control', respectively.</p> <p>2/ The dose-response curve obtained for the test sample corresponds to a sigmoid curve with upper and lower plateaus corresponding to 'cell only control' and 'cell + TNF-alpha control', respectively.</p> <p>3/ The coefficient of determination calculated for the standard curve (<math>r^2</math>) is not less than 0.97.<sup>2</sup></p> <p>4/Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum 3.0.<sup>2</sup></p> <p>5/ Adherence to sample suitability assessment criteria</p>

## ICH Q14 Guideline

Established conditions	ICH Q12 Reporting Category	Justification/rationale
<b>Sample Preparation and product specific reference solution preparation</b>		
Preparation of the test sample and reference solutions: suitable amount of the solutions per well to meet the control strategy elements	NL	<p>The suitability of the readout and of the dose response curve is ensured by the control strategy elements:</p> <ol style="list-style-type: none"> <li>1/ The dose-response curve obtained for the reference standard curve corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively.</li> <li>2/ The dose-response curve obtained for the test sample corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively.</li> <li>3/ The coefficient of determination calculated for the standard curve (<math>r^2</math>) is not less than 0.97<sup>2</sup>.</li> <li>4/Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum 3.0<sup>2</sup>.</li> <li>5/ Adherence to sample suitability assessment criteria</li> </ol> <p>And by: Adherence to ATP ensured by bridging strategy and PQS<sup>3</sup></p>
<b>Assay Execution Step</b>		
Preparation of the positive control wells: Suitable Amount of TNF-alpha added	NL	<p>The suitability of the readout and of the dose response curve is ensured by the control strategy elements:</p> <ol style="list-style-type: none"> <li>1/ The dose-response curve obtained for the reference standard curve corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively.</li> <li>2/ The dose-response curve obtained for the test sample corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively.</li> <li>3/ The coefficient of determination calculated for the standard curve (<math>r^2</math>) is not less than 0.97<sup>2</sup>.</li> <li>4/Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum 3.0<sup>2</sup>.</li> </ol>
Addition of the TNF-alpha solution to the wells: Suitable Amount of TNF-alpha solution per well	NL	
Amount of cells added Add suitable amount of the cell suspension to each well maintaining the cells in a uniform suspension during addition	NL	
Pre-incubation temperature and duration allowing to meet the control strategy elements Conditions (temperature, duration, %CO <sub>2</sub> )	NL	

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Established conditions	ICH Q12 Reporting Category	Justification/rationale
Incubation temperature and duration allowing to meet the control strategy elements Condition (temperature, duration, %CO <sub>2</sub> )	NL	5/ adherence to sample suitability assessment criteria  And by: Adherence to the ATP ensured by the bridging strategy and PQS <sup>3</sup>
<b>Dose response curve construction</b>		
Reconstitute the Tetrazolium salt WST-8 (5-(2,4-disulfophenyl)-3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetrazol-3-ium sodium)	NL	The suitability of the readout of the quantification of the effect of the drug on the cell is ensured by the control strategy elements: 1/ The dose-response curve obtained for the reference standard curve corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively. 2/ The dose-response curve obtained for the test sample corresponds to a sigmoid curve with upper and lower plateaus corresponding to ‘cell only control’ and ‘cell + TNF-alpha control’, respectively. 3/ The coefficient of determination calculated for the standard curve (r <sup>2</sup> ) is not less than 0.97 <sup>2</sup> . 4/Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum 3.0 <sup>2</sup> . 5/ adherence to sample suitability assessment criteria  And by: Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes <sup>3</sup>
Add a suitable amount of the reconstituted tetrazolium salt to each well to meet the control strategy elements	NL	
Incubation conditions (temperature, duration) allowing to meet the control strategy requirements:	NL	
Wavelength: 450 nm and 650 nm	NL	
Four parameter logistic curve model	NL	

1499 PA: Prior Approval, NM: notification moderate; NL: notification low (as per ICH Q12 definitions)

1500 <sup>1</sup> NM if no impact of the change on specification, PA if there is an impact on the specification (see case 1 and 2 below). Note, however, that regulatory agreement may differ by region.

1501 <sup>2</sup> Individual values are just an example and can differ from product to product.

