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ICH TRAINING MATERIALS

VALIDATION OF ANALYTICAL PROCEDURES ICH Q2(R2)

AND

ANALYTICAL PROCEDURE DEVELOPMENT ICH Q14

MODULE 7

ADDITIONAL CASE STUDIES AND EXAMPLES

ICH Q2(R2) / Q14 Training Module 7

Additional Case Studies and Examples

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ICH Q2(R2) / Q14 Training Module 7

Additional Case Studies and Examples

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1. INTRODUCTION

Module 7 is part of the training materials for ICH Q2(R2) and ICH Q14. The module aims to provide additional case studies and examples in order to facilitate the understanding of the concepts described in both guidelines.

The case studies on stereoisomer determination, potency, multi-attribute method (MAM) by liquid chromatography–mass spectrometry (LC-MS) and near-infrared spectroscopy (NIR) exemplify how elements of the enhanced approach can be applied to support risk based development of analytical procedures, the establishment of the analytical procedure control strategy and the validation of analytical procedures following the principles of ICH Q2(R2). They further provide examples on the identification of established conditions (ECs) for analytical procedures and related reporting categories when performance requirements are defined in an analytical target profile (ATP) and the relationship between the analytical procedure control strategy and the parameter settings/ranges is understood. Examples for post-approval changes to analytical procedures are discussed considering an agreed set of ECs and related reporting categories.

The example on platform analytical procedures describes principles, including the use of prior knowledge, relating to the establishment of a platform analytical procedure and considerations for the application to a new product.

The method operable design region (MODR) example describes the application of elements of the enhanced approach to the establishment of an MODR and provides options for validation.

2. CASE STUDY - MEASUREMENT OF STEREOMERS AS SPECIFIC PROCESS RELATED IMPURITIES IN A SMALL MOLECULE DRUG SUBSTANCE

Note: This case study reflects the case study described in Annex A of ICH Q14. Additional content was added to provide further background and explanations to the case study.

Introduction and Background

“Sakuratinib Maleate” is a small molecule drug substance (DS) with multiple chiral centres. The chirality of the molecule, its degradation pathway and the impurities are well characterised. From this knowledge and the established manufacturing process controls the six stereoisomers (Impurities A - F) were found to be potentially present in the final product. Based on toxicological considerations, Impurities A - E were specified at not more than (NMT) 0.1% and Impurity F was specified for release and re-test at NMT 0.5%. Impurities G - J were other process-related impurities, of which process impurity J was found to be also a degradation product of the DS. Impurities G - J are quantified through a separate procedure. All specified impurities are isolated and available as well-characterised substances for procedure development and validation.

Table 1: Analytical Target Profile

Intended Purpose		
Quantitation of the six stereoisomers A - F in Sakuratinib Maleate DS for release testing		
Link to critical quality attribute (CQA) (Stereoisomeric Purity)		
The analytical procedure should allow for the quantitation of the individual stereoisomers A - F and determination of the total sum to verify the CQA Stereoisomeric Purity $\geq 99.0\%$		
Characteristics of the Reportable Results		
Performance Characteristics	Acceptance Criteria*	Rationale
Accuracy	80 - 120% average recovery of spiked DS with Impurities A - E (specified at NMT 0.1% each) 90 - 110% average recovery of spiked DS with Impurity F (specified at NMT 0.5%)	For example, 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. 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
Precision	Intermediate Precision relative standard deviation (RSD): Impurities A - E $\leq 15\%$ Impurity F $\leq 10\%$	
Specificity	Analytical procedure should be able to quantitate impurities A - F in presence of other likely process related substances or DS degradation products with an acceptable bias of not more than 0.02%	Potential interference with quantitation of specified impurities by other regular components in the sample
Reportable Range	Impurities A - E: at least 0.05 - 0.12% Impurity F: at least 0.05 - 0.6%	Reporting threshold to 120% of specification limit

* The above ATP targets are illustrative for this example – other targets may be justified

Technology Selection

Multiple analytical technologies for chiral separations were available: Chromatographic analytical procedures such as gas chromatography (GC), liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and thin-layer chromatography (TLC) were considered, along with capillary zone electrophoresis (CZE) and capillary electrochromatography (CEC) as alternatives to chromatographic analytical procedures. Besides meeting the desired performance characteristics, further practical criteria were considered in the technology selection for development, based on general technical knowledge, operational needs, availability of equipment and capabilities in the company at the time:

- Complexity and robustness of technology
- Time and costs of analysis
- Standardisation of technology and availability of multiple instrument suppliers
- Existing expertise in the company

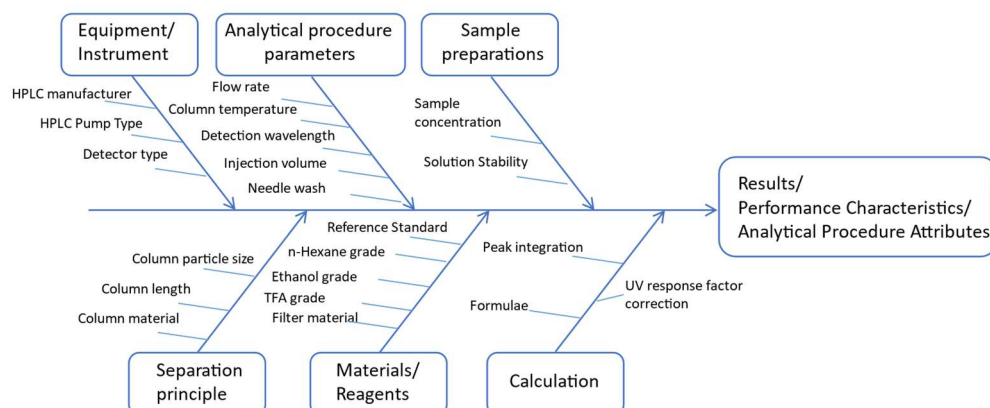
It was concluded to start analytical procedure development with Chiral HPLC because studies showed good potential for separation of stereoisomers and equipment was available at all testing sites. As detection mode, UV detection was selected as it was known that the molecule had sufficient UV absorption properties.

Analytical Procedure Development

The chiral HPLC procedure for quantitation of stereoisomers was developed using enhanced principles. Below is a summary of the activities conducted during development.

- An understanding of the chemistry, process, and impurities that have potential to be present in the drug substance was established;
- Reference materials were made available for development and validation;
- Conducted risk assessment and evaluating prior knowledge to identify the analytical procedure parameters that can impact performance of the procedure;
- Built retention time models and conducted design of experiment (DoE) experiments including robustness testing to explore ranges and interactions between identified analytical procedure parameters;
- Defined analytical procedure control strategy based on procedure understanding including set-points for relevant analytical procedure parameters and System Suitability Test (SST).

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

Figure 1: Ishikawa-Diagram

Analytical Procedure

For the purpose of this example, a summary of the analytical procedure is provided below. This does not reflect the entirety of the analytical procedure description in the dossier. System suitability criteria have been established as a link to performance characteristics, as outlined in ICH Q2(R2) and are indicators of the performance of the procedure at the time of use. Established system suitability tests are described below and in the procedure description in the dossier.

Table 2: Summary of the Analytical Procedure Description

Column:	Chiral column, amylose tris-(3,5-dimethylphenylcarbamate), immobilised on porous, spherical, silica particles, 4.6 mm ID x 250 mm, 3 µm
Mobile Phase:	<i>n</i> -hexane / ethanol / TFA (80/20/0.1)
Flow Rate:	1 mL/min
Column Temperature:	30°C
Detection	UV 214 nm
Injection Volume	5 µL
Standard/Sample Concentration	1.0 mg/mL
Analytical Procedure Control Strategy	
<i>System Suitability Tests</i>	<i>Controlled Parameters</i>
Resolution between Critical Peak Pair: DS Main Peak and Impurity D ≥ 2.0	Column, Temperature*, Mobile Phase, Flow Rate
S/N at quantitation limit (QL); DS at 0.05% >10	Injection Volume, Column, Mobile Phase, Standard/Sample Concentration, Detection Wavelength
Repeatability of Injection of DS at 0.5% Level $\leq 5\%$	Injection Volume, Mobile Phase

* For example, the retention time models built from data collected during analytical procedure development screens were used to assess the robustness of temperature and other parameters, that could potentially affect the performance characteristics (e.g., specificity). The *in silico* robustness was verified experimentally by confirming resolution at the centre point and design points that generated the minimum and maximum main peak retention time.

Analytical Procedure Validation

After the analytical procedure description was finalised based on development studies, prior knowledge, risk assessment, and robustness studies, a technology-specific validation study was planned. Performance characteristics to be demonstrated in the context of the validation study have been identified following ICH Q2(R2) guidance. A technology- and procedure-specific set of attributes and criteria were derived from the performance characteristics. After the performance of the validation study, the results were summarised in a validation report, which concluded that the analytical procedure met the acceptance criteria for the validation tests and hence the performance requirements described in the ATP. The analytical procedure was concluded to be fit for the intended purpose.

Table 3: Validation Summary

Technique	Separation techniques (e.g., HPLC, GC, CE) for impurities	Validation Results
Performance characteristic	Validation study methodology	
Specificity/ Selectivity	<p><u>Absence of relevant interference:</u> With product, buffer, or appropriate matrix, and between individual peaks of interest</p> <p>Spiking with known impurities/ excipients</p> <p>or</p> <p>By comparison of impurity profiles by an orthogonal analytical procedure</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples, if necessary</p>	Demonstrated by spiking all 6 stereoisomers to the drug substance 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 sample diluent were compared with a sample to demonstrate no interference with the analyte detection.
Precision	<p><u>Repeatability:</u> Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering peak(s) of interest</p> <p><u>Intermediate precision:</u> e.g., different days, environmental conditions, analysts, equipment</p>	Six separate preparations of the 6 stereoisomers were made at specification limit. Acceptable precision was obtained for both, Impurities A - E and Impurity F. Confidence interval was determined and assessed to be compatible with the validation acceptance criteria for precision. Intermediate precision between operators, days and instruments were performed and evaluated in an ANOVA experiment.
Accuracy	<p><u>For impurities or related substances:</u></p> <p>Spiking studies with impurities</p> <p>or</p> <p>Comparison of impurity profiles with an orthogonal procedure</p>	<p>Measured by spiking three levels, 0.05 (QL), 0.1 and 0.12% for impurities A - E, 0.05 (QL), 0.5 and 0.6% for impurity F (suitably characterised materials) in presence of drug substance 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.</p> <p>Confidence interval was determined and assessed to be compatible with the validation acceptance criteria for accuracy.</p>

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Technique	Separation techniques (<i>e.g.</i> , HPLC, GC, CE) for impurities	Validation Results
Performance characteristic	Validation study methodology	
Reportable Range	<p><u>Validation of calibration model across the range:</u></p> <p><u>Linearity:</u> Dilution of the analytes of interest over the expected procedure range, at least 5 points</p> <p><u>Validation of lower range limits (for purity only):</u> QL, detection limit (DL) through a selected methodology (<i>e.g.</i>, signal-to-noise determination)</p>	<p><u>Validation of calibration model across the range:</u></p> <p>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.</p> <p>QL was confirmed by demonstrating the RSD of the corrected peak areas for the stereoisomers at the reporting threshold was NMT 10%</p> <p>DL was confirmed to be above a signal to noise ratio of 3:1 for all stereoisomers</p>
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<p><u>Deliberate variation of relevant parameters, <i>e.g.</i>,</u></p> <p>Sample preparation: extraction volume, extraction time, temperature, dilution</p> <p>Separation parameters: column/capillary lot, mobile phase/buffer composition and pH, column/capillary temperature, flow rate, detection wavelength</p> <p>Stability of sample and reference material preparations</p> <p>Relative Response Factors</p> <p>If the analyte has a different response from the reference material (<i>e.g.</i>, a different specific UV absorbance), relative response factors should be calculated using the appropriate ratio of responses. This evaluation may be performed during validation or development, and should use the finalised analytical procedure conditions and be appropriately documented</p>	<p>Conducted modelling and multi-variate experiments including robustness testing to explore ranges and interactions between identified analytical procedure parameters</p> <p>Stability of sample and reference material preparations assessed</p> <p>No relative response correction factors implemented as linearity slopes of the stereoisomers were compared to the linearity of drug substance to demonstrate a UV response factor of between 0.8 and 1.2 for each stereoisomer versus the drug substance</p>

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

The applicant proposed and justified established conditions and reporting categories, as part of the submission. For the purpose of this example, Table 4 describes the proposed ECs, their proposed reporting categories and examples of parameters that are not ECs.

Note: The extent of ECs and associated reporting categories listed in this table depend on the extent of knowledge gained, information and justification provided in the dossier. The dossier is subject to regulatory review. The information provided in this example is only part of the knowledge available that will be submitted and is provided for illustrative purposes only. The extent of ECs (EC or not EC designation), actual reporting categories, and data requirements may differ by region. Depending on the nature and extent of the change (e.g., change to a different technology), a post-approval change management protocol (PACMP) may be required.

Table 4: Evaluated risk, proposed established conditions and proposed reporting categories

Established Condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comments
Performance Characteristics and Criteria as described in the ATP: Accuracy, Precision, Specificity, Range (see Annex A, Table 1)	High	PA	The performance characteristics and criteria ensure the quality of the reportable result and link to the CQA. If widening of the performance criteria is necessary, it will be reported as PA.
Technology: Chiral Liquid Chromatography Suitable chiral separation technique to meet performance characteristics defined in ATP	Medium	NM	A technique that meets the performance characteristics and criteria ensures the quality of the reportable result and link to the CQA. There is a strong understanding between product knowledge, intended purpose, and the analytical procedure performance established to enable the design of future bridging studies. A change resulting in a widening of the specification acceptance criteria might require a higher reporting category
System Suitability Test and parameter-control relationship (see Impurity case study in ICH Q14, Annex A, Table 2)	Medium	NL/NM	SST was developed for the LC procedure based on a risk analysis and ensures adherence to the performance characteristics and criteria. Control relationships were established through prior knowledge (general principles of technique) and during procedure development. If the SST criteria are widened the reporting category would be higher.
LC Column: Amylose tris-(3,5-dimethylphenylcarbamate), immobilised on porous, spherical, silica particles Mobile Phase Components: <i>n</i> -Hexane, Ethanol, TFA Method of detection: UV 214 nm	Low	NL/NM	The LC column, mobile phase components and mode of detection are the main parameters, defining the separation mechanism and detection. Changing these parameters may result in the need to adapt the SST
The following conditions are examples of parameters that are not ECs²⁾:			
Ratio of mobile phase components: <i>n</i> -Hexane/Ethanol/TFA (80/20/0.1) Instrumental conditions: Temperature: 30°C	Low	Not reported	These parameters are controlled by the SST. Robustness testing supported by modelling was performed at the centre point and the extrema that generated the minimum and maximum main band retention time.

Established Condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comments
Column length, packing particle size			
Preparation of test solutions and reference materials: 1 mg/mL DS in mobile phase	Low	Not reported	The performance over the working range was demonstrated through the linearity experiments during validation.

1) PA: Prior Approval, NM: Notification Moderate; NL: Notification Low (as per ICH Q12 definitions)

2) Depending on the region, some of this information is included in an approval letter

Change Management and Bridging Strategy

For every change, the Marketing Authorisation Holder (MAH) will perform a risk assessment to evaluate potential impact on the performance characteristics and the link to CQA (purity) which in this example is defined in the ATP. The outcome of the risk assessment informs the extent of the bridging studies used to demonstrate adherence to the performance characteristics and associated criteria. These can include, if necessary, full or partial revalidation of the analytical procedure performance characteristics affected by the change and/or comparative analysis of representative samples and reference material.

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

Change Description and Management

The following illustrates two independent examples of post-approval changes that could occur during the lifecycle of an analytical procedure and illustrate the steps a MAH would follow when changing an approved analytical procedure.

Change #1: adjustment to mobile phase ratio and column temperature

Background

The company has monitored and trended the retention times of the stereoisomers during routine use and found that the retention times between the critical peak pair have been eluting more closely than during development and could be reproduced in a more stable manner by lowering the mobile phase ratio to n-hexane/ethanol/TFA (75/25/0.1) and increasing the column temperature to 35°C.

Risk assessment

The intended change was a change of analytical procedure parameters, and these parameters were agreed to be managed within the company's quality system following the adherence to commitments made (i.e., the parameters were not ECs).

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

The current control strategy of the product is considered sufficient and would not be impacted by the change. The specifications for the chiral impurities remain unchanged.

b) Complexity of the technology:

HPLC is a well-established technology and the relationship of organic solvent and temperature on the retention of the analytes on the chromatographic column is well understood.

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

The extent of the change is low as it is a minor adjustment of the mobile phase composition and temperature.

Estimated Risk: Low

Development approach and application of enhanced understanding

Elements of the enhanced approach (ATP, prior knowledge, modelling, robustness studies using design of experiments) were used to define a control relationship between mobile phase, temperature, flow rate and the resolution system suitability requirement, as communicated in the submission.

Re-Confirmation Question: Are relevant performance criteria defined as ECs to ensure the post-change quality of the measured result and is sufficient understanding available to design appropriate future bridging studies?

Answer: Yes

Demonstration of analytical procedure performance after the change

Based on the established control relationship between analytical procedure parameters and the SST, demonstration of meeting the SST criteria was considered as appropriate along with meeting the relevant performance characteristics and associated criteria in the ATP through validation studies.

Conclusions

Based on the initial risk assessment and the additional SST controls, the risk of changing the mobile phase composition and column temperature was considered to be low.

Regulatory reporting

The original agreement with the regulator that this parameter is not an EC was confirmed as a result of the steps that were performed to implement the actual change. Thus, no regulatory reporting was needed. The company documented this change within the pharmaceutical quality system (PQS).

Change #2: from chiral HPLC to chiral SFC

Background

As supercritical fluid chromatography (SFC) has become more common with systems at manufacturing facilities and could meet the intended purpose, the company decided to implement SFC as an alternative procedure. This well-established technology, SFC, is targeted in the alternative development to allow the use of a more environmentally friendly technology for separation of the chiral impurities. The intended change is not related to any quality issues of the product or the established HPLC procedure and the company will not modify the specifications for the chiral impurities.

Risk assessment

The intended change is a change in technology, and this was agreed as an EC with notification moderate (NM) during approval of the product by the regulatory health authority.

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

The current analytical control strategy of the product is considered as sufficient and would not be impacted by the change. The specifications for the chiral impurities remain unchanged.

b) Complexity of the technology:

Only well-established separation technologies (HPLC and SFC) are in scope.

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

The performance of the analytical procedure for the intended purpose was described through accuracy, precision, specificity, and range. The intended change may have an impact on the analytical procedure performance. Therefore, the company used an analytical target profile as upfront control element to minimise the risk of the change.

Estimated Risk: High

Development approach and application of enhanced understanding

The change will neither impact the already established product understanding nor the expected analytical procedure performance, as described in the ATP. Additionally, the fundamentals of the analytical techniques are well understood as general methodology and described in pharmacopoeias. Technology and analyte behaviour are predictable. The product, analytes, and sample preparation are well characterised and understood. Elements of the enhanced approach were applied to develop, validate, and establish an analytical procedure control strategy. Below is a summary of the activities conducted during development.

- Prior knowledge of the chemistry, process, and impurities that have potential to be present in the drug substance;
- Reference materials were available for development and validation;
- Evaluated prior knowledge and conducted studies to confirm that the stationary phase from chiral LC procedure was suitable for separation of stereoisomers by SFC;
- Screened gradient levels, CO₂ and methanol concentrations to identify analytical procedure parameters that can impact performance of the procedure;
- Built retention time models and conducted modelling and DoE experiments including robustness testing to explore ranges and interactions between identified analytical procedure parameters;
- Defined analytical procedure control strategy based on procedure understanding including set-points for relevant analytical procedure parameters and SST.

Re-Confirmation Question: Are relevant performance criteria defined as ECs to ensure the post-change quality of the measured result and is sufficient understanding available to design appropriate future bridging studies?

Answer: Yes

Further evaluation performed following ICH Q14 Figure 2, resulted in an overall risk level of medium and confirmed the risk level that was agreed to in the Product Lifecycle Management (PLCM) document at the time of initial approval.

Demonstration of analytical procedure performance after the change

The procedure was validated in alignment with ICH Q2(R2). A technology specific validation protocol was established. The acceptance criteria for validation was derived from the ATP. Additionally, a bridging study was completed, comparing the two procedures through analysis of the same drug substance batch.

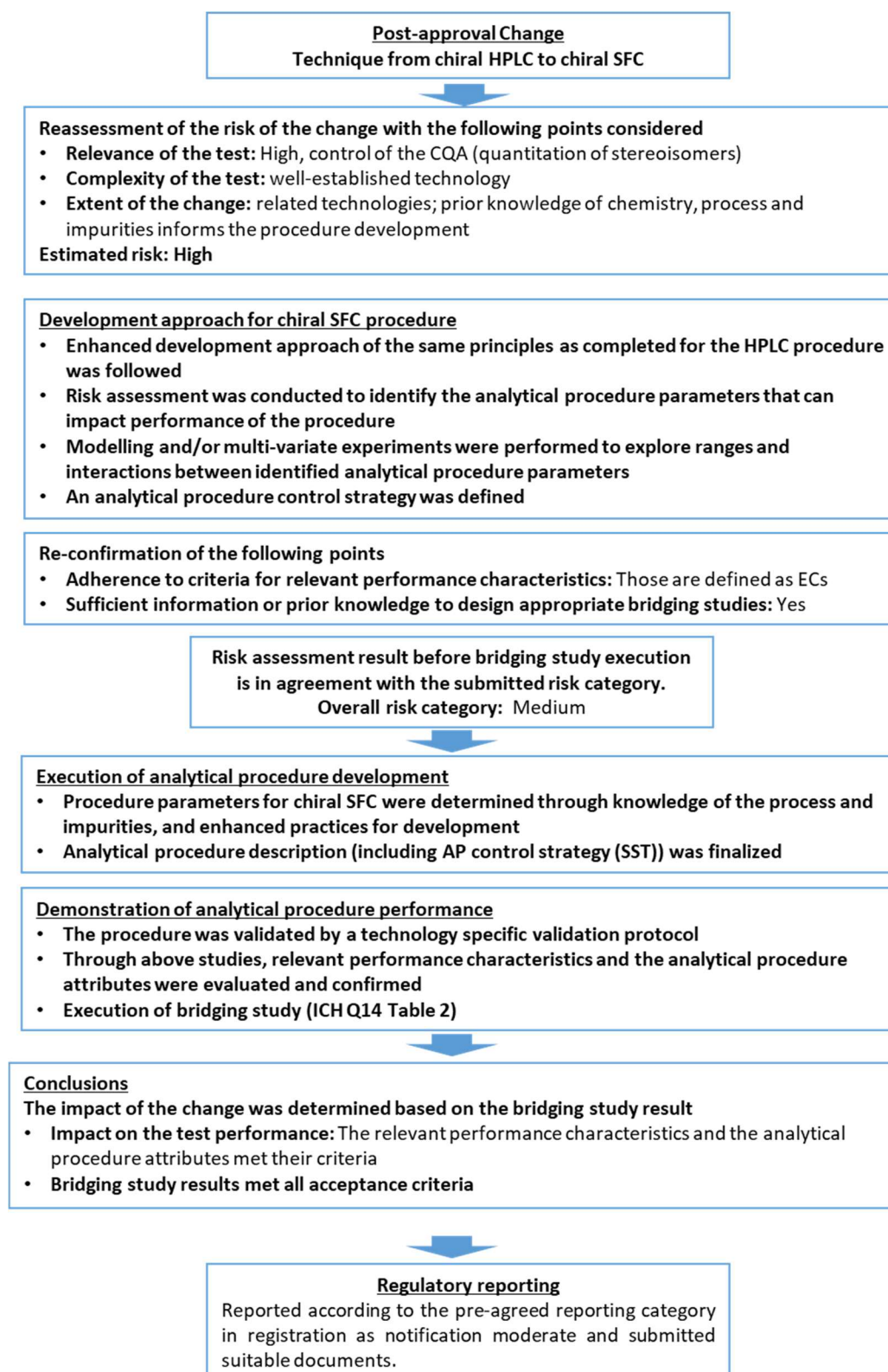
Conclusions

Based on the initial risk assessment, enhanced development studies, the additional controls in place and the bridging study, the risk of using a chiral SFC procedure as a replacement for the HPLC procedure was considered medium. The original agreed reporting category of NM was confirmed as a result of the additional assessment and development/validation data.

Regulatory reporting

The original EC with associated reporting category as agreed with the health authority per Table 3 was confirmed as a result of the steps that were performed to change the approved analytical procedure and thus the change was submitted as notification moderate.

Figure 2: Example of work process of applicant to change an approved analytical procedure



3. CASE STUDY - MEASUREMENT OF POTENCY FOR AN ANTI-TNF-ALPHA MONOCLONAL ANTIBODY

Note: This case study reflects the case study described in Annex A of ICH Q14. Additional content was added to provide further background and explanations to the case study.

Introduction and Background

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

Assumptions for the example:

- Mode of action: the neutralisation of the biological activity of soluble TNF-alpha by preventing TNF-alpha from binding to the TNF-alpha receptor;
- Fc-effector functions are out of scope;
- Specification limits for the relative potency: 80% to 125% compared to reference material;
- Potency assay to be developed is able to detect a change and/or a shift in potency upon forced degradation.

Table 1: Analytical Target Profile

Intended Purpose		
Measurement of the potency of an anti-TNF-alpha monoclonal antibody in drug substance and in drug product at release and for stability testing.		
Link to CQA (Biological Activity)		
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. Target acceptance criteria: 80% to 125% relative potency ¹⁾		
Characteristics of the Reportable Result		
Performance Characteristics	Acceptance criteria	Rationale
Accuracy	Accuracy 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% ¹⁾	Parameters are assessed based on compendial guidance The acceptance criteria are determined considering the intended purpose of the measurement Selected performance characteristic ensures that the intended analytical procedure delivers the quality of the reportable result
Precision	Upper 95% confidence interval for the average intermediate precision across levels across the reportable range (95% CI % geometric coefficient of variation ²⁾ is not more than 20% ¹⁾	
Specificity	Analytical procedure 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>i.e.</i> , capable of detecting a change in potency and/or a change in the shape of the dose-response curve, confirmed using forced degraded samples	To ensure that the product remains within specification over the shelf-life

Reportable Range	The potency range is the range that meets accuracy and precision. It should include the specification range (in this case, 80 to 120% of the specification range corresponds to a relative potency range of 64 to 150% for a specification of 80 to 125% relative potency ¹⁾)	Stated range for which the required accuracy and precision characteristics are demonstrated
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1) Individual values are just an example and can be different from product to product

2) Calculation of geometric coefficient of variation (CV) is required only if logarithmic transformation of the data is performed

Technology Selection

Binding assays and cell-based bioassays are suitable technologies for the measurement of the relative potency of an anti-TNF-alpha monoclonal antibody when considering the ATP above. The two assays rely on the binding of the anti-TNF-alpha monoclonal antibody to the soluble TNF-alpha. While the signal of a binding assay directly measures the binding, the cell-based assay may target a later stage event in the signalling cascade. Out of different formats of cell-based assay, the cell-based proliferation assay was chosen as it is widely used and a well characterised cell line was available.

This assay is based on the ability of the product to block TNF-alpha induced inhibition in a responsive cell line (e.g., murine fibrosarcoma WEHI-164). The assay compares the dose-response of a test sample with a designated reference material to provide a quantitative measurement of relative potency. The cells are incubated with varying dilutions of test sample and reference material in presence of TNF-alpha. The cell growth is assessed by a staining method using a tetrazolium salt which is converted by cellular dehydrogenases to a coloured formazan product. The release of formazan is measured using a spectrophotometer and the amount is determined by subtracting the absorbance value at 450 nm from the absorbance value at 650 nm. The spectrophotometric response is directly proportional to the number of living cells.

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

Analytical Procedure Development

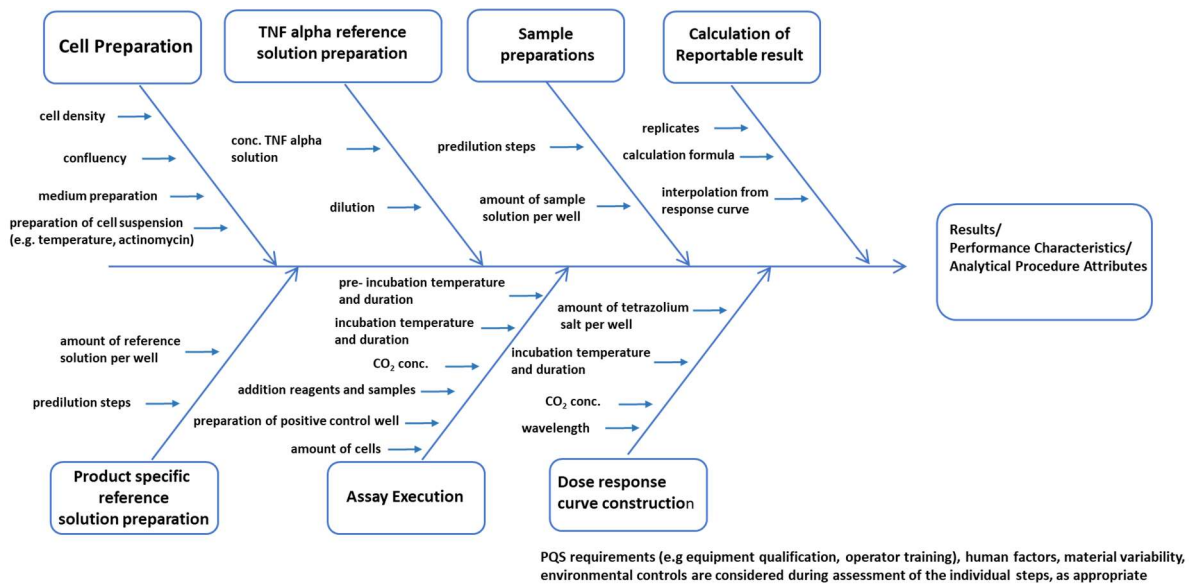
The development of the analytical procedure described has been performed using an enhanced approach and was based on extensive knowledge of the product and on knowledge on relative potency assays, considering the following points for example:

- Performance characteristics and associated criteria for the analytical procedure defined in the ATP based on product knowledge:
 - The applicant has extensive knowledge about relevant factors that could impact the CQA (biological activity) based on CQA assessment and process characterisation and has established the link between the mode of action (MOA) and the clinical performance. Based on these data, the appropriate cell line and antigen binding conditions for the potency assay were selected.
 - The monoclonal antibody has been characterised with other functional and/or physicochemical assays that contribute to understanding of the molecule and binding properties (e.g., Fc effector function). The other characterisation assays are also continuously used in the lifecycle of the drug.

- A well-characterised material (*e.g.*, a reference material) is available. The reference material was demonstrated to be suitable for the intended purpose (*e.g.*, through calibration against the international standard). Relative potency is calculated for samples by comparison to the signal from a well-characterised material (*e.g.*, a reference material) generated in the same analysis.
- Extensive analytical procedure understanding gained from prior knowledge and development studies guided by quality risk management (QRM) principles.
 - The **cell line** and **cell line performance** (*e.g.*, sterility, viability, cultivation conditions, cell density, cell line stability including minimum and maximum number of passages) are well understood. Robustness of the cell cultivation conditions ensuring suitable cell metabolism has been confirmed during the development of the analytical procedure. A qualified cell bank was implemented and stored appropriately, with adequate stability testing in place.
 - Criteria for confluence and cell viability have been defined during development to ensure the required cell metabolism, resulting in an appropriate signal amplitude and dose-response curve.
 - Extensive studies have been done to identify the appropriate **TNF-alpha solution** (antigen) leading to **a spectrophotometrically measurable sigmoidal dose-response curve** in the presence of the reference samples or test samples, with lower and upper asymptotes corresponding to negative and positive controls, respectively.
 - The assay conditions such as incubation times and amount of cells per well have been studied and the parameters which influence the assay performance have been identified.
 - Serial dilution levels were developed to optimise the dose-response curve, *e.g.*, to ensure minimally three points in the linear segment of the dose-response curve and two in each asymptote.
 - Detection and calculation methods were optimised, and system suitability criteria and sample suitability criteria were determined.
 - The relative potency of the reference material used in the procedure was qualified, and criteria around the analytical procedure performance were established to ensure run-to-run variability remains within suitable limits.
 - An additional assay control sample can be used to monitor long-term consistency of the assay.
- Robustness evaluation was conducted and its outcome was reflected in the analytical procedure control strategy.

Factors considered during risk assessment are shown in Figure 1. A summary of the development data and the outcome of risk assessment is listed in Table 2.

Figure 1: Ishikawa diagram ¹⁾



¹⁾ Ishikawa diagram was derived from ICH Q14 Annex A, Potency case study, Figure 2

Table 2: Summary of development data and risk assessment

Unit Operation	Procedure Parameter*	Defined Target or Range	Investigated Range during Studies	Rationale	Risk**
Cell preparation	Cell Density (cells/mL)	1x10 ⁶ 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
	FBS concentration in the medium	5%	1 - 9%	To ensure appropriate sensitivity of the assay	low
TNF-alpha solution preparation	Concentration of the TNF-alpha solution	Targeted working concentration	50 to 150% of targeted working concentration	To ensure appropriate potency determination of the anti-TNF mAb	low
Reference material/Control Sample	Dilution factor	Target	Target	To ensure appropriate potency determination of the anti-TNF mAb	low
Assay execution	Amount of cells added (µL)	50 µL	25 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
	Pre-incubation CO ₂ 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 selected as target	low
	Incubation temperature	37°C	35 - 38°C	Combination of incubation conditions to allow generation of an appropriate dose-response curve	low
	Incubation CO ₂ 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 - 15 µL	Salt needed to perform the colourimetric 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

* Parameters are provided as an example only and are not an exhaustive list of parameters

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

Analytical Procedure

For the purpose of this example, a summary of the analytical procedure is provided below. This does not reflect the entirety of the procedure description in the dossier.

Table 3 Summary of analytical procedure description

Unit Operation	Description
Cell preparation	Prepare a suspension of WEHI-164 cells containing 1×10^6 cells/mL, using assay medium containing 2 µg/mL of actinomycin D
Reference solution and test solution preparation	Included in analytical procedure description in the dossier but not listed in this table
Plate preparation	
Plating cells	
Absorbance measurement	
Calculations	
Solutions & reagents preparation	WEHI-164 cells (ATCC), TNF-alpha solution of suitable concentration, assay and culture medium including components and concentrations - Actinomycin D, Tetrazolium salt WST-8
Analytical Procedure Control Strategy	
System suitability test	<ol style="list-style-type: none"> 1. The dose-response curve obtained for the reference solution 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 solution 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 each reference solution curve (R^2) is not less than 0.97 * 4. Maximum value (cell only) to minimum value (TNF-alpha control) ratio: minimum 3.0*
Sample suitability assessment	Assessment of similarity/parallelism: - The upper asymptote ratio (A_{std}/A_{test}): 0.8 - 1.2* - The lower asymptote ratio (D_{std}/D_{test}): 0.8 - 1.2* - The Hill slope ratio (B_{std}/B_{test}): 0.8 - 1.2* - The upper to lower asymptote ratio $((D - A)_{std}/(D - A)_{test})$: 0.8 - 1.2*

* The ways of assessing of similarity/parallelism as well as individual values are just examples and can be different from product to product

Analytical Procedure Validation

After the analytical procedure was finalised based on development studies, prior knowledge, risk assessment, and robustness studies, a technology-specific validation study was planned. Performance characteristics to be demonstrated in the context of the validation study have been identified following ICH Q2(R2) guidance. A technology- and procedure-specific set of attributes and criteria were derived from the performance characteristics. After the performance of the validation study, the results were summarised in a validation report, which concluded that the analytical procedure met the acceptance criteria for the validation tests and hence the performance requirements described in the ATP. The analytical procedure was concluded to be fit for the intended purpose.