1502 <sup>3</sup> Reporting category was initially NM but has been downgraded to NL based on the justification provided

1503 **The following parameters are not ECs:**

- 1504 • Preparation of the negative control wells
- 1505 • Plating format

1506 **Change assessment and bridging strategy**

1507  
1508 The assumption is that the information in the table above (ECs and reporting categories) has  
1509 been agreed upon up front with the regulatory agency.

1510  
1511 For every change, the MAH will perform a structured risk assessment to evaluate potential  
1512 impact on the performance characteristics and the link to CQA (biological activity) as defined  
1513 in the respective ATP. As a potential outcome of the risk assessment, experimental bridging  
1514 studies to demonstrate adherence to the performance characteristics and associated criteria will  
1515 be performed. These can include, if necessary, partial or full (re-)validation of the analytical  
1516 procedure performance characteristics affected by the change and/or comparative analysis of  
1517 representative samples and standards.

1518  
1519 The MAH commits to not implement the modified analytical procedure using the predefined  
1520 reporting category if adherence to the performance characteristics and associated criteria  
1521 defined in the ATP cannot be demonstrated during the bridging studies.

1522 .

1523 **Change Description and Management**

1524 The following scenarios illustrate examples of post- approval changes and illustrate the steps a  
1525 MAH would follow when actually implementing the change.

1526

1527 **Change #1: from classical cell culture (continuous cell culture) to ready to use cells (frozen**  
1528 **cells)**

1529 **i) Background of change**

1530 Change from continuous cell culture to ready to use cells for cell-based potency assay using the  
1531 same cell line. This change affects only the analytical procedure step cell preparation.  
1532 Conditions of freezing and thawing of the cells are the key parameters to control (cell  
1533 metabolism of responsive cell line) for the success of this change, while the rest of the analytical  
1534 procedure is unchanged. This change is inside the technology and is not expected to have an  
1535 impact on the specifications.

1536 **ii) Summary of structured risk assessment:**

1537 **The relevance of the test** is classified as high as there is a direct link to the CQA potency,  
1538 which is key for ensuring the efficacy of the drug. The change is not expected to impact the link  
1539 to the CQA (same cell line used, same readout) and has low criticality in this respect.

1540 The cell-based assay used for the measurement of potency represents a **complex technology** as  
1541 such assays have multiple sources of variability. Factors contributing to variability are well  
1542 understood (based on prior knowledge and enhanced development data) and addressed in the  
1543 analytical procedure control strategy.

1544 **The extent of the change** is restricted to the preparation of the cells (change in analytical  
1545 procedure step cell preparation), with potential impact on only one analytical procedure

1546 attribute (cell metabolism). Factors contributing to the cell performance are understood,  
 1547 investigated as part of development of the ready to use cell preparation and monitored by the  
 1548 SST.

1549 The initial risk assessment proposed a moderate risk. Further evaluation was performed  
 1550 following Step 2 of ICH Q14 Figure 2.

1551 **iii) Adherence to criteria for relevant performance characteristics**

1552 The understanding of the analytical procedure and link to the CQA allowed the definition of  
 1553 criteria for relevant performance characteristics which ensure the post change quality of the  
 1554 measured result after the change (please refer to Table 4). The change can potentially affect cell  
 1555 metabolism and hence the method performance characteristics accuracy and precision. Before  
 1556 implementation of the change, adherence to these performance characteristics should be  
 1557 demonstrated. This change does not impact the performance characteristics specificity and  
 1558 reportable range as the same cell line is used and the potency is measured against the same  
 1559 reference standard.

1560

1561 **iv) Demonstration of Analytical Procedure performance after change**

1562 *Evaluation of impact on performance characteristics*

1563 Based on analytical procedure understanding the following parameters that could potentially  
 1564 impact the performance have been evaluated and defined in the analytical procedure  
 1565 description: Cell freezing and thawing conditions/cell metabolism are the key parameters to  
 1566 control (freezing medium, freezing conditions, growth/assay medium). The SST of the method  
 1567 covers the suitability of the cell preparation (e.g., confluency, cell density, cell viability, signal  
 1568 amplitude, shape of the response curve).

1569 *Experimental Bridging Study Results*

1570 In accordance to Table 2 of ICH Q14 a partial revalidation of the analytical procedure was  
 1571 performed to demonstrate the affected analytical procedure attributes are met after the change.  
 1572 Comparative analysis of a set of representative samples with pre- and post-change analytical  
 1573 procedure will be performed to ensure that the achieved results are comparable or that observed  
 1574 differences are acceptable and do not impact the established specification.

1575 **v) Conclusion**

1576 Evaluation of performance characteristics demonstrated that defined criteria could be met. The  
 1577 result of the studies confirmed the expected cell performance post change. The purpose of the  
 1578 method has not changed and its capability to generate the reportable result is unchanged.  
 1579 Method bridging was successfully performed. The risk associated with the change is considered  
 1580 low taking into account the outcome of the initial risk assessment, the evaluation of the  
 1581 performance characteristics and the bridging study results.

1582 **vi) Regulatory reporting:**

1583 The original EC with associated reporting category as agreed upon with the regulator per Table  
 1584 6 was confirmed as a result of the steps performed, thus the change is proposed as notification  
 1585 low. The revised analytical procedure description together with the analytical validation report  
 1586 and the outcome of the bridging study will be submitted accordingly. The SST criteria of the  
 1587 analytical procedure including those ensuring sufficient cell performance remain unchanged.

1588 Appropriated development data demonstrating suitable absence of impact on cell performance  
1589 upon preparation and handling frozen cell will be provided.

1590

1591 **Change #2: from binding ELISA to cell-based assay**

1592 Another example considers a development scenario where the MAH has initially developed a  
1593 binding assay (ELISA) to determine the relative potency of the anti TNF alpha recombinant  
1594 protein and plans to implement a cell-based assay post approval. The measurement requirement  
1595 as defined in the ATP (Table 4) and included in the initial marketing authorization remained  
1596 unchanged and were used to support assay development and implementing the change.

1597 **i) Background of change:**

1598 Change from binding ELISA to cell-based assay. Both methodologies evaluate the relative  
1599 potency of the drug in comparison to a reference standard. However, the evaluation of the  
1600 mechanism of action is usually different: Binding ELISA targets early-stage event (binding  
1601 activity only), while cell-based assay targets late stage event, i.e., downstream event in the  
1602 signaling cascade. The change from ELISA to a cell-based assay is outside the technology and  
1603 a potential impact on the specifications acceptance criteria cannot be excluded.

1604 **ii) Summary of structured risk assessment:**

1605 The **relevance of the test** is classified as high as there is a direct link to the CQA potency, which  
1606 is key for ensuring the efficacy of the drug. The change could impact the measurement of the  
1607 CQA potency as the change is from an immunochemical binding assay to a cell-based assay  
1608 where also downstream event cascades can be targeted. However, this change is expected to  
1609 better reflect the mode of action of the product.

1610 The cell-based assay proposed to be used for the measurement of potency represents a **complex**  
1611 **technology** as it is related to multiple sources of variability. Analytical procedure parameters  
1612 have been evaluated following a risk-based approach and it could be demonstrated that factors  
1613 contributing to variability are well understood (based on prior knowledge and enhanced  
1614 development data) and addressed in the analytical procedure control strategy.

1615 **The extent of the change** is high as a change in technology from an immunochemical binding  
1616 assay to a cell-based assay is foreseen. The functional properties of the molecule and related  
1617 mode of action are well understood and supported by preclinical and clinical data. Different  
1618 responsive cell line candidates have been screened. The WEHI 164 cell line and the assay  
1619 format (cell proliferation) have been chosen based on predefined selection criteria and the mode  
1620 of action of the molecule. To address the mode of action of the molecule (anti-TNF), a TNF-  
1621 alpha standard is used to measure the impact of its addition on the proliferation of the cells in  
1622 presence of the drug. Optimal amounts of TNF-alpha and of drug have been identified and are  
1623 described in the analytical procedure. Relevant SST criteria have been defined to ensure the  
1624 proper control of the analytical procedure (refer to analytical procedure description). The initial  
1625 risk assessment proposed a high risk. Further evaluation was performed following Step 2 of  
1626 ICH Q14 Figure 2.