Table 4: Validation Summary

Technique	Cell-based assay for determination of potency relative to a reference	
Performance characteristic	Validation study methodology ¹	Results
Specificity/ Selectivity	<p><u>Absence of interference:</u> Dose-response curve fulfils the response criteria demonstrating the similarity of the analyte and reference material, as well as no dose-response from the cell line alone</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary</p>	<p>The analytical procedure is specific for the intended mechanism of action</p> <ul style="list-style-type: none"> - Similarity of analyte and reference material (similar dose-response curves for analyte and reference material) - No dose-response obtained for cell line alone and other biological products (no other anti-TNF-alpha monoclonal antibody) tested - No interference from relevant process related impurities or matrix components <p>The assay is stability indicating as demonstrated with forced degradation samples</p>
Precision	<p><u>Repeatability:</u> Repeated sample analysis on a single day or within a short interval of time covering the reportable range of the analytical procedure (at least 3 replicates over at least 5 levels)</p> <p><u>Intermediate Precision:</u> Different analysts, multiple independent preparations over multiple days at multiple potency levels through the analytical procedure's reportable range, inclusive of normal laboratory variation</p>	<p><u>Repeatability:</u> Demonstrated by analysis of 3 replicates at 5 levels in a range of 64 - 150%. Max GCV²⁾ per level: 12% GCV across all levels: 10% Upper limit of 95% CI of GCV across all levels³⁾: 15%</p> <p><u>Intermediate Precision:</u> Demonstrated by 6 independent determinations at 3 levels over the range 64 - 150% with 2 analysts at 3 days using 2 different plate suppliers: max GCV per level: 14% GCV across all levels: 11% Upper limit of 95% CI of GCV across all levels⁴⁾: 16%</p>
Accuracy	<p><u>Reference material comparison:</u> Assess recovery <i>versus</i> theoretical activity for multiple (at least 3) independent preparations at multiple (at least 5) levels through the analytical procedure's reportable range</p>	<p>Demonstrated by analysis of 3 replicates at 5 levels in a range of 64 - 150%. Min.-Max Geometric Mean Recovery (for all levels) 91 - 118% Overall Geometric Mean Recovery: 107% The 95% Confidence Interval of Overall Geometric Mean Recovery²⁾: 101 - 113% The 95% CI of the slope of the fitted regression line between theoretical and measured potency is 0.91 to 1.20</p>
Reportable Range	<p><u>Validation of range, including lower and higher range limits:</u> The lowest to highest relative potency levels that meet accuracy, precision, and response criteria, determined over at least 5 potency levels.</p>	<p><u>Reportable range established from 64 - 150% relative potency.</u></p>
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<p><u>Deliberate variation of parameters, e.g.,</u> Plate type, buffer components, incubation times, incubation conditions, instruments, reaction times, reagent lots including controls, cell density, effector/target cell ratio, cell generation number</p>	<p><u>Deliberate variation of parameters, as shown in Table 2</u></p>

¹⁾ Validation study methodology was derived from ICH Q2(R2) Annex 2 Table 7

²⁾ % Geometric Coefficient of Variation (%GCV) is utilised as the measure of variability in order to preserve continuity using the log transformation

³⁾ It is assumed in this example that all 15 determinations (3 replicates at 5 levels) are independent and belong to the same population regardless of the level and the replicate

⁴⁾ It is assumed that all 18 (6 x 3) determinations are independent and belong to the same population regardless of the level

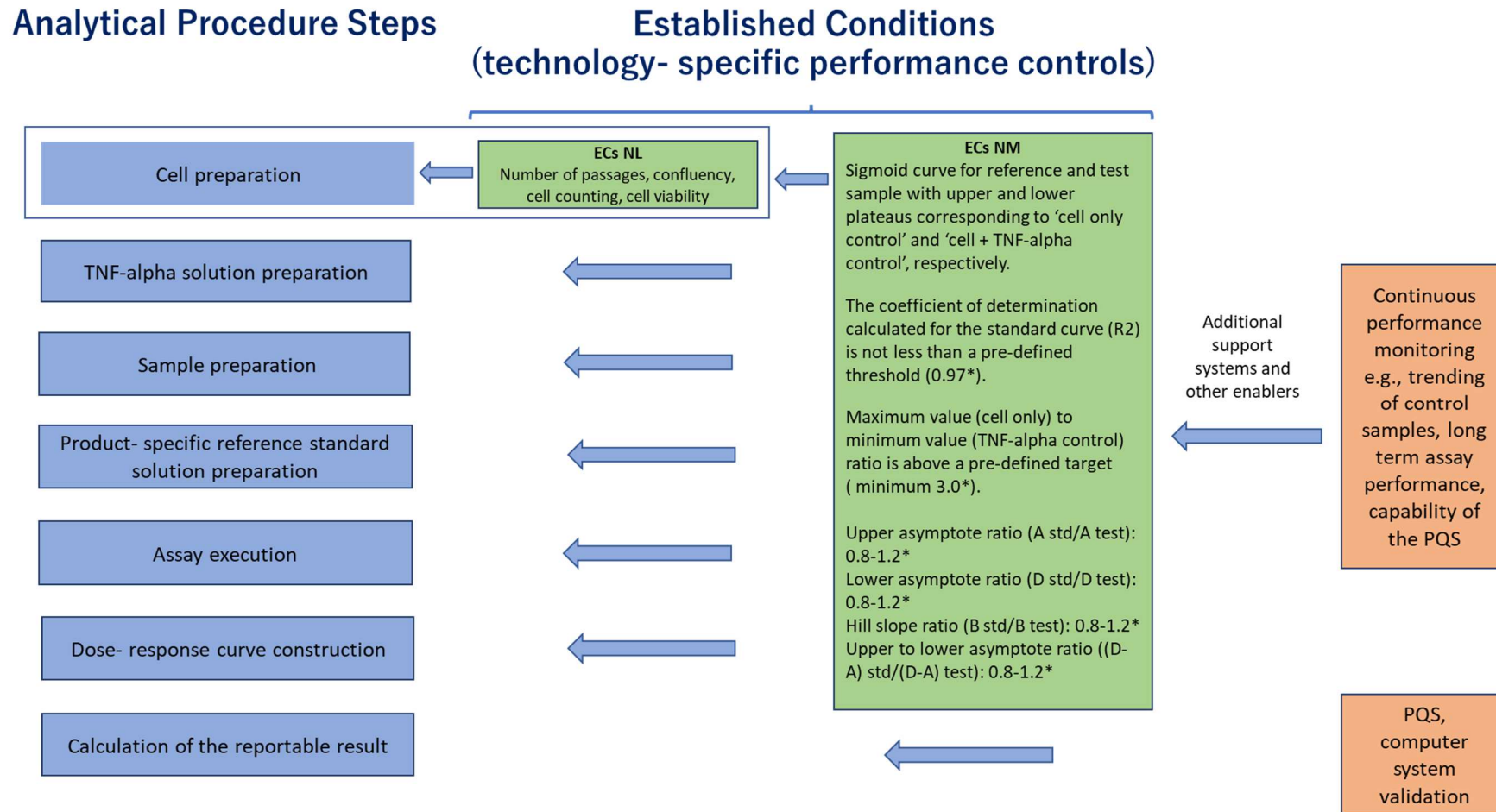
Description of Established Conditions (ECs), Reporting Categories, and Justifications

An overview of how the individual analytical process steps are controlled by the analytical procedure control strategy is shown in Figure 2. This provides the basis for the justification that a certain parameter or parameter value may not necessarily need to be defined as an EC or may be assigned a lower reporting category.

The applicant proposed and justified ECs and reporting categories, as part of the submission. For the purpose of this example, Table 5 describes a selection of the proposed ECs, their proposed reporting and an example of a parameter that is not an EC.

Note: The extent of ECs and associated reporting categories listed in this table depend on the extent of knowledge gained, information and justification provided in the dossier. The dossier is subject to regulatory review. The information provided in this example is only part of the knowledge available that will be submitted and is provided for illustrative purposes only. The extent of ECs (EC or not EC designation), actual reporting categories, and data requirements may differ by region. Depending on the nature and extent of the change (e.g., change to a different technology), a PACMP may be required.

Figure 2: Overview of the performance control strategy of the analytical procedure



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

Table 5: Evaluated risk, proposed established conditions and proposed reporting categories

Established condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comment
Performance characteristics and associated criteria as defined in the ATP (Table 1)	high	PA	The performance characteristics and criteria ensure the quality of the reportable result and link to the CQA. Widening of performance characteristics and criteria could have an impact on the control of the CQA
Technology (principle) Cell Based Assay	high or medium	PA or NM	Adherence to performance characteristics and criteria ensured by control strategy and defined bridging strategy (see below) to assess impact of changes Change would be reported as Notification Moderate if no impact of the change on the specification acceptance criteria and as Prior Approval if there is an impact on the specification acceptance criteria
Analytical procedure control strategy elements (SST 1 - 4, sample suitability assessment)			
System suitability test (Table 3)	medium	NM ²⁾	Performance of the analytical procedure is ensured by • Direct control of individual analytical procedure steps through analytical procedure control strategy elements listed in Table 3 (and the dossier) • Defined analytical procedure control strategy elements which ensures the adherence to the ATP • Adherence to the performance characteristics and criteria after a change of analytical procedure control strategy elements If assurance of performance of the analytical procedure cannot be demonstrated, the change needs to be reported as Prior Approval
Sample suitability assessment (Table 3)	medium	NM ²⁾	
Cell Preparation			
Cell line: WEHI-164 cells (ATCC)	medium	NM	Based on demonstrated 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 System suitability test ensures the suitability of the cell line and its performance (number of passages, confluency, cell counting, cell viability, signal amplitude, shape of the response curve)

Established condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comment
Preparation of cells: sub culturing	low	NL	<p>Sufficient cell performance to detect changes in the quality of the drug is ensured by:</p> <ul style="list-style-type: none">• System suitability 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 analytical procedure 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 <p>Analytical procedure control strategy ensures adherence to performance characteristics and criteria. The extent of the bridging study will depend on the extent of the change</p>
Medium composition: RPMI 1640, L-glutamine, heat-inactivated foetal bovine serum, and a suitable antibiotic	low	NL	
Preparation of a suspension of WEHI-164 cells containing 1x10 ⁶ cells per millilitre, using assay medium containing 2 µg/mL of actinomycin D.	low	NL	
The other analytical procedure parameters defined as ECs are omitted for the purpose of this example			
Example of a parameter that is not an EC:			
Plating format	low	Not reported	No impact on assay output based on development data

1) PA: Prior Approval, NM: Notification Moderate; NL: Notification Low (as per ICH Q12 definitions)

2) Based on regional requirements the proposed reporting category may need to be elevated to PA

Change Assessment and Bridging Strategy

For every change, the Marketing Authorisation Holder (MAH) will perform a risk assessment to evaluate potential impact on the performance characteristics and the link to CQA (biological activity) which in this example is defined in the ATP. The outcome of the risk assessment informs the extent of the bridging studies used to demonstrate adherence to the performance characteristics and associated criteria. These can include, if necessary, full or partial revalidation of the analytical procedure performance characteristics affected by the change and/or comparative analysis of representative samples and reference material.

The marketing authorisation holder (MAH) should not implement the new analytical procedure using the predefined reporting category unless adherence to the performance characteristics and associated criteria defined in the ATP are demonstrated during the bridging studies. If the precondition of adherence to the ATP cannot be met, a higher reporting category would apply.

Change Example:

The example in Figure 3 illustrates a post-approval change in the cell preparation from subculture to ready-to-use cells and includes the steps an applicant would follow when changing an approved analytical procedure.

Background

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

Summary of structured risk assessment:

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

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

The extent of the change is restricted to the preparation of the cells (change in analytical procedure step cell preparation), with potential impact on only one analytical procedure attribute (cell metabolism). Factors contributing to the cell performance are understood, investigated as part of development of the ready to use cell preparation and monitored by the SST.

The initial risk assessment proposed a moderate risk. Further evaluation performed following ICH Q14 Figure 2, resulted in an overall risk level of low and confirmed the risk level that was agreed to in the PLCM document at the time of initial approval.

Adherence to criteria for relevant performance characteristics

The understanding of the analytical procedure and link to the CQA allowed the definition of criteria for relevant performance characteristics which ensure the post change quality of the measured result after the change (refer to Table 1, ATP). The change can potentially affect cell metabolism and hence the analytical procedure performance characteristics accuracy and precision. Adherence to these performance characteristics should be demonstrated before implementation of the change. This change does not impact the performance characteristics specificity and reportable range as the same cell line is used and the potency is measured against the same reference material.

Demonstration of Analytical Procedure Performance after Change***Evaluation of impact on performance characteristics***

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

Experimental Bridging Study Results

In accordance to Table 2 of ICH Q14 a partial revalidation of the analytical procedure was performed (accuracy and precision) to demonstrate the affected analytical procedure attributes are met after the change. Comparative analysis (including statistical analysis) of a set of representative samples with pre- and post-change analytical procedures were performed to ensure comparable results.

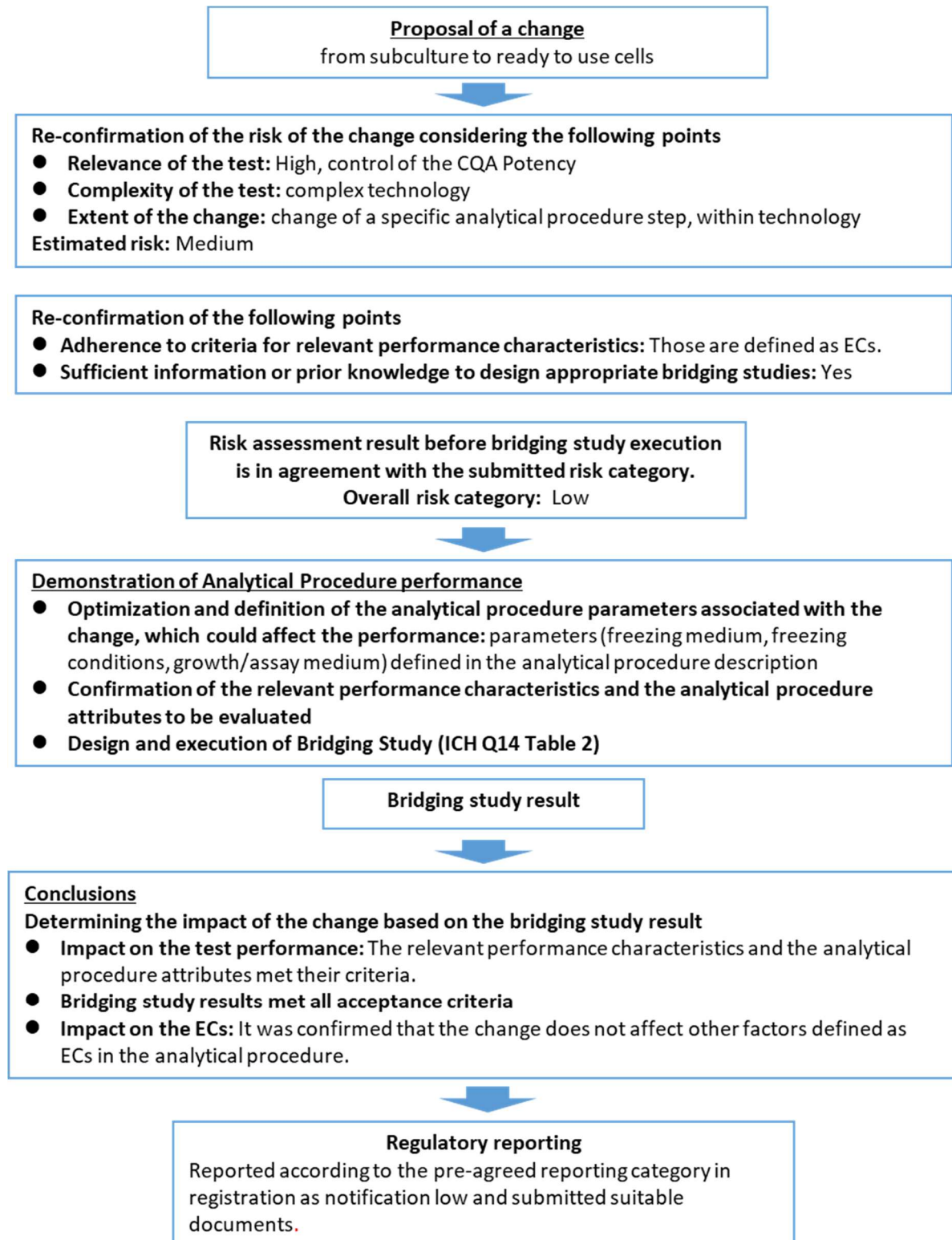
Conclusion

Evaluation of performance characteristics demonstrated that defined criteria could be met. The result of the studies confirmed the expected cell performance post change. The purpose of the analytical procedure has not changed and the capability of the analytical procedure to generate the reportable result is unchanged. Bridging studies were successfully performed. The risk associated with the change is considered low taking into account the outcome of the initial risk assessment, the development data, the evaluation of the performance characteristics and the bridging study results.

Regulatory reporting

The original EC with associated reporting category as agreed upon with the regulatory agencies per Table 5 was confirmed as a result of the steps performed. Thus the change was submitted as “notification low”. The revised analytical procedure together with the analytical validation report and the outcome of the bridging study was submitted accordingly. The SST criteria of the analytical procedure including those ensuring sufficient cell performance remained unchanged. Appropriate development data demonstrating suitable absence of impact on cell performance upon preparation and handling frozen cells was provided.

Figure 3: Example of work process of applicant to change an approved analytical procedure

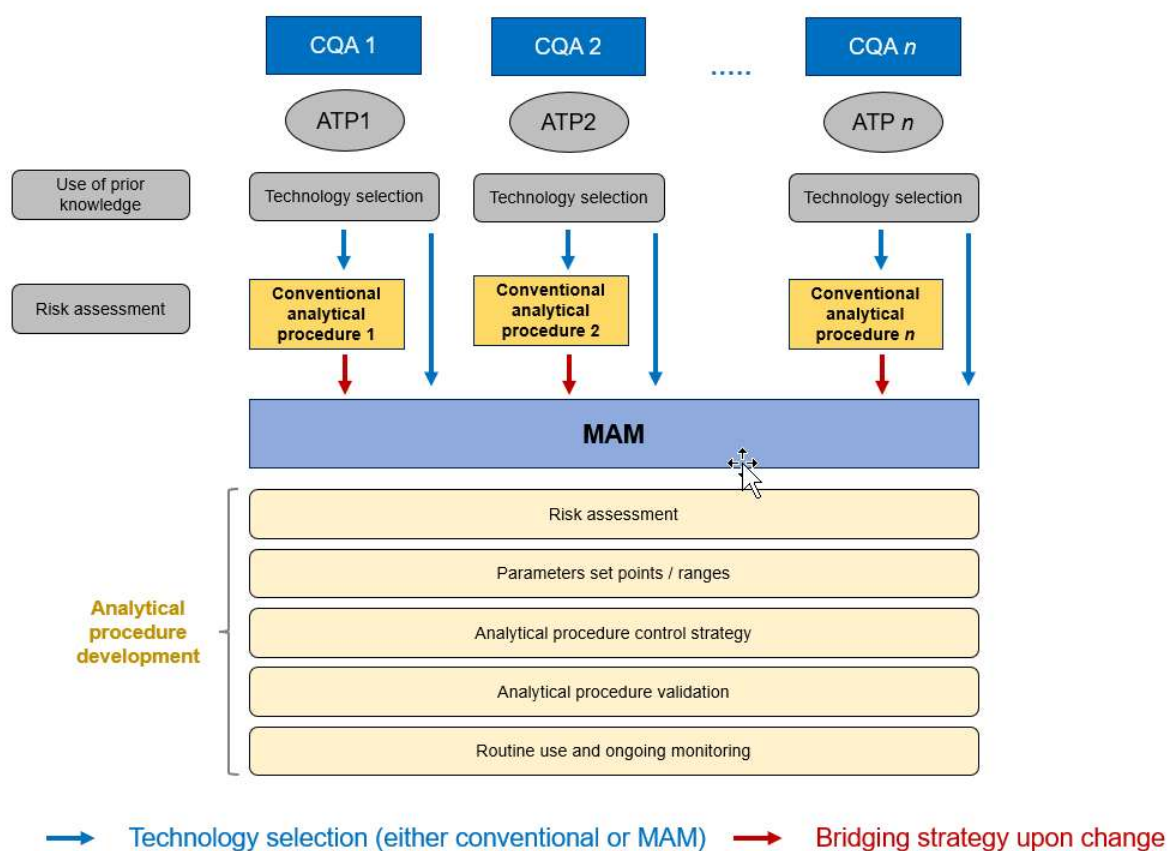


4. CASE STUDY - MAM BY PEPTIDE MAPPING LC-MS

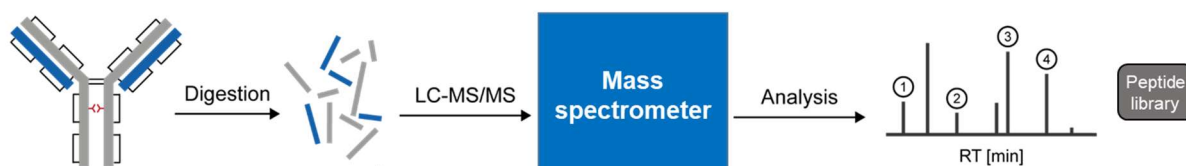
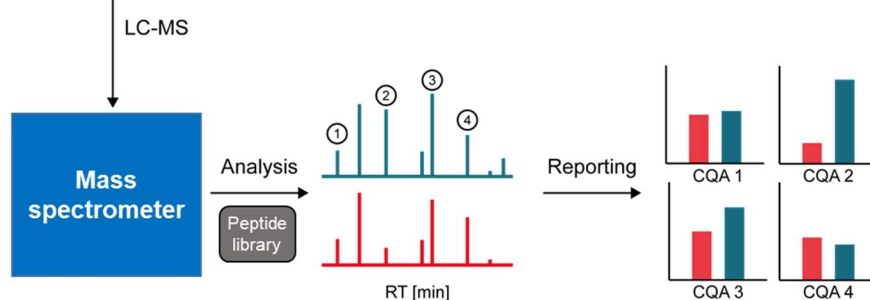
Introduction and Background

This case study illustrates the application of ICH Q14 principles for the development and lifecycle management of multi-attribute methods (MAM) by peptide mapping LC-MS for therapeutic proteins and for the measurement of several critical quality attributes (CQA) using a single analytical procedure.

Graphical abstract:



A typical workflow for the MAM by peptide mapping LC-MS is illustrated in Figure 1. The workflow is based on the digestion of the target protein by a suitable endoprotease and subsequent analysis of the resulting peptides by LC-MS and LC-MS/MS. After the initial identification and characterisation of product related variants by LC-MS/MS a peptide library is built, which contains all relevant information to calculate the relative product CQA abundance during routine targeted monitoring by LC-MS. This is achieved by comparison of the LC-MS signals observed with a reference material (red) in comparison to a test sample (turquoise); new peaks can be identified.

Figure 1: Typical workflow for the MAM by peptide mapping LC-MS**1. Product characterization****2. Targeted monitoring**

This example refers to the measurement of three CQAs of an IgG1 monoclonal antibody (mAb A), in drug substance (DS) and drug product (DP) at release and for stability testing, as appropriate.

The three CQAs are:

- The deamidation of asparagine N_x in the complementarity-determining region (CDR) of mAb A, measured at release and for stability (on DS and DP),
- The oxidation of methionine M_z in the CDR of mAb A, measured at release and for stability (for DS and DP),
- The relative abundance of mannose-5-type glycan on asparagine N_y at the canonical glycosylation site in the constant domain of mAb A, measured at release (on DS).

Furthermore, this case study illustrates the different possible strategies to measure these CQAs, either using one individual analytical procedure for each of them or using the capabilities of MAM to measure all three CQAs in a single analytical procedure.

The assumptions are the following:

For CQA1 – deamidation of asparagine at N_x in the CDR of mAb A

- Asparagine X has been shown during product development to be prone to deamidation.
- Asparagine X is located in the CDR of the molecule, and it has been shown that its deamidation impairs target binding and therefore negatively impacts the biological activity of mAb A.
- For the purpose of this case study, it is assumed that the level of deamidation of asparagine at N_x should be no more than 5.0%.
- The assay to be developed should be able to detect a change in level of deamidation of asparagine at N_x under the intended storage conditions, accelerated stability as well as forced degradation conditions, as appropriate.

For CQA2 – oxidation of methionine M_z in the CDR of mAb A

- Methionine Z has been shown during product development to be prone to oxidation.
- Methionine Z is located in the CDR of the molecule, and it has been shown that its oxidation impairs target binding and therefore negatively impacts the biological activity of mAb A.
- For the purpose of this case study, it is assumed that the level of oxidation of methionine at M_z should be no more than 3.0%.
- The assay to be developed should be able to detect a change in level of oxidation of methionine M_z under intended storage conditions, accelerated stability as well as forced degradation conditions as appropriate.

For CQA3 – Mannose-5-type glycan (Man5) on asparagine N_y

- During development of the product, it was found that Man5 is the most abundant of the high mannose glycans and the level was observed at 2% or higher.
- Due to its potential impact on pharmacokinetics (PK), it is considered as a CQA.
- For the purpose of this case study, it is assumed that the level of Man5 should be no more than 4.0%.
- As Man5 is a glycosylation variant incompletely processed to complex N-glycan forms by the cells during cultivation in the bioreactor and as it does not evolve during storage of the DS (or DP), the assay to be developed does not need to be on the stability assay panel. It will be applied to DS only.

The example here and the assumptions made are illustrative and the numerical values provided throughout the document are merely suggestions to provide an idea of how these impact certain steps in the procedure. Relative levels of post-translational modifications tend to vary from molecule to molecule and from position to position in the amino acid sequence. Consequently, the (relative) variance observed in individual measurements of levels of CQAs can differ quite substantially, depending on the nominal value of the modification observed. In addition, the attributes selected here may not be a CQA in real therapeutic products depending on their specificities (structure, mode of action, etc.).

Table 1: Analytical Target Profile (ATP) for CQA1 - Deamidation of Asparagine at N_x

Intended Purpose		
Measurement of the level of deamidation of asparagine at N _x in mAb A DS and DP at release and for stability testing.		
Link to CQA (Level of Deamidation of Asparagine at N_x)		
Any analytical procedure selected must ensure that the level of deamidation of asparagine at N _x can be determined within the assumed specification range: not more than 5.0%.		
Characteristics of the Reportable Result		
Performance Characteristics	Acceptance Criteria	Rationale
Accuracy	<p>The recovery at each level covering the range of the reportable result is between 80%* and 120%*.</p> <p>Note: Recoveries are defined as the ratio of the measured deamidated species value to the expected value.</p> <p>Alternatively, accuracy can be inferred once precision, response within the range and specificity have been established.</p>	Ensures that the intended analytical procedure delivers the quality reportable result, taking into account prior knowledge obtained for other similar molecules and aligning with the release/stability acceptance criterion.
Precision	The intermediate precision (RSD) of the measurement of deamidated species is not more than 20%*.	
Specificity	Analytical procedure is specific for the attribute measured (deamidation of asparagine at N _x).	Ensures that the intended analytical procedure delivers the quality reportable result for the modification of interest (deamidation) at the specific location (asparagine N _x) in the amino acid sequence.
	No interference from relevant process-related impurities or matrix components.	
	Assay is stability indicating <i>i.e.</i> , analytical procedure is capable of detecting a change in the level of deamidation of asparagine at N _x using samples subjected to accelerated/stressed stability studies and/or to conditions known to induce deamidation of asparagine (<i>e.g.</i> , high pH).	
Reportable Range	The range covers at least the reporting threshold up to 6.0%*.	Range for which the required accuracy and precision characteristics are demonstrated according to ICH Q2(R2) (120% of the specification limit).

Note: a combination of precision and accuracy is also possible;

* numerical values are merely illustrative and must not be understood as general guidance

Table 2: Analytical Target Profile (ATP) for CQA2 - Oxidation of Methionine M_z

Intended Purpose		
Measurement of the level of oxidation of methionine at M _z in mAb A DS and DP at release and for stability testing.		
Link to CQA (Level of Oxidation of Methionine at M_z)		
Any analytical procedure selected must ensure that the level of oxidation of methionine M _z can be determined within the assumed specification range: not more than 3.0%.		
Characteristics of the Reportable Result		
Performance Characteristics	Acceptance Criteria	Rationale
Accuracy	<p>The recovery at each level covering the range of the reportable result is between 80%* and 120%*.</p> <p>Note: recoveries are defined as the ratio of the measured oxidised species value to the expected value.</p> <p>Alternatively, accuracy can be inferred once precision, response within the range and specificity have been established.</p>	Ensures that the intended analytical procedure delivers the quality reportable result, taking into account prior knowledge obtained for other similar molecules and aligning with the release/stability acceptance criterion.
Precision	The intermediate precision (RSD) of the measurement of oxidised species is not more than 30%*.	
Specificity	Analytical procedure is specific for the attribute measured (oxidation of methionine at M _z).	Ensures that the intended analytical procedure delivers the quality reportable result for the modification of interest (oxidation) at the specific location (methionine M _z) in the amino acid sequence.
	No interference from relevant process-related impurities or matrix components.	
	Assay is stability indicating <i>i.e.</i> , analytical procedure is capable of detecting a change in the level of oxidation of methionine at M _z using samples subjected to accelerated/stressed stability studies and/or to conditions known to induce methionine oxidation (<i>e.g.</i> , increased temperature, incubation with H ₂ O ₂).	
Reportable Range	The range covers at least the reporting threshold up to 3.6%*.	Range for which the required accuracy and precision characteristics are demonstrated according to ICH Q2(R2) (120% of the specification limit).

Note: a combination of precision and accuracy is also possible;

** numerical values are merely illustrative and must not be understood as general guidance*

Table 3: Analytical Target Profile (ATP) for CQA3 - Man5 on Asparagine N_y

Intended Purpose		
Measurement of the level of mannose-5-type glycans on asparagine N _y in mAb A DS at release.		
Link to CQA (Level of Mannose-5-type Glycans on N_y)		
Any analytical procedure selected must ensure that the level of mannose-5-type glycans on asparagine N _y can be determined within the assumed specification range: not more than 4.0%.		
Characteristics of the Reportable Result		
Performance Characteristics	Acceptance Criteria	Rationale
Accuracy	<p>The recovery at each level covering the range of the reportable result is between 80%* and 120%*. Expected value can be determined from a well-established orthogonal analytical procedure such as HILIC of released glycans.</p> <p>Note: recoveries are defined as the ratio of the measured Man5 value to the expected value.</p> <p>Alternatively, accuracy can be inferred once precision, response within the range and specificity have been established.</p>	Ensures that the intended analytical procedure delivers the quality reportable result taking prior knowledge obtained for other, similar, molecules into account and to align with the release/stability acceptance criterion.
Precision	The intermediate precision (RSD) of the measurement of mannose-5-type glycosylated species is not more than 20%*.	
Specificity	Analytical procedure is specific for the attribute measured (mannose-5-type glycosylated N _y).	Ensures that the intended analytical procedure delivers the quality reportable result for the modification (glycosylation with Man5) of interest at the specific location (asparagine N _y) in the amino acid sequence.
	No interference from relevant process-related impurities or matrix components.	
	Analytical procedure is capable of detecting varying levels of mannose-5-type glycosylated species at N _y using samples generated under different bioreactor conditions.	
Reportable Range	The range covers at least the reporting threshold up to 4.8%*.	Range for which the required accuracy and precision characteristics are demonstrated according to ICH Q2(R2) (120% of the specification limit).

Note: a combination of precision and accuracy is also possible;

** numerical values are merely illustrative and must not be understood as general guidance*

Technology Selection

Several analytical technologies are suitable for the measurement of the 3 CQAs when considering the three ATPs above.

For CQA1 – deamidation of asparagine at N_x in the CDR of mAb A

Separative analytical technologies, like **cation exchange chromatography (CEX)**, **capillary zone electrophoresis (CZE)** or **imaged capillary isoelectric focusing (iCIEF)** with UV or fluorescence detection, tend to be non-specific. The measure of charge variants includes N_x, asparagine residues in the constant region, C-terminal truncation, aggregation and is not limited to deamidated species. These methodologies cannot differentiate the type of charge variant and/or the position of the modified amino acid residue, and therefore, are not able to quantify deamidation of asparagine at N_x in the CDR of mAb A.

Peptide mapping LC-UV and/or LC-MS involve enzyme digestion followed by separation of the resulting peptides and analysis by UV and/or mass spectrometry detection. When only UV detection is used, a more reliable quantitation of deamidation of asparagine at N_x can only be obtained if the UV peak areas of deamidated and non-deamidated peptides can be accurately measured. It requires high chromatographic resolutions of the deamidated and non-deamidated peptides containing asparagine, aspartic acid or iso-aspartic acid at N_x respectively from all other peptides. This requires significant efforts in analytical procedure development for mAb A to achieve satisfactory resolution and specificity. In contrast, peptide mapping LC-MS analytical procedure has high specificity and resolution. It allows measurement of individual chemical modifications on the polypeptide chain of the mAb such as deamidation of asparagine at N_x, without interference from other product quality attributes or other co-eluting peptides. In addition, it is able to quantify other quality attributes of the molecule simultaneously as a MAM, such as oxidation of methionine at M_z and glycosylation of asparagine with Man5.

For CQA2 – oxidation of methionine M_z in the CDR of mAb A

Hydrophobic interaction chromatography (HIC) or **reverse phase (RP) chromatography** with UV detection are commonly used to analyse mAb variants based on their hydrophobicity. HIC operates under non-denaturing conditions, whereas RP chromatography is typically performed under denaturing conditions. These analytical procedures are not specific, as they separate and detect variants with reduced hydrophobicity, often appearing as pre-peaks. Such variants may result from modifications like clipping, oxidation of tryptophan and oxidation of methionine residues, including – but not limited to - M_z oxidation in mAb A.

Subunit analysis approach, which involves the specific cleavage of mAb A at the hinge region under reducing condition followed by RP separation and UV and/or mass spectrometry detection, can be used to monitor methionine oxidation at the domain level, though it is not site-specific. In addition, for some mAbs, the measured mass can be confounded with other prevalent modifications, such as N-terminal pyroglutamate or oxidation of other residues.

Peptide mapping LC-UV and/or LC-MS. When only UV detection is used, a more reliable quantitation of oxidation of methionine at M_z can only be obtained if the resolutions of the chromatographic peaks of both the oxidised and the non-oxidised peptides from all other peptides are sufficiently high. This requires significant efforts in analytical procedure development for mAb A to achieve satisfactory resolution and specificity. In contrast,

peptide mapping LC-MS analytical procedure has high specificity and resolution. It allows measurement of individual chemical modifications on the polypeptide chain of the mAb such as oxidation of methionine at M_z without interference from other product quality attributes or other co-eluting peptides. In addition, it is able to quantify other quality attributes of the molecule simultaneously as a MAM, such as deamidation of asparagine at N_x and glycosylation of asparagine with Man5.

For CQA3 – Man5 on asparagine N_y

Chromatography techniques (reverse phase (RP), hydrophilic interaction (HILIC), normal phase and mixed mode) **or capillary gel electrophoresis** with fluorescence detection can be used for the analysis of N-glycosidase F (PNGase F) or fluorophore-derivatised glycans. The specificity of the analytical procedure relies on separation of Man5 from the other glycans, as well as on confident identification of the glycan peak by *e.g.*, mass spectrometry. These analytical procedures only provide relative quantitation of glycans, including Man5 on **asparagine N_y** of mAb A, but not other attributes on mAb A such as deamidation of asparagine at N_x or oxidation of methionine at M_z .

LC-MS analysis of released glycans with or without labeling. RP, HILIC and mixed mode chromatography separation methods coupled with mass spectrometry can be applied to analyse released glycans to overcome issues related to co-elution of glycan species.

Peptide mapping LC-MS allows measurement of individual chemical modifications on the polypeptide chain of the mAb simultaneously as a MAM, such as glycosylation of asparagine at N_y including Man5 form, deamidation of asparagine at N_x and oxidation of methionine at M_z .

Selected technology:

Amongst the different possible technologies being able to measure deamidated, oxidised and glycosylated species including Man5, MAM by peptide mapping LC-MS was selected for the following reasons:

- MAM can quantify multiple types of post translational modifications (*e.g.*, deamidation of asparagine, oxidation of methionine, asparagine glycosylation, etc.) simultaneously that usually require multiple conventional analytical procedures. Significantly more effort is involved in developing and maintaining the analytical procedures,
- MAM is able to address specifically the CQA in scope at the amino acid level enabling better discrimination of CQAs from low criticality quality attributes of the same type,
- Mass spectrometry-based release and stability testing assays in quality control (QC) settings have been gaining more acceptance by both applicants and regulatory agencies, benefiting from the advancement of automation and mass spectrometry data processing software,
- Additional quality attributes *e.g.*, identity could also be addressed by the MAM approach. Those are not discussed further in this case study.

For the sake of simplification and illustration of the application of ICH Q14 principles, it is assumed here that the MAM by peptide mapping LC-MS is used as a targeted method for multi-attribute monitoring. This means that aspects related to New Peak Detection (NPD) are out of

scope of this example. In practice, MAM by LC-MS methodology could cover purity-related attributes when using NPD features.

Analytical Procedure Development

The peptide mapping LC-MS analytical procedure (MAM) was developed using an enhanced approach. The development was based on a thorough understanding of the molecules' structure and degradation pathways through in-depth characterisation by biological and physico-chemical analytical procedures, including LC-MS, and extensive knowledge of the complete peptide mapping LC-MS workflow.

The unit operations of the peptide mapping LC-MS workflow and the factors related to these were listed and risk-assessed using QRM principles (see Ishikawa diagram in Figure 2 and an example risk assessment outcome for the unit operation "sample preparation" in Table 4). Finally multi-variate experiments were performed to explore ranges and interactions between identified analytical procedure parameters and to define an analytical procedure control strategy including set-points and/or ranges for relevant analytical procedure parameters.

During sample preparation, caution should be taken to prevent occurrence of artefactual post-translational modifications, such as methionine oxidation or asparagine deamidation, as these influence the levels intrinsically present on the analyte protein (see Table 4). Although not described in Table 4, long incubation times in the autosampler during execution of lengthy sample sequences can also induce artefactual methionine oxidation. The extent of this phenomenon can be monitored using bracketing reference material injections.