1627 **iii) Adherence to criteria for relevant performance characteristics**

1628 The understanding of the analytical procedure and link to the CQA allowed the definition of  
1629 criteria for relevant performance characteristics which ensure the quality of the measured result  
1630 after the change (please refer to ATP table above). In spite of analytical method principle being

1631 different between the immunochemical binding ELISA and the cell-based assay methods, in  
1632 both procedures the reportable result is measured and calculated relative to the same reference  
1633 standard allowing data normalisation (RS used as “internal calibrator”). Consequently, the  
1634 reportable result is expressed using the same approach (% relative potency). However, based  
1635 on the extent of change a validation of the new procedure including data driven assessment of  
1636 adherence to the performance characteristics as defined in ATP is required.

1637

1638 **iv) Demonstration of Analytical Procedure performance after change**

1639 The cell-based assay was developed based on the criteria defined in the ATP. After development,  
1640 validation of the analytical procedure was performed.

1641 If adherence to the performance characteristics as defined in the ATP can be demonstrated and  
1642 no change to the specification acceptance criteria is needed, then the bridging studies will be  
1643 initiated.

1644 However, due to the complex nature of the cell-based assay, the performance characteristics  
1645 may be affected compared to the binding ELISA (e.g., precision). An assessment should be  
1646 done to determine if the performance of the assay still meets the criteria described in the ATP  
1647 and supports the specification acceptance criteria. In case a change of the performance criteria  
1648 described in the ATP and/or the specification acceptance criteria is needed, the change should  
1649 follow a pre-approval pathway.

1650 ***Experimental Bridging Study Results***

1651 In accordance to Table 2 of ICH Q14 a full validation of the cell-based procedure was performed  
1652 to demonstrate the suitability for its intended purpose. The cell-based procedure was found to  
1653 satisfy the requirements of the ATP. Comparative analysis of a set of representative samples  
1654 with the ELISA and cell-based analytical procedures was performed including representative  
1655 degraded samples (forced degraded samples able to detect a loss of potency or end of shelf-life  
1656 samples). The studies were designed to demonstrate continuity of the results generated with the  
1657 two methods (e.g., abnormal results should be detected as non-conforming by both methods).

1658 **v) Conclusions**

1659 Validation of the cell-based procedure and evaluation of performance characteristics  
1660 demonstrated that the defined criteria were met. The result of the studies demonstrated the  
1661 ability of both the ELISA and cell-based procedures to measure relative potency with the  
1662 required levels of accuracy, precision and specificity. The purpose of the analytical procedure  
1663 had not changed and its capability to generate the reportable result was unchanged.

1664 Method bridging was successfully performed. The change evaluation showed that the extent of  
1665 change had no impact on the ATP nor on specifications. In addition, the bridging evaluation of  
1666 the two methods had confirmed that the relative potency specification remained unchanged.  
1667 The risk associated with the change was considered moderate taking into account the outcome  
1668 of the initial risk assessment, the evaluation of the performance characteristics and the bridging  
1669 strategy.

1670 **vi) Regulatory reporting**

1671 The original EC with associated reporting category as agreed upon with the regulator per Table  
1672 6 was confirmed as a result of the steps performed, thus the implementation of the change will

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1673 be submitted to the relevant regulatory authorities using “Notification moderate” category. The  
1674 revised analytical procedure description together with the analytical validation report and the  
1675 outcome of the bridging study will be submitted.

1676

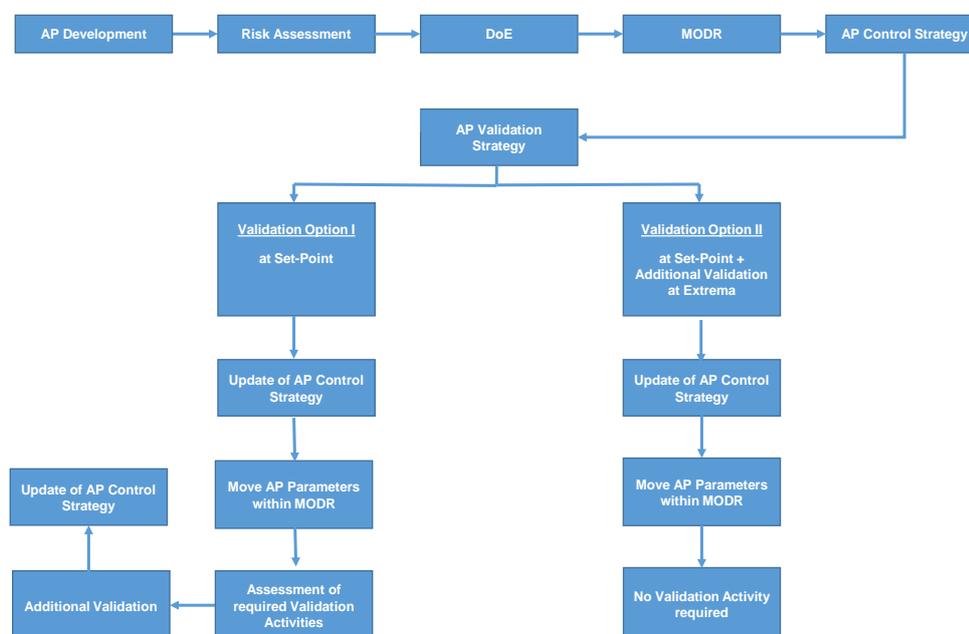
1677 **13.2 Annex B: Validation Strategies for MODRs**

1678 This annex describes validation strategies for MODRs and includes an example table to present  
 1679 the performance characteristics combined with the attribute acceptance criteria, parameter  
 1680 ranges, control strategy and validation strategy.

1681 *ICH Q2* provides the concepts for analytical procedure validation. Generally, the operating  
 1682 space needs to be covered by validation data. The extent of validation activities and the  
 1683 respective operational flexibility associated requires to be assessed and justified on a case-by-  
 1684 case basis. Performance characteristics whose validation is already comprised by development  
 1685 are not considered. Two options below represent examples of typical approaches, allowing also  
 1686 in-between solutions.

- 1687
- 1688 Option 1: For validation, at minimum, a single set of univariate operating parameters of  
 1689 the MODR is selected (typically the intended operational conditions or the set  
 1690 point). For future changes of the parameters within the MODR an assessment  
 1691 with regard to additional validation activities should be performed. The strategy  
 1692 for determining the extent of additional validation should be described in the  
 1693 submission
- 1694 Option 2: The validation of the set point, e.g., center point, and the extrema of the MODR  
 1695 allows full operational flexibility within the MODR without demand for further  
 1696 validation activities.

1697 Figure 1 gives an overview on the lifecycle steps of an analytical procedure showing the impact  
 1698 of the two different validation options.



1699

1700 **Figure 1:** Analytical Procedure Lifecycle following different validation options

1701 Table 1 represents an approach to summarize the basic knowledge on an analytical procedure  
 1702 and can be used as a consulting resource for changes. It is an example how to compile the core  
 1703 information of an analytical procedure based on the ATP (col. B) and the DoE results (columns

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1704 D, E, F), leading to the definition of the MODR (col. D) as well as the individual ranges which  
 1705 are shown to fulfil the criteria of specific analytical procedure attributes (col. E). The MODR  
 1706 (col. D) originates common overlap of these individual ranges (col. E), whereas the existing  
 1707 information (col. F) defines the entire investigated range covered by the experiments. At the  
 1708 same time, Table 1 allows to align the acceptance criteria of the analytical procedure attributes  
 1709 (col. B) with the analytical procedure control strategy (col. G) and even to set up an analytical  
 1710 procedure validation strategy (col. H) for the analytical procedure performance characteristics  
 1711 (col. A) derived from ICH Q2. The experimental scheme for future movements of parameters  
 1712 within an MODR can be predefined in the analytical procedure control strategy (col. G).