Figure 22: Critical procedure parameters of the MAM by peptide mapping LC-MS

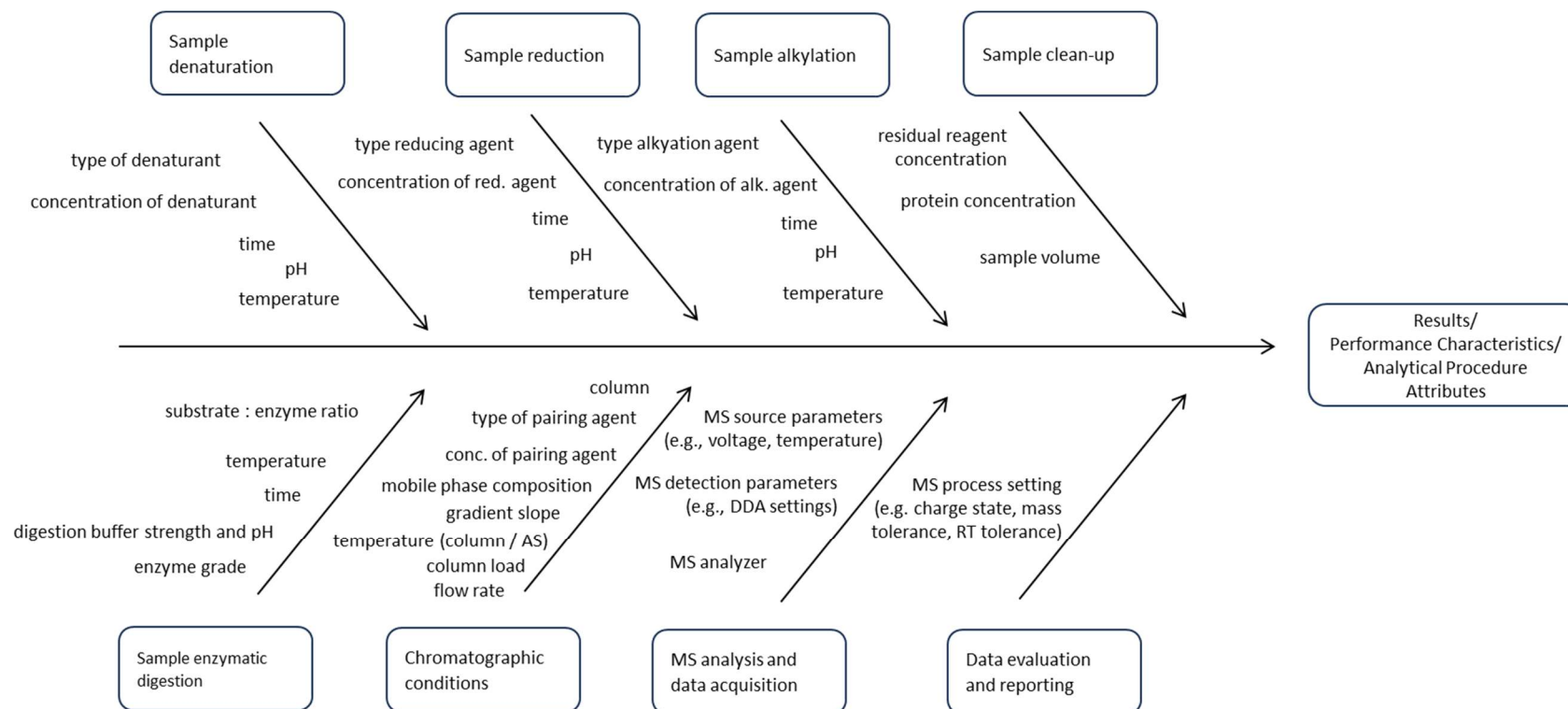


Table 4: Summary of development data and risk assessment

Unit operation	Procedure parameter	Defined target or range	Investigated range	Rationale	Risk* of impacting results CQA1/CQA2/CQA3
Sample denaturation, reduction and alkylation	chaotropic agent concentration (e.g., guanidine HCl)	6.0 M	5.5 - 6.5 M	- to achieve complete denaturation enabling reproducible digestion of the polypeptide chain - investigated range justified considering worst case weighing/pipetting accuracy	medium/medium/medium
	Reducing agent concentration (e.g., DTT)	10 mM	9 - 11 mM	- to achieve complete reduction enabling reproducible digestion of the polypeptide chain - investigated range justified considering worst case weighing/pipetting accuracy	medium/medium/medium
	pH	7.4	7.2 - 7.6	- to achieve complete reduction enabling reproducible digestion, while minimising artefactual post-translational modifications - investigated range justified considering accuracy of the pH adjustment step and qualified range of pH-meter	high/medium/low
	Alkylation agent concentration (e.g., iodoacetamide)	22 mM	21 - 23 mM	- to achieve quantitative alkylation of the free cysteines enabling reproducible digestion, while minimising artefactual post-translational modifications - investigated range justified considering worst case weighing/pipetting accuracy	medium/medium/medium
	temperature	25°C	24of - 26°C	- to achieve complete reduction, alkylation enabling reproducible digestion, while minimising artefactual post-translational modifications - investigated range justified considering qualified range of the incubator	medium/medium/medium
	time	30 min (denat., red.) 20 min (alkyl.)	27 - 33 min 18 - 22 min	- to achieve complete reduction, alkylation enabling reproducible digestion, while minimising artefactual post-translational modifications - investigated range justified considering expected variability of incubation duration	high/high/low
Sample clean-up	etc.				

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

Only the first unit operation is discussed in this table to exemplify the methodology

Analytical Procedure

The example in the tables below and the assumptions are purely illustrative and do not provide recommendations for performance of a MAM. For the purpose of this example, a summary of the analytical procedure is provided below. This does not reflect the entirety of the procedure description in the dossier.

Sample preparation and LC-MS Analysis

Denaturation, reduction alkylation and clean-up	<ol style="list-style-type: none"> 1. Samples (100 µg) were denatured and reduced using final concentration of 1 mg/mL antibody, 6.0 M guanidine HCl, 10 mM DTT, 50 mM Tris at pH 7.4 and incubated at 25°C for 30 min. 2. Alkylation with iodoacetamide (IAM) at a final concentration of 22 mM IAM at 25°C for 20 min in the dark. 3. Alkylation was quenched by adding another aliquot of DTT equivalent to 12 mM final concentration. 4. The solutions were buffer exchanged into 50 mM Tris at pH 7.4 using centrifugal diafiltration units. Protein concentrations were determined by A280.
Enzymatic digestion	<ol style="list-style-type: none"> 1. After clean-up, samples were incubated with trypsin at a substrate:enzyme ratio of 1:10 at 37°C for one hour. 2. Digestion was stopped by adding trifluoroacetic acid (TFA) to a final concentration of 0.5 v/v%.
Chromatographic conditions	<ol style="list-style-type: none"> 1. Mobile phase A containing 0.02 v/v% TFA in water and mobile phase B contains 0.02 v/v% TFA in acetonitrile. 2. Column: C18 (2.1 x 150 mm, 1.7 µm, 300 Å) 3. Flow rate: 0.2 mL/min. 4. Gradient: 0 - 5 min at 1% B; 5 - 7 min 5-10% B; 7 - 57 min 10 - 35% B; 57 - 58 min 35 - 90% B; 58 - 63 min at 90% B; 63 - 64 min 90 - 1% B; 64 - 75 min at 1% B. 5. Column temperature: 60°C 6. Column load: 5 µg 7. Autosampler temperature: 5°C
MS analysis and data acquisition	<ol style="list-style-type: none"> 1. Source parameters: spray or cone voltage - 3500 V, sheath gas - 30 arb, aux gas 10 arb, sweep gas - 1 arb, transfer tube temp - 180°C, vapouriser or desolvation temp - 200°C. 2. Detection parameters: resolution - 120,000, scan range - m/z 300 - 2,000, time 5 - 60 min
Data evaluation and reporting	<ol style="list-style-type: none"> 1. MS detection: extracted ion chromatogram using charge states as defined in the peptide library. 2. MS settings: number of decimal points - 4, mass tolerance - 5 ppm, retention time (RT) tolerance - 0.5 min 3. Calculation of %CQA relative abundance: $\%CQA1 = \frac{\sum A_{P1QA}}{\sum A_{P1QA1} + A_{P1}} \times 100\%$ $\%CQA2 = \frac{A_{P2QA2}}{A_{P2QA2} + A_{P2}} \times 100\%$ $\%CQA3 = \frac{A_{P3Man}}{\sum A_{P3Glycans} + A_{P3}} \times 100\%$

	<p>with</p> <p>A_{Px} = Peak area of unmodified peptide x</p> <p>A_{PxQA_x} = Peak area of peptide x carrying quality attribute / modification x</p> <p>ΣA_{P1QA1} = sum of peak areas of peptide 1 carrying all modifications related to quality attribute 1 <i>e.g.</i>, for deamidation of asparagine N_x, <i>i.e.</i>, D_x and iso-D_x</p>
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Analytical procedure control strategy

System suitability test	<p>Acceptance criteria of LC-MS system using selected peptides from pre-digested general reference material:</p> <ol style="list-style-type: none"> 1. Resolution of peptide 3 and 4 ≥ 2.0 2. Delta RT of peptides 1 - 7 between the first and last run ≤ 0.5 min 3. Mass accuracy of peptides 1 - 7 ≤ 5 ppm 4. Signal intensity of peptide 3 $\geq 1E6$ <p>Acceptance criteria of LC-MS system using product-specific control sample prepared in the same experiment as test sample:</p> <ol style="list-style-type: none"> 1. The extracted ion chromatogram obtained should be comparable to the example shown in the analytical procedure 2. Resolution between modified peptides and corresponding unmodified peptides ≥ 1.5 3. The intensity of non-modified reference peptide $\geq 1E7$ 4. %CQA1, %CQA2 and %CQA3 are within 80 - 120% of expected values reported for the control sample <p>Acceptance criteria of digestion blank (prepared in the same experiment as test sample): No interference peaks in the RT ranges of modified peptides or unmodified peptides with intensity $> 5E4$ in the extracted ion chromatogram.</p>
Sample suitability assessment	<ol style="list-style-type: none"> 1. Mass errors of the modified peptides and unmodified peptides ≤ 5 ppm 2. Resolution between modified peptides and corresponding unmodified peptides ≥ 1.5 3. The intensity of non-modified reference peptide $\geq 1E7$

Analytical Procedure Validation

The analytical procedure was validated according to ICH Q2(R2), considering a validation protocol with predefined acceptance criteria to address:

- Performance characteristics as defined in the ATP (specificity, accuracy, precision, reportable range)
- Technology dependent analytical procedure attributes (linearity, quantification limit)

The results were summarised in a validation report, which concluded that the performance characteristics across the reportable ranges met the acceptance criteria. In summary, the analytical procedure was demonstrated to be suitable for the intended purpose.

Table 5: Summary of analytical procedure validation approach

Performance characteristic	Validation study methodology and validation acceptance criteria
Specificity	<p>Technology inherent justification:</p> <ol style="list-style-type: none"> 1. Analytical procedure specificity for deamidated N_x, oxidised M_z and Man5 on N_y inferred through accurate <i>m/z</i> value of specific peptides, in combination with their chromatographic retention time. 2. No interference from sample matrix components. Demonstrated through absence of response from analysis of <i>e.g.</i>, sample formulation buffer, mobile phase blank. 3. Demonstrate assay is stability indicating for CQA1 and CQA2 by detecting a change in deamidation of asparagine at N_x and oxidation of methionine at M_z using samples subjected to relevant stressed conditions <i>e.g.</i> high pH for N_x deamidation and hydrogen peroxide treatment for oxidation of methionine at M_z.
Precision	<p>For CQA1 and CQA2: Establish series of samples with varying levels of asparagine deamidation or methionine oxidation by co-mixing unstressed material with varying levels of highly stressed (asparagine-deamidated or methionine-oxidised) material.</p> <p>For CQA3: Establish series of samples with varying levels of Man5 by co-mixing Man5-containing IgG with another non Man5-containing IgG sample.</p> <p>A minimum of three CQA levels to be generated, covering the QL to at least 120% of the upper specification limit (USL). The upper limit of the range for the different CQAs equates to:</p> <ul style="list-style-type: none"> • CQA1: 6.0% based on a USL of 5.0% for deamidated species, • CQA2: 3.6% based on a USL of 3.0% for oxidised species, • CQA3: 4.8% based on a USL of 4.0% for Man5 <p>Repeatability: perform measurement of a minimum of three replicates at a minimum of three CQA levels under the same operating conditions over a short period of time or a minimum of 6 replicates at the 100% level.</p> <p>Intermediate precision: perform measurement of independently prepared samples under varying conditions such as different days, different analysts, different batches of critical reagents <i>e.g.</i>, columns, enzymes, different LC-MS systems.</p> <p><u>Repeatability acceptance criteria:</u></p> <p>CQA1: For each of the levels, the repeatability of the measurement of deamidated species is ≤ 10% CV.</p> <p>CQA2: For each of the levels, the repeatability of the measurement of oxidised species is ≤ 10% CV.</p> <p>CQA3: For each of the levels, the repeatability of the measurement of Man5 is ≤ 10% CV.</p> <p><u>Intermediate precision acceptance criteria:</u></p> <p>CQA1: The intermediate precision of the measurement of deamidated species is ≤ 20% CV.</p> <p>CQA2: The intermediate precision of the measurement of oxidised species is ≤ 30% CV.</p> <p>CQA3: The intermediate precision of the measurement of Man5 is ≤ 20% CV.</p>
Accuracy	<p>For CQA1 and CQA2: Determine recovery by establishing the ratio of the measured deamidated species (CQA1), or oxidised species (CQA2), and the expected value, based on the known spike level (see precision). Determine recovery at a minimum of three CQA levels covering the QL to at least 120% of the USL.</p>

	<p>For CQA3: Determine recovery by establishing the ratio of the measured Man5 species and the expected value based on the co-mix level (see precision). Determine recovery at a minimum of three CQA levels covering the minimum and maximum range of Man5 observed across batches. Expected value can be determined from a well-established orthogonal analytical procedure such as HILIC of released glycans.</p> <p>For the three CQAs, alternatively, the accuracy can be inferred from precision, response within the range and specificity.</p> <p>Acceptance criteria: For deamidated species, the recovery at each level covering the range of the reportable result is between 80 and 120%. For oxidised species, the recovery at each level covering the range of the reportable result is between 80 and 120%. For Man5, the recovery at each level covering the minimum and maximum range of Man5 observed across batches is between 80 and 120%.</p>
Reportable range	<p><u>Validation of calibration model across the range:</u></p> <p>Linearity: experimental demonstration of the linear relationship between analyte concentrations and peak responses at 5 or more concentration levels appropriately distributed across the range: the coefficient of determination of the measured-expected values curve should be ≥ 0.98 and there should be no systematic pattern in the residuals plot through visual examination.</p> <p><u>Validation of lower range limits:</u> QL: acceptable accuracy and precision.</p> <p>For the three CQAs, the reportable range extends from, and includes, the QL to the highest evaluated level, with acceptable accuracy, precision and response. The range of the measurement covers at least the reporting threshold up to 120% of the USL.</p>
Robustness	<p>Deliberate variations of critical procedure parameters (see Figure 22 and Table 4), e.g., by using a design of experiment (DoE) approach confirmed adherence of performance characteristics to predefined acceptance criteria.</p>

Note 1: numerical values in this table are merely illustrative and must not be understood as general guidance.

Note 2: a combination of precision and accuracy is also possible

Established Conditions (ECs), Reporting Categories and Justifications

The applicant proposed and justified ECs and reporting categories as part of the submission. In Table 6 some of the proposed EC, their risk categories and their proposed reporting categories are described. The evaluation of the risk categories and associated reporting categories depends on the extent of knowledge gained and information/justification described in the dossier. In this example, it is assumed that the applicant has developed the analytical procedure using the enhanced approach and has a deep understanding and knowledge, both of the molecule and of the analytical peptide mapping LC-MS technique.

Note: The dossier is subject to regulatory review. The information provided in this example is only part of the knowledge available that will be submitted and is provided for illustrative purposes only. The extent of ECs (EC or not EC designation), actual reporting categories, and data requirements may differ by region. Depending on the nature and extent of the change (e.g., change to a different technology), a PACMP may be required.

Table 6: Evaluated risks, proposed established conditions and proposed reporting categories

Established Condition*	Overall Risk Category	Proposed Reporting Category	Comment
Performance characteristics and associated criteria as defined in the ATP	High	PA	The performance characteristics and criteria ensure the quality of the reportable results and link to the CQAs. Widening of performance characteristics and criteria could have an impact on the control of the CQAs.
Technology (principle) Peptide mapping LC-MS	High or medium	PA or NM	Adherence to performance characteristics and criteria ensured by control strategy and defined bridging strategy to assess the impacts of changes. Changes would be reported as NM if no impact on the specification acceptance criteria and as Prior Approval PA if there is an impact on the specification acceptance criteria.
Analytical procedure control strategy elements			
System suitability test (SST)	High or medium	PA or NM**	The performance of the analytical procedure is ensured by: <ul style="list-style-type: none">- Direct control of the analytical procedure steps through the analytical procedure control strategy.- Defined analytical procedure control strategy elements that ensure adherence to the three ATPs.- Adherence to the performance characteristics and acceptance criteria after a change of analytical procedure control strategy elements. If assurance of performance of the analytical procedure cannot be demonstrated, the change needs to be reported as PA.
Sample suitability assessment	High or medium	PA or NM**	
Sample denaturation, reduction, alkylation and clean-up			
Concentration of denaturant, alkylation and reducing agent, reaction temperature, pH and duration	Low	NL	The concentration of denaturant and reducing agent are important to ensure reproducible digestion of the polypeptide chain and achieve required signal intensity. For the CQAs measured, pH, temperature and duration of the reaction are important to not artefactually impact the levels of N _x -deamidated species or M _z -oxidised species. The SST and more specifically the presence of a reference material in each run, ensures the suitability of the analytical conditions for sample denaturation, reduction, alkylation and clean-up and their performance.

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Established Condition*	Overall Risk Category	Proposed Reporting Category	Comment
Sample enzymatic digestion			
Reaction temperature, pH and duration; substrate:enzyme ratio	Low	NL	The extent of digestion is important to ensure suitable evaluation of the data. The SST, and more specifically the inclusion of a reference material in each run, ensures the suitability and the performance of the analytical conditions for sample digestion as well as adequate digestion efficiency.
Chromatographic conditions			
Chromatography column, eluent composition, elution gradient and temperature (column, autosampler)	Low	NL	The SST and the sample suitability assessment ensure adherence to performance characteristics and acceptance criteria.
MS analysis and data acquisition			
Mass analyser – use of different technology	High or medium	PA or NM	Adherence to performance characteristics and criteria ensured by control strategy and defined bridging strategy to assess the impacts of changes. Changes would be reported as NM if no impact on the specification acceptance criteria and as PA if there is an impact on the specification acceptance criteria.
MS source parameters	Low or medium	NM or NL	The SST and the sample suitability assessment ensure adherence to performance characteristics and acceptance criteria. Upon change of the mass spectrometer instrument, there could be a difference in specificity or sensitivity that could impact the detectability and limit of quantification of the analytical procedure and therefore could impact the reportable results. If assurance of performance of the analytical procedure cannot be demonstrated, the change needs to be reported as NM.
MS detection parameters	Low or medium	NM or NL	
Data - evaluation and reporting			
MS data processing parameters (charge states, RT and <i>m/z</i> detection windows)	Low	NL	The SST and the sample suitability assessment ensure adherence to performance characteristics and acceptance criteria.
An example of a parameter that is not an EC			

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Established Condition*	Overall Risk Category	Proposed Reporting Category	Comment
Buffer composition in process step - sample denaturation, reduction, alkylation and clean-up:	Low	Not reported	As long as concentration of denaturant, reducing and alkylation agent as well as pH are defined, the composition of the sample buffer is not expected to impact procedure performance and hence is considered a non-EC with an overall low risk category

PA: Prior Approval, NM: Notification Moderate; NL: Notification Low (as per ICH Q12 definitions);

** all other elements shown in Figure 22 are non-EC;*

*** Based on regional requirements the proposed reporting category may need to be elevated to PA.*

Change Assessment and Bridging Strategy

Change 1: Change of MS model equipment to a newer MS instrument generation

Background of change:

This section refers to the exchange of a current model mass spectrometer to one from a newer generation (no change in mass analyser technology, but improved resolution, acquisition performance, etc.). This change affects the “MS analysis and data acquisition” step of the analytical procedure. The rest of the analytical procedure remains unchanged. This change remains within the same technology.

Summary of structured risk assessment

The relevance of the test is classified as high as there is a direct link to multiple CQAs: deamidation of asparagine at N_x, oxidation of methionine at M_z and mannose-5-type glycosylation on asparagine N_y that need to be controlled to ensure the safety and efficacy of the drug.

The extent of the change is limited to the change of the mass spectrometer and could in theory impact the detection of CQA-related peptides and/or the measurement of the lower levels of the three CQAs in case the resolution and sensitivity of the new instrument are better than those of the current one. Considering the improved resolution and sensitivity of the new instrument as specified by the vendor, the residual risk of the change is considered as medium. Other mass spectrometer parameters (*e.g.*, source parameters) are well understood and monitored by SST.

Adherence to criteria for relevant performance characteristics

The understanding of the analytical procedure and link to the CQAs allowed for the definition of criteria for relevant performance characteristics which ensure the quality of the results after the change (see

Table 1 to Table 3 for the ATPs of the three CQAs measured by the MAM). The analytical procedure remains unchanged up to the MS analysis. Despite the specified improved resolution and sensitivity of the new instrument, an impact on the specificity and reportable range is excluded as the resolution and sensitivity are controlled by SST upon injection of a product-specific control sample. The reporting limit will remain the same, despite a potentially lower QL. Furthermore, it is not expected that there will be any impact on the accuracy and precision. Following ICH Q14 Figure 2 performance characteristics and related criteria are defined in the ATPs and sufficient understanding is available to design the bridging study. Therefore the risk category before implementation of the change was low and aligned with risk category in the PLCM document.