1713

1714 **Table 1:** Comprehensive compilation of analytical procedure information

A AP Performance Characteristic	B AP Attributes based on ATP	C AP Parameters with potential influence on the AP Attribute (based on AP Risk Assessment)	D Parameter Range			G AP Control Strategy	H AP Validation Strategy
			MODR	shown to fulfil the specific AP Attribute	Existing Information *		
Specificity / Selectivity	separation of impurities A and B: $R_s \geq NNN$	column temperature	35 - 42°C	32 - 60°C	20 - 60°C	- MODR - $R_s \geq NNN$ for impurity A and B for SST solution	validation covered by MODR and SST
		gradient slope	3.0 - 4.5% eluent B/min	2.5 - 5.0% eluent B/min	1.0 - 10.0% eluent B/min		
		flow rate	0.8 - 1.2 ml/min	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min		
Precision	TAE $\leq$ NNN% for impurity A	column temperature	35 - 42°C	32 - 60°C	20 - 60°C	- validation - instrument qualification - SST: RSD of reference solution (impurities) $\leq$ NNN%	validation of precision: - repeatability (n = NN): RSD $\leq$ NNN% - intermediate precision (n = NN): RSD $\leq$ NNN% - intermediate precision: $\Delta$ vs. repeatability $\leq$ NNN%
		gradient slope	3.0 - 4.5% eluent B/min	2.5 - 5.0% eluent B/min	1.0 - 10.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	85 : 15 - 95 : 5	85 : 15 - 95 : 5	75 : 25 - 100 : 0		
		flow rate	0.8 - 1.2 ml/min	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min		
		injection volume	4 - 6 $\mu$ l	3 - 20 $\mu$ l	1 - 20 $\mu$ l		

NNNNN ... values to be defined and justified  
 \* e.g. based on DoE performed

1715

1716

1717 **13.3 Annex C: Example of Multivariate Model Lifecycle Components**

Model Description	On-line NIR to determine blending ranges to achieve blend uniformity during development	Measurement of Content Uniformity and Assay of uncoated tablets by NIR used for product release	Glucose Raman model used for qualitative identification testing on incoming raw material release for GMP use
	Model Category – Low Impact	Model Category - High impact	Model Category – High impact
	User requirements	Defined model requirements (e.g., ATP)	Defined model requirements (e.g., ATP)
Risk Assessment	Initial assessment based on existing knowledge, laboratory and pilot studies, or DOE, as appropriate.	Formal risk assessment based on knowledge gained during initial development.	Formal risk assessment with knowledge gained during initial development
Model Development - Calibration	Scientifically sound approach based on laboratory and pilot data and previous experience.	Formal design-based approach (e.g., DOE) covering appropriate ranges of relevant variability sources with established acceptance criteria that are suitable for intended use.	Formal design-based approach covering appropriate ranges of relevant variability sources (raw material, lots, packaging, instruments-to-instrument, user, software limitation) with established acceptance criteria that are suitable for intended use. Establish an identification threshold that has the same probability of detection as the existing method and a suitable alternative testing method should the Raman method fail.
Validation (Verification)	Assess specificity and robustness, optionally assess linearity and/or precision	Full validation covering applicable performance characteristics across reportable ranges with established acceptance criteria (ICH Q2).	Full validation covering applicable performance characteristics across reportable ranges with established acceptance criteria (ICH Q2). Include establishing suitable comparability of Raman method to existing method for release (can be reference method)
Performance Monitoring	Routine monitoring – maintain data sources (instruments), automation connectivity, and data integrity.	Routine monitoring – maintain data sources (instruments), automation connectivity, and data integrity.	Routine monitoring – maintain data sources (instruments), automation connectivity, and data integrity.
	Real-time diagnostics – implement initial diagnostics to confirm model performance in real-time.	Real-time diagnostics – implement routine diagnostics to confirm model performance in real-time.	Real-time diagnostics – implement routine diagnostics to confirm model performance in real-time.
	Periodic monitoring – if applicable, compare model predicted results to reference method at a frequency that is scientifically justified or on an event driven basis as needed.	Periodic monitoring – compare model predicted results to reference method at a frequency that is scientifically and statistically justified or on an event driven basis.	Periodic monitoring – compare model predicted results to reference method at a frequency that is scientifically and statistically justified or on an event driven basis.
Model Maintenance	Model Update - updates are common during the process development stage as new experimental data becomes available	Model Update - updates should be triggered based on Model Monitoring and Maintenance Strategy.	Model Update - updates should be triggered based on Model Monitoring and Maintenance Strategy.
	Change Management per PQS	Change Management per PQS	Change Management per PQS

1718