Demonstration of analytical procedure performance after change

Evaluation of impact on performance characteristics:

Based on the analytical procedure development, the analytical procedure parameters related to MS source and detection that could potentially impact the performance have been evaluated and defined in the analytical procedure description. The SST of the analytical procedure covers the MS acquisition step, in particular the sensitivity with a measurement of the mass accuracy and signal intensity of certain key peptides.

Experimental bridging study results:

In accordance with Table 2 of ICH Q14, a partial revalidation of the analytical procedure was performed to demonstrate the suitability of the MS analysis with the new instrument to confirm that the acceptance criteria for the performance characteristics were met. In practice, the partial revalidation covered accuracy, precision and specificity as defined in the ATPs and demonstrated that they remained unchanged. A comparative analysis (including appropriate statistical analysis) of a set of representative samples covering the reportable range of the analytical procedure, with the current and the new mass spectrometer was performed confirming that the measured relative abundance of the three CQAs remain within a predefined variability when analysed with both mass spectrometers.

Conclusion:

The analytical procedure bridging was successfully performed. The capacity of the analytical procedure to generate the reportable results remained unchanged. The risk associated with the change was considered low, taking the outcome of the initial risk assessment, the evaluation of the performance characteristics and the bridging strategy into account.

Regulatory reporting:

Based on the above, the change evaluation showed that the extent of the change neither impacted the ATP, nor the specifications, including reporting limit. The risk was confirmed to be low and the change was submitted as notification low as agreed in the PLCM document. Accordingly, the analytical validation report and the outcome of the bridging study was submitted. The SST criteria of the analytical procedure including those ensuring the correctness of the sample preparation unit operations remained unchanged.

Change 2 - Change of sample preparation from manual to fully automated system

Background of change:

This section refers to the change of sample preparation from manual to fully automated system. This change affects the steps “sample denaturation, reduction and alkylation” as well as “sample clean-up” and “sample enzymatic digestion”. The same reagents, consumables and analytical conditions will be used and implemented on a robotic system. The rest of the analytical procedure remains unchanged. This change remains within the same technology and is not expected to have an impact on the specifications.

Summary of structured risk assessment

The relevance of the test is classified as high as there is a direct link to multiple CQAs: deamidation of asparagine at N_x, oxidation of methionine at M_z and mannose-5-type glycosylation on asparagine N_y that need to be controlled to ensure the safety and efficacy of the drug. The change is not expected to impact the link to the CQAs (same sample preparation unit operations, same readout) and has low criticality in this respect. The MAM used for the measurement of aforementioned CQAs represents a complex technology, as such an assay exhibits multiple sources of variability. Factors contributing to variability are well understood (based on prior knowledge and enhanced development data) and are addressed in the analytical procedure control strategy. In addition, it is expected that by moving from manual to automated sample preparation, the range of critical procedure parameters such as reagent concentration, incubation time and temperature will decrease, thereby increasing reproducibility of sample denaturation, reduction, alkylation and digestion.

The extent of the change is restricted to the preparation of the sample (change in analytical procedure step sample preparation), with potential impact on several analytical procedure attributes (denaturation, reduction, alkylation, clean-up and proteolytic digestion). Factors contributing to the sample preparation are understood and were investigated as part of development of the sample preparation unit operations and monitored by the SST. The initial risk assessment suggested the risk to be medium.

Adherence to criteria for relevant performance characteristics

The understanding of the analytical procedure and link to the CQAs allowed the definition of criteria for relevant performance characteristics which ensure the post-change quality of the reportable result obtained following the change. The change can potentially affect protein denaturation, reduction, alkylation and digestion and hence the analytical procedure performance characteristics accuracy and precision. Prior to changing the approved analytical procedure, adherence to these performance characteristics should be demonstrated. This change neither impacts the performance characteristic specificity, nor reportable range as the same sample preparation unit operations are used and the CQAs are determined relative to the same reference material. Following ICH Q14 Figure 2 performance characteristics and related criteria are defined in the ATPs and sufficient understanding is available to design the bridging study. Therefore the risk category before implementation of the change was low and aligned with the risk category in the PLCM document.

Demonstration of analytical procedure performance after change

Evaluation of impact on performance characteristics:

Based on analytical procedure development, analytical procedure parameters related to protein denaturation, reduction, and alkylation as well as sample clean-up and proteolytic digestion that could potentially impact the performance have been evaluated and defined in the analytical procedure description. The SST of the analytical procedure remains the same and covers the suitability of the sample preparation (*e.g.*, digestion efficiency, signal intensity and peak ratios in the overall

chromatogram as well as the recovery of relative abundance of CQA1, CQA2 and CQA3 as reported for the control sample).

Experimental bridging study results:

In accordance with Table 2 of ICH Q14, a partial revalidation of the analytical procedure was performed to demonstrate the impacted analytical procedure attributes met the pre-defined criteria after the change. Comparative analysis (including appropriate statistical analysis) of a set of representative samples with pre- and post-change analytical procedures was performed to ensure comparable results.

Conclusion:

Evaluation of performance characteristics demonstrated that defined criteria were met. The result of the studies confirmed the expected performance of the sample preparation unit operations post-change. The purpose of the analytical procedure has not changed and its capability to generate the reportable result remained unchanged. Analytical procedure bridging was successfully performed. The risk associated with the change is considered low, taking the outcome of the initial risk assessment, the evaluation of the performance characteristics and the bridging study results into account.

Regulatory reporting:

Evaluation of the impact of the change on the performance characteristics and the experimental bridging study confirmed that the risk category was low and the change could be submitted as notification low as agreed in the PLCM document. Accordingly, the revised analytical procedure description was submitted together with the validation report and the outcome of the bridging study. Development data demonstrating no impact on sample preparation and the corresponding reported results for the CQAs were provided.

5. CASE STUDY - AT-LINE ASSAY OF CORE TABLETS BY MULTIVARIATE ANALYTICAL PROCEDURE IN CONTINUOUS MANUFACTURING OF A DRUG PRODUCT

Note: This case study reflects the illustrative example described in Annex 2 of ICH Q2(R2), example 2 of Annex B of ICH Q14 and example 2 of ICH Q2(R2) / Q14 training module 6.

Introduction and Background

Real-time monitoring of assay of core tablets during a continuous manufacturing process of product X is used to conduct real time release for assay of product X. The tablets of product X are captured in discrete containers following compression. After results from NIR testing, the compliant containers are mixed and presented to the tablet coating process.

The monitoring procedure for assay is an at-line NIR procedure utilising a multivariate partial least squares (PLS) model. The NIR instrument is integrated into the process line with an automated sampling mechanism that allows for real time measurement of a minimum of 30 tablets evenly distributed across each container (which is subsequently processed in a batch manner downstream). The procedure associated PLS model is considered a high impact model based on ICH definition (ICH Q8/Q9/Q10 Q&As (R2) Points to Consider).

Table 1: Analytical Target Profile:

Intended Purpose		
Real time release of continuously manufactured tablets for the Critical Quality Attribute (CQA) assay.		
Link to CQA (Assay)		
The analytical target profile is defined as “the obtained assay by the analytical procedure needs to be equal to the true assay value within (\pm) a maximum allowable difference of 3.3%”. The maximum allowable difference is determined as one third (1/3) of the total assay specification range [95.0 - 105.0%].		
Characteristics of the Reportable Results		
Characteristic	Acceptance Criteria*	Rationale
Performance Characteristics		
Accuracy	Root mean square error of prediction (RMSEP)** $\leq 3.3\%$	Set aligned with target requirement of the spectroscopic analytical procedure and to ensure equivalence with reference analytical procedure
Precision	RSD $\leq 2.0\%$	
Specificity	Spectral range and PLS loadings and model regression vector should demonstrate API unique spectral features.	To ensure quantitative model is relevant to analyte of interest

Reportable Range	70.0 - 130.0%	To encompass potential analytical results that would be considered acceptable within the pharmacopeial expectations for assay
Response	Correlation coefficient $r \geq 0.970$	Set to ensure range is determined through a linear quantitation

**The above ATP targets are illustrative for this example – other targets may be justified*

***RMSEP represents combined accuracy and precision*

Technology Selection

The user requirement were defined as:

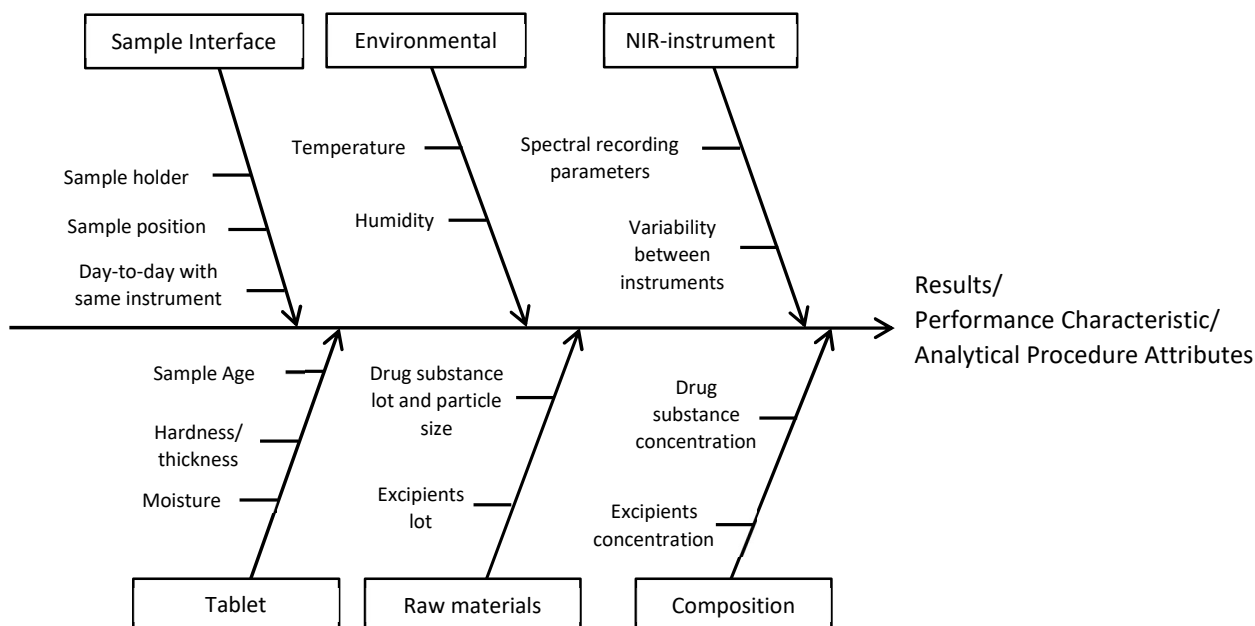
1. Procedure needs to be suitable for oral solid dosage form
2. In-line or at-line analysis
3. Rapid analysis
4. Minimal sample preparation (non-destructive)
5. Existing organisational knowledge available.

Based on the above, NIR spectroscopy was chosen.

Analytical Procedure Development

An enhanced development approach leveraging knowledge and risk management concepts, as well as considerations defined in ICH Q14 was used. Risk assessment was used to determine which factors could impact the overall performance of the NIR procedure (see Ishikawa diagram shown in Figure 1). Based on development outcomes, an analytical procedure was defined.

Figure 1: Ishikawa Diagram



It is usually not possible to foresee every source of variation that can occur during the lifecycle of the product during the development of the PLS model. Therefore, when conducting a routine NIR analysis of a specific batch, a parameter may cause spectral variation that was not incorporated into the model. To prevent this, data quality checks are incorporated which assess quality attributes for each recorded NIR spectrum against a threshold, defined during analytical procedure development and validation.

Analytical Procedure

For this example, a summary of the analytical procedure is provided below. This does not reflect the entirety of the analytical procedure description in the dossier.

Table 2: Summary of the Analytical Procedure Description

NIR Method Parameter		Value
NIR Instrument	Spectrometer	FT-NIR with a transmission unit
	Spectral collection range	12500 - 5800 cm ⁻¹
	Spectral collection mode	Transmission
Data Acquisition	Number of scans	At least 32
	Resolution	At least 16 cm ⁻¹
Sample interface	Sample presentation	At least 30 tablets/container are measured via an automated diversion mechanism from the outlet of the tablet press to an at-line NIR spectrometer. Tablets are presented to the spectrometer in a specific sample holder, ensuring a representative and precise positioning of the tablet in the NIR radiation
Software	Model development, spectral recording and analysis software	Software name + version
	Tablet press interface	Software name + version
Calculation	Chemometrics algorithm	Partial Least Squares (PLS) model
Analytical procedure range	%Label claim	70.0 - 130.0
Reference analytical procedure	Off-line HPLC	Liquid chromatography analytical procedure X
PLS Model	Spectral pre-processing	Standard Normal Variate (SNV) followed by 17 points Savitzky-Golay 1st derivative
	PLS model spectral range	12500 - 8950 cm ⁻¹
	Number of latent variables	3
Data quality checks ^a		Mahalanobis distance ≤ 0.74
		Residuals ≤ 0.078

^a The data quality check is a real time test to verify the incoming spectra are within the PLS model space. The test will reject outlier spectra that are out of the established model space. Spectra failing the data quality check are not necessarily out of specification or out of control limits. The purpose of the data quality check is to flag outliers and potential model maintenance needs.

Description of Analytical Procedure Performance Criteria Monitoring

Qualification, routine use and maintenance of the NIR analytical procedure, including the NIR instrument, and NIR PLS model are managed within the company quality system, and are subject to inspection.

Analytical Procedure Validation

Validation of the defined analytical procedure was conducted as per ICH Q2(R2). Performance characteristics were identified prior to execution of validation activities. The procedure met all pre-defined acceptance criteria as outlined in Table 1 and is deemed validated.

Table 3: Summary of the Analytical Procedure Validation

Performance characteristic	Validation study methodology	Validation Results
Specificity/ Selectivity	<u>Absence of interference:</u> Comparison of drug substance spectrum and the loading plots of the model Rejection of outliers (e.g., excipient, analogues) not covered by the multivariate procedure	An overlay of spectra of drug substance, a core tablet and a placebo tablet are made. Furthermore, plots of the regression coefficients and the relevant PLS components as a function of wavenumbers are reported. Out-of-scope samples are challenged and rejected by the model. Specificity/selectivity was adequate.
Precision	<u>Repeatability:</u> Repeated analysis with removal of sample from the holder between measurements	RSD of 1.6% at target level (100%). Repeatability was adequate.
Accuracy	<u>Comparison with an orthogonal procedure:</u> Demonstration across the range through comparison of the predicted and reference values using an appropriate number of determinations and concentration levels (e.g., 5 concentrations, 3 replicates) Accuracy is typically reported as the standard error of prediction (SEP or RMSEP)	RMSEP of 2.3%. Accuracy was adequate.
Reportable Range	<u>Response:</u> Demonstration of the relationship between predicted and reference values <u>Error (accuracy) across the range:</u>	69.3 - 132.9%. A linear response, with a correlation coefficient r of 0.998 is obtained. A plot of the residuals of the model prediction versus the actual data was provided. The response was found to be linear across the reportable range.

Performance characteristic	Validation study methodology	Validation Results
	Information on how the analytical procedure error (accuracy) changes across the calibration range, <i>e.g.</i> , by plotting the residuals of the model prediction <i>versus</i> the actual data	
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<u>Deliberate variation of parameters</u> , <i>e.g.</i> , Chemical and physical factors that can impact NIR spectrum and model prediction should be represented in data sets. Examples include various sources of drug substance and excipients, water content, tablet hardness, and orientation in the holder	Variability within and between instruments, tablet hardness and thickness variability, moisture content of tablets, batch-to-batch variability, drug substance particle size variability, tablet relaxation, sample position variability, tablet composition, and environmental conditions of temperature and humidity were successfully demonstrated.

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

The ECs of the NIR analytical procedure are identified based on the procedure principle, robustness studies, and ATP. In this example, selected elements of measurement principle, measurement location and chemometrics are used to illustrate the approach to identification of ECs and their associated reporting categories (Table 4). The reporting categories are based on the level of potential risk associated with the change management of each established condition, considering their impact on analytical procedure performance and the overall drug product control strategy. Table 4 also exemplifies other analytical procedure conditions/parameters that were considered in the risk analysis and determined not to be ECs.

Note: This table is for illustrative purposes only and is not an exhaustive list. For all NIR procedures, ECs and their associated reporting categories should be derived and justified according to ICH Q12. Designations (EC or not EC), reporting categories and data requirements may differ by region.

Table 4: Evaluated risk, proposed established conditions and proposed reporting categories

Established Condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comments
Performance Characteristics and Criteria as described in the ATP: Accuracy, Precision, Specificity, Range, Linearity	High	PA	The performance characteristics and criteria ensure the quality of the reportable result and link to the CQA. If widening of the performance criteria is necessary, it will be reported as PA.
Analytical Procedure Principle: <ul style="list-style-type: none"> NIR transmission spectroscopy with PLS (partial 	High	PA	Changing technology <i>e.g.</i> , going from NIR spectroscopy to Raman spectroscopy will be reported as PA.

Established Condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comments
<p>least squares) model.</p> <ul style="list-style-type: none"> • A FT- NIR spectrometer • Minimal Spectral Collection Range: 12500 - 5800 cm⁻¹ • Measuring location: Tablet press outlet 	Medium	NM	<p>Within the same technology principle (NIR) but:</p> <ul style="list-style-type: none"> • Other acquisition modes than transmission (<i>i.e.</i>, reflectance, transreflectance) would probably result in markedly different spectra • Change of key NIR instrument characteristics may impact spectral resolution, acquisition speed, and scale of scrutiny • Changes to spectral range can affect specificity and spectral noise • Change to measurement location (<i>e.g.</i>, upstream) could affect post-analysis segregation. <p>Adherence to ATP ensured by control strategy and defined bridging strategy (see below) to assess impact of changes. Changes to the analytical procedure principle will be reported as NM. There is a deep understanding between product knowledge, intended purpose, and the analytical procedure performance established.</p>
Sampling Frequency (less than 30 tablets/container)	Medium	NM	Must satisfy sampling volume requirement
Software vendor with PLS capability	Low	NL	Able to manage the NIR spectrometer and tablet press interface to perform NIR measurement and trigger diversion in real-time (same functionality as the existing system).

Established Condition	Overall Risk Category	Proposed Reporting Category ¹⁾	Comments
Detailed Model parameters: <ul style="list-style-type: none"> • Preprocessing: Standard Normal Variate (SNV) followed by 17 points Savitzky-Golay 1st derivative • PLS model range: 12500 - 8950 cm⁻¹ • Number of latent variables: 3 • Data quality checks: Mahalanobis distance ≤ 0.74 and Residuals ≤ 0.078 	Low	NL	The detailed model parameters and data quality attributes thresholds are optimised/changed as the consequence of model updates. Validation results reported against the performance criteria should demonstrate that the revised procedure is suitable for the intended purpose. For the data quality check thresholds specifically: dependent on the size of change of these thresholds, documentation in PQS is sufficient on the condition that the calculation principle remains the same.
The following conditions are examples of parameters that are not ECs²⁾:			
NIR instrument Change of instrument (with no change of measurement principle, <i>i.e.</i> , NIR transmission spectroscopy, a FT-NIR spectrometer.	Low	Not reported	All changes to any parameters are re-evaluated, confirmed, and verified to ensure performance (regardless of reporting category) Model Validation will be conducted where appropriate.
Sampling Frequency (more than 30 samples/container)	Low	Not reported	All changes to any parameters are re-evaluated, confirmed, and verified to ensure quality (regardless of reporting category). Model validation will be conducted where appropriate.
Number of scans more than 32	Low	Not reported	These parameters should be defined based on outcomes during robustness studies. Detector integration time multiplied by the number of averaging scans, to achieve approximate unit dose level sampling volume. Instrument operation is described in the established procedure.
Resolution higher than 16 cm ⁻¹	Low	Not reported	
Software version	Low	Not reported	The software version and upgrade are normal operational changes and do not require any submission.

1) PA: Prior Approval, NM: Notification Moderate; NL: Notification Low (as per ICH Q12 definitions)

2) Depending on the region, some of this information is included in an approval letter

Change Assessment and Bridging Strategy

For every change, a structured risk assessment is performed, evaluating the potential impact on the performance characteristics and the link to CQA (as defined the respective ATP). As required by the risk assessment, experimental bridging studies to demonstrate adherence to the performance characteristics and associated criteria may be required. These can include, if necessary, partial or full (re-)validation of the analytical procedure performance characteristics affected by the change and/or comparative analysis of representative samples and standards.

Where adherence to the performance characteristics and associated criteria defined in the ATP cannot be demonstrated during the bridging studies, the changes to the procedure should not be implemented until any impact of reporting category has been confirmed.

Change description and management

The following scenarios are illustrative examples of post-approval changes and their implementation.

Change #1: Introduction of an additional NIR instrument

Background:

An additional NIR instrument is introduced as an alternate instrument for use. The new instrument is identical to that already in use. This instrument will be used for routine product monitoring in case the first instrument goes down and needs to be removed from service.

Risk assessment:

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

The current control strategy of the product is considered sufficient and will not be impacted by the change. As a result, the specifications remain unchanged.

b) Complexity of the technology:

NIR is a complex procedure that has a well-established use in oral solid dosage forms. The process for developing a multivariate procedure for evaluating the assay of tablets is well understood within the company.

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

The extent of the change is low as there is no change to the analytical procedure principle. The new instrument falls within the EC description for the NIR method.

Estimated risk: Low

Re-confirmation Question: Are relevant performance criteria defined as ECs to ensure the post-change quality of the measured result and is sufficient understanding available to design appropriate

future bridging studies?

Answer: Yes

Demonstration of analytical procedure performance after the change

From the risk assessment, it was determined that the PLS model did not require an update with additional data from the new instrument. All performance criteria were met using the new NIR instrument.

Conclusions

Based on the initial risk assessment and analytical procedure control strategy, the risk of introducing a new instrument is low.

Regulatory Reporting

The original agreement with the regulator that inclusion of a new instrument is not an EC was confirmed. Thus, no regulatory reporting is needed. The company will document this change within the PQS.

Change #2: Change in drug substance particle size and excipient leads to model update

Background:

Changes to drug substance particle size distribution and excipient Loss on Drying (LOD) were planned, and were confirmed to have no impact on any CQA of the drug product intermediates or the finished product. No changes to the manufacturing process were necessary. However, these changes resulted in an out-of-model space spectral response detected by a failure of the data quality check. During the subsequent investigation, a model prediction bias was confirmed, with the result that the PLS model required updating to include new sources of variability.

Risk assessment:

This is a change of the multivariate model, and this was agreed as an EC with NL following approval of the product by the regulatory health authority.

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

The current control strategy of the product is considered sufficient and will not be impacted by the change. As a result, the specifications remain unchanged.

b) Complexity of the technology:

NIR is a complex procedure that has a well-established use in oral solid dosage forms. The process for developing a multivariate procedure for evaluating the assay of tablets is well understood within the company.

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

The extent of the change is low as there is no change to the analytical procedure principle, but a model update was needed.

Estimated risk: **Low**

Development Approach and Application of Enhanced Understanding

Elements of the enhanced approach (ATP, prior knowledge, modelling) were used to assess the impact of the drug substance particle size distribution and excipient loss on drying and further described below:

- There is no change to the ATP and analytical procedure principle.
- The enhanced analytical procedure understanding and the results from the parameter assessment during development confirmed that minor changes in raw material attributes are drivers for NIR model updates. This could be resolved by a model update by adding the spectra containing the new sources of variability and removing outdated spectra to ensure the sample library is reflective of the current product.

Re-Confirmation Question: Are relevant performance criteria defined as ECs to ensure the post-change quality of the measured result and is sufficient understanding available to design appropriate future bridging studies?

Answer: **Yes**

Demonstration of analytical procedure performance after the change

From the risk assessment, it was determined that the PLS model should be updated based on the variability of the drug substance particle size distribution and excipient loss on drying to enhance robustness. The number of latent variables for the PLS model changed from 3 to 4, based on the mathematical optimisation. The spectral quality thresholds were re-calculated based on the updated calibration and validation datasets. All performance criteria were met when updating the model and the model is considered fit for use.

Conclusions

The performance criteria were met with the model update. Because the detailed PLS model parameters (change in number of latent variables) and model suitability thresholds were changed to incorporate additional raw material variability the risk remains low. Regulatory Reporting

The original agreement on reporting category with the regulator that a change to the detailed PLS model parameters and model suitability threshold ECs were confirmed as a result of the steps that were performed to implement the actual change. Thus, the change was submitted as notification low.

6. EXAMPLE - PLATFORM ANALYTICAL PROCEDURE

Determination of High Molecular Weight Species (HMWS) in Monoclonal Antibody Products

Note: The following example describes the development and application of a platform analytical procedure for the determination of high molecular weight species in monoclonal antibody products by size exclusion chromatography during release and stability. This approach can also be applied for other technologies and modalities (e.g., residual solvents for synthetic molecules)

Abbreviated development/validation/transfer of a platform analytical procedure for a new analyte needs to be justified based on prior knowledge. The scientific principles in this example are applicable, irrespective of regional legislative frameworks.

Introduction and Background

ICH Q2(R2) defines a platform analytical procedure as an analytical procedure that is suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure. This type of analytical procedure can be used to analyse molecules that are sufficiently alike with respect to the attributes that the platform analytical procedure is intended to measure.

For biological products, HMWS in the form of dimers and/or oligomers constitute product-related substances/impurities that could alter bioactivity and/or safety/immunogenicity profiles. HMWS may form and/or be cleared during production of the biological product and may also be impacted by the formulation, storage and handling of the product. Consequently, HMWS represent a critical quality attribute which is often controlled for batch release and stability. The amount of HMWS is commonly reported as % HMWS, a sum of HMWS (e.g., dimers, oligomers) relative to the sum of all species which can include also higher order aggregates.

Due to the structural characteristics of monoclonal antibodies (mAbs), the size variants of different mAbs products are often sufficiently alike and can be quantitated by the same analytical procedure without significant change to its operational conditions, system suitability and reporting structure.

This example describes the establishment and use of a platform analytical procedure to measure the HMWS in mAb products during release and stability. The example is focused on the technical aspects for the implementation and extension of platform analytical procedures. It is assumed that documentation including change management and risk assessments is enabled by and contained in the applicant's PQS as described in ICH Q14.

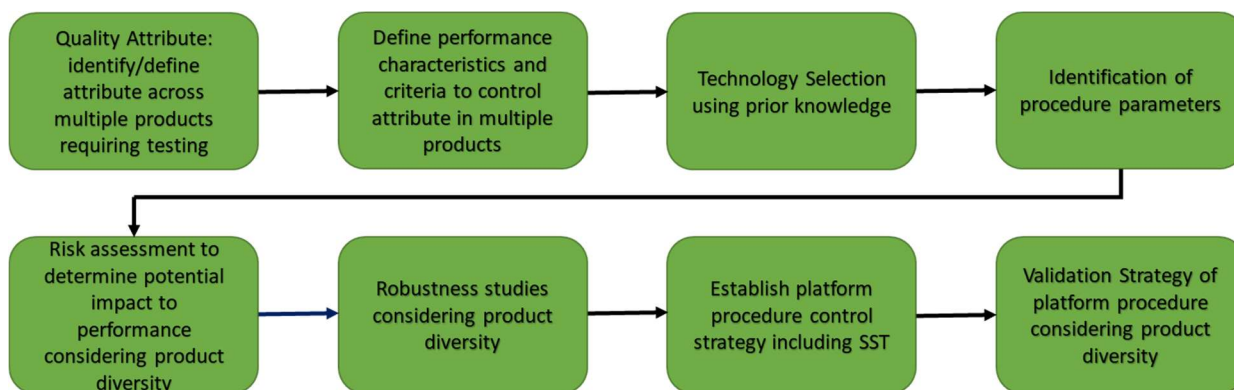
Establishment of a Platform Analytical Procedure

The workflow exemplifying the establishment of a platform analytical procedure is described in Figure 1. Platform analytical procedures can be established using a prospective or a retrospective approach. The prospective approach is the development and validation of a new analytical procedure with the aim to establish a platform analytical procedure. The retrospective approach is the establishment of a platform analytical procedure based on an existing procedure which has been previously validated for one or more products. In that case existing data is used to demonstrate the suitability as a platform analytical procedure. In this example the prospective approach for the establishment of a platform analytical procedure is described. The fundamental principles for the demonstration of the suitability of a platform analytical procedure are the same in the prospective and

the retrospective approaches.

Additional information on the establishment and lifecycle management of platform analytical procedures can be found in Module 3 Part B of the ICH Q2(R2) / Q14 training material.

Figure 1: Example of a workflow for the establishment of a platform analytical procedure



During stability and forced degradation testing, the company has generated data that established upper and lower ranges for the expected changes in proportion of HMWS under long term and accelerated storage conditions. The specification limits for HMWS were defined individually for each product based on acquired knowledge from the clinical history and other sources, such as characterisation data, data from biological assays, literature and experience from similar molecules. The specification criteria consider the criticality of HMWS and their potential impact on safety and efficacy.

The ATP presented below (Table 1) refers to the measurement of the HMWS of different mAbs. The performance characteristics of the reportable result are accuracy, precision, specificity and range of the reportable result for HMWS. The definition of the performance characteristics consider specification limits usually set for HMWS and the expected change at recommended and accelerated storage conditions. The reportable range defined in the ATP should allow the quantitation of HMWS in mAb products with an end of shelf-life specification acceptance criterion for HMWS up to $\leq 5.0\%$.

Table 1: Analytical target profile of the platform analytical procedure (optional element of the enhanced approach):

Intended Purpose		
Measurement of the relative amount of HMWS (dimers and oligomers) of monoclonal antibody products for the release and stability testing of drug substance and drug product.		
Link to CQA		
The analytical procedure should allow the determination of the relative amount of HMWS reported as “Sum of HMWS” relative to the sum of all species (sum of all species can include higher order aggregates potentially formed under stress conditions). The analytical procedure should support the specification limits of the respective products.		
Required characteristics of the reported results		
Performance Characteristic	Acceptance criteria*	Rationale
Accuracy	Individual recoveries of HMWS for samples at each level covering the range of the reportable result, are within 70 and 130%	Selected performance characteristic ensures that the intended analytical procedure delivers the quality of the reportable result.
Precision	Repeatability: RSD for HMWS at the limit of quantitation must be $\leq 20\%$ At HMWS amounts that cover 25% to 120% of the specification acceptance criterion, precision (RSD) of the HMWS measurement is not more than 10% Intermediate Precision: At HMWS amounts that cover 25% to 120% of the specification acceptance criterion, precision (RSD) of the HMWS measurement is not more than 15%	
Specificity	No interference from matrix or other species when quantifying the species of interest. Confirmation that the analytical procedure is stability-indicating.	Confirmation of the capability to quantify HMWS in the presence of the components expected to be present. To ensure that potential changes in the amount of HMWS over shelf life can be detected.
Reportable Range	Quantitation Limit (QL): The analytical procedure is sensitive enough to achieve a target QL of $\leq 0.2\%$ HMWS The quantitation of HMWS vs. expected value is proportional across the range from QL to 7.5% HMWS. The total peak area is proportional to the sample load	Based upon prior knowledge from other mAb products regarding the potential criticality of the HMWS attribute, the ability to quantify HMWS amounts as low as 0.2% is targeted. Based on prior knowledge from other monoclonal antibody products within the applicant’s portfolio an upper range limit of 5.0% HMWS is sufficient to also cover changes occurring during accelerated stability studies (considered as worst case during stability).

* The above ATP targets are illustrative for this example – other targets may be justified

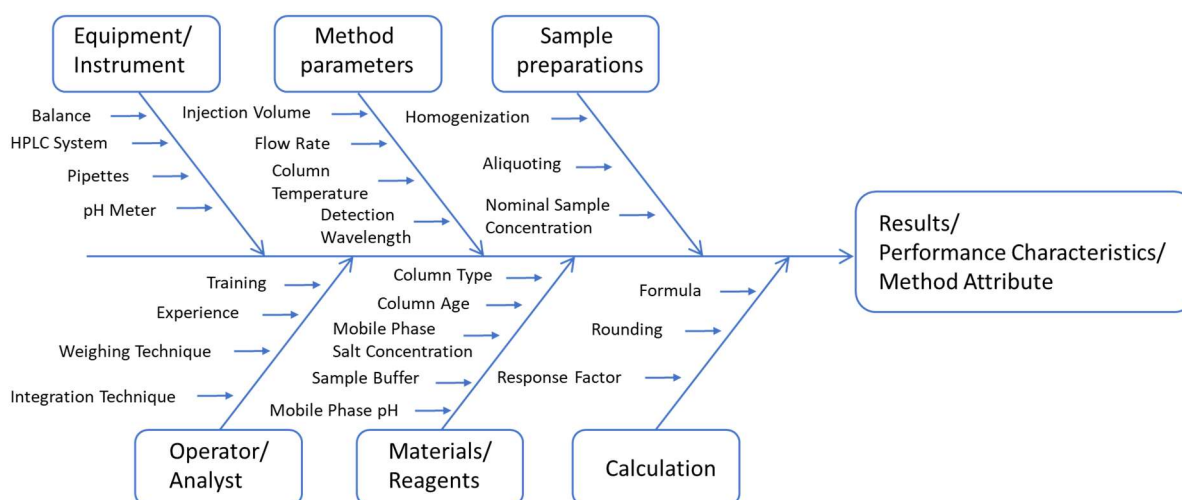
Technology selection

There are numerous technologies suitable for quantifying HMWS in mAb products, such as analytical ultracentrifugation (AUC), size exclusion chromatography (HPLC or UPLC) with UV or multi angle light scattering (MALS) detectors, analytical field flow fractionation (FFF) and electrophoresis (PAGE and capillary electrophoresis) that could potentially meet the performance requirements defined in the ATP in Table 1. For the intended purpose of control system testing under quality control conditions, the company decided to use Size Exclusion Chromatography (SEC) with UV detection, as SEC is well established in the commercial and development laboratories and is routinely used to quantify HMWS in biotechnology products such as mAb products in a QC setting with minimal sample manipulation. Based on prior knowledge, SEC is likely capable of fulfilling the ATP requirements, is generally robust, and is widely available in development and quality control laboratories.

Identification of procedure parameters (Analytical Procedure Development)

The analytical procedure was proactively developed with three mAbs (mAb-A, mAb-B, and mAb-C) using a risk-based approach that leveraged prior knowledge. These mAbs were selected as representative of the type of analytes for which the platform analytical procedure could be applied. Factors potentially affecting the performance of the analytical procedure have been categorised using an Ishikawa diagram (Figure 2) and assessed considering their impact and the likelihood of occurrence.

Figure 2: Ishikawa diagram



To ensure accurate and precise quantitation of HMWS, the critical separation was considered to be between the monomer and dimer species, so the procedure was optimised to maximise monomer-to-dimer resolution across all analytes of interest. Preparations of mAb-A (10 mg/mL), mAb-B (20 mg/mL), and mAb-C (50 mg/mL) drug substance, drug product, and aged material samples (with elevated HMWS amounts) were included in screening experiments and compared to formulation buffer blanks where appropriate.

Mobile phase ionic strength and pH were optimised from targets based on prior knowledge from other mAb products. Additionally, different column loads were evaluated throughout screening; the target column load was determined to be appropriate for mAb-A, mAb-B, and mAb-C and also in consideration of the wide range of analyte concentration across potential future mAb candidates. Column screening was limited to two HPLC columns that have shown reliable performance and minimal lot-to-lot variability based on prior knowledge. Flow rate and column temperature were varied, and sample stability in the autosampler was confirmed for up to 48 hours.

Risk assessment considering multiple products to determine potential impact to procedure parameters

The establishment and utilisation of the HMWS platform analytical procedure was supported by a risk assessment that evaluates the potential impact of several factors on the performance of the procedure from a multi-product perspective. The risk assessment is provided in Table 2. The outcome was used to design the robustness studies and the validation strategy for the platform analytical procedure.

Table 2: Risk assessment to determine analytical parameter impact

(This risk assessment has been provided for the purpose of exemplifying the approach, and is not considered to be a fully comprehensive example)

	Factor	Nominal Value and Variation	Expected Effect on Analytical Procedure Performance/Results/Robustness	Risk	Recommendation
Instrumentation	Instrument Vendor/Model	Vendor 1 and Vendor 2	Dwell volumes can be different from instrument to instrument and can affect peak retention times and resolution.	Medium	At least two different instruments
	Column temperature	Room Temperature	Inaccuracy due to change in peak shape, resolution or retention time.	Low	Keep at nominal. Variance due to column temperature is expected to be low.
	Detection Wavelength	214 nm \pm 3 nm	Inaccuracy due to change in peak response. Sensitivity, baseline noise inaccuracy due to change in peak response. Less than 50% variation is expected in peak responses ranging from 211 nm to 217 nm based on the method qualification. Variance in detection wavelength is expected to have minimal impact on method robustness.	Medium	Vary nominal \pm 3 nm
	Flow rate	1.0 mL/min \pm 5%	Change in flow rate may impact resolution or retention time	Medium	Vary nominal \pm 5%
Column	Column Type	Column Type 1 and Column Type 2	Different SEC column can impact peak shape, resolution, and retention time.	High	Use an alternate column in the study, including all three proposed molecules
	Column Age	New (<0 injections) to aged (>100 injections)	Peak shape and resolution can change as column ages.	Medium	Use a new lot and an aged column in study, including all three proposed molecules
Sample Preparation	Nominal Sample Concentration	8 mg/mL	Changes in column load (mass) may impact analytical procedure performance. Analytical procedure qualification data for all three molecules demonstrated	Low	Keep at nominal

	Factor	Nominal Value and Variation	Expected Effect on Analytical Procedure Performance/Results/Robustness	Risk	Recommendation
			linearity and repeatability around the nominal concentration		
	Sample Buffer	6.5 mM sodium dihydrogen phosphate 13.5 mM disodium hydrogen phosphate 150 mM sodium chloride	pH and ionic strength may affect sample binding on the column. Samples will be diluted in the corresponding mobile phase for each run as per study design.	Medium	Vary pH by ± 0.2 . (will need separate study, including all three proposed molecules)
Mobile Phase	Mobile Phase – Salt Concentration	150 mM	Potential for aggregation at alternate salt concentration. The salt concentration may affect the electrostatic interaction of the protein with the column and flow path.	Medium	Vary nominal $\pm 10\%$ Include all three molecules
	Mobile Phase pH	7.1 \pm 0.1 pH units	Potential for aggregation at alternate pH. pH may affect the electrostatic interaction of the protein with the column and flow path	Medium	Vary pH by ± 0.2 . (will need separate study, including all three proposed molecules)
Analyst	Sample Preparation (weighing)	Not applicable	Minimal - relative area% quantitation based analytical procedure is not expected to be sensitive to the $\pm 10\%$ weighing allowances.	Low	At least two different analysts

Robustness studies using multiple products

Based on the outcome of the risk assessment, robustness of the platform analytical procedure was evaluated using mAb-A, mAb-B, and mAb-C or a subset thereof for those analytical procedure parameters assigned medium and high risk. Because higher order aggregates may be more adhesive, stressed samples should be evaluated as well as unstressed samples. Unstressed samples may not contain higher order aggregates, which would be generated under accelerated stability testing.

Table 3: Robustness Study Design

Analytical Procedure Parameter	Product(s) during study	Rationale
Instrument Vendor/Model (at least two)	mAb-C	mAb-C has shown the lowest resolution between HMWS and monomer and is therefore considered as the worst case
Detection wavelength	mAb-A	Because of the similarity of the framework of the mAbs no impact of detection wavelength expected
Flow rate	mAb-C	mAb-C has shown the lowest resolution between HMWS and monomer and is therefore considered for the parameter that impacts resolution
Column (type and vendor)	mAb-A, mAb-B and mAb-C	Impact of column type as most critical factor evaluated on all mAbs
Column age	mAb-C	Column age could lead to change of resolution between HMWS and other species. Therefore mAb-C was selected
Sample homogeneity	mAb-A and mAb-C	The mAb with the highest and lowest concentration were selected
Sample buffer	mAb-A, mAb-B and mAb-C	All three mAb products selected to evaluate potential molecule specific impact
Mobile phase buffer concentration	mAb-A, mAb-B and mAb-C	
Mobile phase salt concentration	mAb-A, mAb-B and mAb-C	
Mobile phase pH	mAb-A, mAb-B and mAb-C	

The robustness study was used to determine the final analytical procedure parameters which were demonstrated to be suitable across all studied mAb products.

Establishment of platform procedure control strategy including SST

The description of the analytical procedure parameters, the SST and sample suitability assessment are summarised in Table 4. Based on the intended purpose and the technology the SST may consist of platform specific tests and of additional product specific tests as appropriate. The provided content does not reflect the entirety of the analytical procedure description in the dossier.

Table 4: Summary of the Analytical Procedure Description

Technology / Technique:	Size-exclusion chromatography: use relative area quantitation
Column:	Hydrophilic silica gel with a pore size of 25 nm and of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000. 7.8 mm ID x 300 mm, 5 µm
Mobile Phase:	6.5 mM sodium dihydrogen phosphate 13.5 mM disodium hydrogen phosphate 150 mM sodium chloride pH 7.1
Flow Rate:	1.0 mL/min
Column temperature:	Room temperature
Detection:	UV 214 nm
Injection volume:	10 µL
Number of injections:	1 injection per sample
Test solution:	Dilute the preparation to be examined with mobile phase to obtain a concentration of 8 mg/mL.
Reference solution (product specific):	Dissolve the contents of a vial of reference material in mobile phase to obtain a concentration of 8 mg/mL.
Molecular marker solution:	Reconstitute a mixture of thyroglobulin, gamma-globulin, ovalbumin, myoglobin and vitamin B12 in water to obtain an 18 mg/mL solution of molecular mass markers suitable for calibration in the range of 1350-670000 Da. Further dilute 10 µL of the solution with water to obtain a concentration of 0.9 mg/mL.
Relative retention: (with reference to monomer retention time = about 8 min)	HMWS = about 0.88
System suitability tests	The chromatogram obtained with reference solution (product specific) is qualitatively similar to a typical chromatogram of the reference material.
	Resolution: minimum 1.2 between the peaks due to gamma-globulin and

	ovalbumin in the chromatogram obtained with molecular marker solution).
Sample suitability assessment	The retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with reference solution (product specific).
Generation of reportable result	Calculate individual peak areas expressed as a percentage relative to the sum of the areas of all peaks eluting between 5 min and 11 min. <i>NOTE: protein species that elute between 5 min and the monomer peak are classified as high molecular weight species, while those that elute after the monomer peak and before 11 min are classified as low molecular weight species.</i>

Validation of platform analytical procedure using multiple products

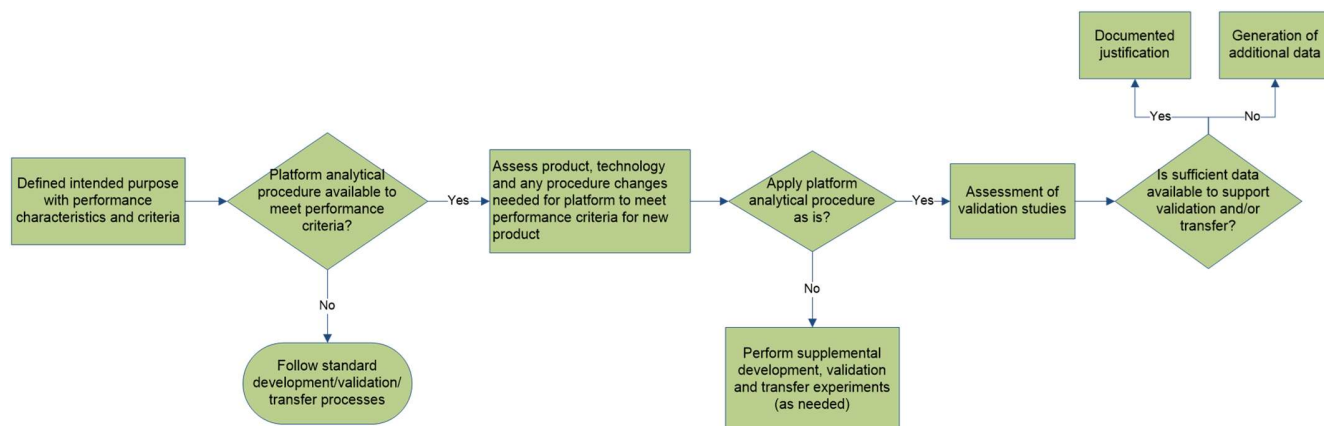
When validating a new platform analytical procedure (prospective approach), risk assessment is used to define the validation strategy. The decision on which samples to use and which performance characteristic(s) to evaluate using which sample is based on the outcome of the risk assessment and also on the need to engage samples that can be reflective of products that are intended to be tested with the platform analytical procedure.

These types of samples can be used to evaluate all, or a subset, of the performance characteristics listed in Table 1 of ICH Q2(R2). For instance, specificity of a chromatographic analytical procedure might require to be evaluated for every mAb product that is intended to be tested with the platform analytical procedure, while its precision and accuracy could be evaluated on a subset of the different products.

Table 5: Validation Summary - Example of application of ICH Q2(R2) Annex 2 Table 3 for SE-HPLC

Performance characteristic	Size-exclusion chromatography using relative area quantitation	
	Validation study methodology	Results
Specificity/ Selectivity	<p><u>Absence of relevant interference:</u> With product, buffer, or appropriate matrix, and between individual peaks of interest</p> <p>Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary</p>	<p>Specificity was demonstrated using unstressed, stressed and mixtures of stressed and unstressed samples from mAb-A, mAb-B, and mAb-C with HMWS amounts in the range of equal or less than 0.2% to up to 7.5%.</p> <p>Sufficient resolution between HMWS and other species was obtained and the HMWS peak was clearly visible and separated in all samples.</p> <p>No interference from buffer or matrix components</p> <p>The analytical procedure is stability indicating as demonstrated with forced degradation samples</p>
Precision	<p><u>Repeatability:</u> Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering peak(s) of interest</p> <p><u>Intermediate precision:</u> <i>e.g.</i>, different days, environmental conditions, analysts, equipment</p> <p><u>Reproducibility:</u> Different laboratories</p>	<p><u>Repeatability:</u> Demonstrated by calculation of the RSD and associated confidence interval for replicate measurements with 3 times 3 levels across the reportable range for mAb-A, mAb-B, and mAb-C</p> <p><u>Intermediate precision:</u> Demonstrated by a repetition of the precision testing (RSD and associated confidence interval) by a second analyst on a second day and on a second equipment with new prepared mobile phase and sample solutions.</p> <p><u>Reproducibility:</u> Demonstrated by a repetition of the precision testing in all laboratories in scope on mAb-A and mAb-C (covering the highest and lowest concentration).</p>
Accuracy	<p>Comparison with an orthogonal procedure and/or suitably characterised material (<i>e.g.</i>, reference material)</p> <p>or</p> <p>Accuracy can be inferred once precision, linearity and specificity have been established</p> <p>or</p> <p>Spiking studies with forced degradation samples and/or suitably characterised material</p>	<p>Demonstrated by calculation of the mean recovery and associated confidence interval of 3 injections of different mixtures of stressed and unstressed samples from mAb-A and mAb-C (covering the highest and lowest concentration)</p>

Figure 3: Example of a workflow for the extension of a platform analytical procedure



Scenario 1 – Documented justification:

A new mAb product with comparable molecular weight to mAb-A, mAb-B, and mAb-C is entering the portfolio and was evaluated to be analysed with the existing platform analytical procedure by assessing the intended purpose, performance characteristics and criteria of the new product against that of the platform analytical procedure. Characterisation studies demonstrate comparable size and comparable chromatographic behaviour of HMWS. The formulation of the new product uses the same formulation components as used for mAb-A, mAb-B, and mAb-C but slightly different concentrations. The protein concentration of the new mAb product is in the concentration range between mAb-A to mAb-C.

The assessment concluded that the product characteristics are comparable to those of mAb-A, mAb-B, and mAb-C based on prior knowledge and characterisation studies, and the platform analytical procedure could be applied as is, without a change in operational conditions or system suitability criteria.

Once the applicability of the platform analytical procedure to the new product was confirmed, an assessment was performed to determine if additional validation experiments would be required. The anticipated specification range for HMWS for the new product does not exceed those for mAb-A, mAb-B and mAb-C and the sample dilution and range have been covered by data from existing validations. The assessment concluded that the performance characteristics demonstrated during the initial validation of the platform analytical procedure were suitable for the measurement of HMWS in the new mAb product and therefore no additional validation experiments were required.

Laboratory 1 performed this assessment that concluded the suitability of the platform analytical procedure and validation. No additional experiments in laboratory 2 and laboratory 3 were required based on the same justification and the fact that these laboratories previously participated in the implementation of the platform analytical procedure and were using it on a routine basis.

Scenario 2 – Supplemental development, validation and transfer experiments

A new product with a molecular weight that is 50 kDa lower compared to mAb-A, mAb-B and mAb-C is entering the portfolio. The intended purpose, performance characteristics and criteria of the new product were assessed against those of the platform analytical procedure. The formulation components for the new product are the same as for the formulations of mAb-A, mAb-B and mAb-C but in slightly different quantities. The protein concentration of the new product is the same as for mAb-B. Characterization studies on the new product revealed that the degradation pathways lead to dimer and oligomer formation. However, the dimers could not be sufficiently resolved from the main species to allow accurate quantitation. The risk assessment conducted in laboratory 1 for the applicability of the platform procedure concluded that additional development studies were required to optimise the parameters of flow rate and mobile phase ionic strength.

The required changes in analytical procedure parameters resulted in an analytical procedure which no longer aligned with the platform analytical procedure. This was therefore considered a product specific analytical procedure which required product specific development, validation, and transfer experiments. As justified, prior knowledge from development and validation of the platform analytical procedure was used as part of the validation data.

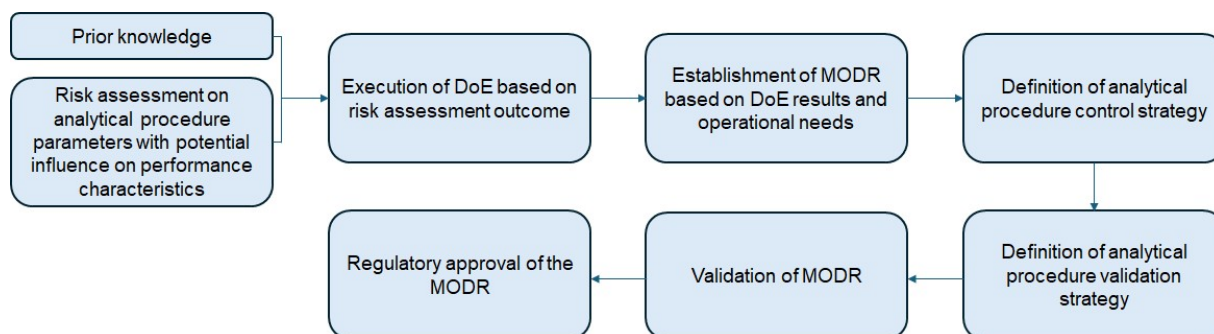
7. EXAMPLE - APPLICATION OF THE ENHANCED APPROACH USING DoE STUDIES - ESTABLISHMENT AND VALIDATION OPTIONS FOR MODRs

Note: This example reflects the content of Chapters 5 and 6 of ICH Q14 guideline – Evaluation of Robustness and Parameter Ranges of Analytical Procedures and Analytical Procedure Control Strategy

Introduction

As described in ICH Q14, robustness evaluation is usually performed as part of analytical procedure development and may be designed according to the minimum approach or the enhanced approach. The outcome of the evaluation of robustness should be documented and reflected in the analytical procedure control strategy. Based on the minimum approach robustness evaluation is usually performed for a relevant analytical procedure parameter by its variation within a certain range around the intended operative value whereas all other analytical procedure parameters are kept constant. Such studies could be used for the establishment of a proven acceptable range (PAR). For the enhanced approach robustness evaluation can be performed by means of a DoE study which includes two or more analytical procedure parameters. Risk assessment and/or prior knowledge may inform on the relevance of analytical procedure parameters to be included in the DoE study. The study results define the ranges within which the analytical procedure performance criteria are fulfilled allowing the establishment of an MODR (Figure 1). A PAR provides data to support the change of one analytical procedure parameter within the approved range, but all other analytical procedure parameters need to be kept constant. In contrast to a PAR, an approved MODR allows changes within the entire multivariate range, *i.e.*, two or more analytical procedure parameters may be changed at the same time. Moving within an approved PAR or MODR does not require regulatory communication. PARs and MODRs are captured in the analytical procedure description. Verification that the intended analytical procedure parameters are covered by the required validation data has to be done before changing analytical procedure parameters within approved ranges (PAR or MODR).

Figure 1: MODR workflow



Illustrative Example

An HPLC procedure intended to determine the assay and an impurity of a drug substance is used to illustrate one possible way for the establishment of an MODR.

Table 1 compares the results of the different robustness evaluations. The first column includes the analytical procedure parameters which have a potential impact on the quality of the test result. These analytical procedure parameters were identified by a risk assessment. The setpoints reflect the intended operational conditions and are identical for both robustness study approaches, but the knowledge gained and the related opportunities for changes are different. The univariate studies (based on experiments when only a single analytical procedure parameter is changed at a time) are leading to five PARs – one for each analytical procedure parameter. In the case of the multivariate study (DoE study with experiments changing two or more analytical procedure parameters at the same time) an MODR with five dimensions is generated – one dimension for each analytical procedure parameter. A PAR allows to change one single analytical procedure parameter while all other analytical procedure parameters remain at the setpoint, *e.g.*, the flow rate is reduced to 0.8 mL/min, but column temperature is fixed at 40°C, injection volume 5 µL, ratio of eluents 90:10 and gradient slope 4.0%/min. In contrast to a PAR, an MODR enables a change to all included analytical procedure parameters at the same time. To set up an MODR, usually appropriate software is applied supporting the corresponding study design definition (multivariate experiments) and the data evaluation based on the study results.

Table 1: HPLC example - impact of different development approaches / robustness studies on the related analytical procedure parameter ranges

Analytical Procedure Parameter	Univariate robustness studies		DoE study	
	Setpoint	PAR	Setpoint	MODR
Flow rate (mL/min)	1.0	0.7 - 1.3	1.0	0.7 - 1.3
Column temperature (°C)	40	28 - 45	40	32 - 45
Injection volume (µL)	5	1 - 20	5	3 - 8
Gradient – starting conditions (ratio eluents A:B)	90:10	75:25 - 100:0	90:10	85:15 - 95:5
Gradient slope (eluent B/min)	4.0%	2.0 - 5.0%	4.0%	2.5 - 5.0%

Table 2 shows how elements of the enhanced approach can be linked and how they interact for the HPLC procedure in this example. The performance criteria for the analytical procedure (column 2) were derived from the ATP. Based on a risk assessment the analytical procedure parameters which could have an influence on the performance (column 3) were derived. In a DoE study these analytical procedure parameters were systematically investigated within a certain range (column 4). The acceptable ranges found for each single ATP performance requirement are shown in column 5. The combined acceptable range considering all analytical procedure parameters in scope is given in column 6 and also represents the MODR. The establishment of the analytical procedure

control strategy (column 7) and the analytical procedure validation strategy (column 8) is shown as an example. The use of analytical procedure development data for validation purposes was justified in the analytical procedure validation strategy.

Table 2: HPLC example - elements of the enhanced development approach and their interaction

1	2	3	4	5	6	7	8
Analytical Procedure Performance Characteristic	Performance criteria based on ATP	AP parameters with potential influence on performance characteristics (based on risk assessment)	Analytical Procedure Parameter Range			Analytical Procedure Control Strategy	Analytical Procedure Validation Strategy
			Range investigated during development (DoE)	Range found suitable in DoE for one performance characteristic	MODR (range found acceptable considering multivariate evaluation) ¹⁾		
Specificity / Selectivity	separation of impurity A and active substance: $R_s \geq 3$	column temperature	20 - 60°C	28 - 45°C	32 - 45°C	- MODR ²⁾ - $R_s \geq 3$ for impurity A and the active substance in SST solution	validation requirements covered by DoE results demonstrated in the MODR and controlled by SST
		gradient slope	1.0 - 10.0% eluent B/min	2.0 - 5.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min	0.7 - 1.3 ml/min		
		injection volume	1 - 20 µl	1 - 20 µl	3 - 8 µl		
Range	sensitivity QL (0.05%)	column temperature	20 - 60°C	20 - 60°C	32 - 45°C	- MODR ²⁾ - S/N ≥ 10 for QL (0.05%, active substance and impurity A) shown in the SST	validation requirements covered by DoE results demonstrated in the MODR and controlled by SST
		gradient slope	1.0 - 10.0% eluent B/min	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
		injection volume	1 - 20 µl	3 - 20 µl	3 - 8 µl		
	linearity assay (80 - 120%)	column temperature	20 - 60°C	20 - 60°C	32 - 45°C	- MODR ²⁾ - analytical procedure validation - instrument qualification - SST: Δ (response) $\leq 1.0\%$ for two independent reference solutions	validation of linearity (15 independent weightings at 5 concentration levels); determination of correlation coefficient (requirement $R \geq 0.999$) and recovery (requirement 99.0 - 101.0% with regard to 100% level); a change within the MODR does not require revalidation
		gradient slope	1.0 - 10.0% eluent B/min	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
	linearity impurity A (0.05 - 0.3%)	column temperature	20 - 60°C	20 - 60°C	32 - 45°C	- MODR ²⁾ - analytical procedure validation - instrument qualification	validation of linearity (3 independent weightings and dilutions at 5 concentration levels); determination of correlation coefficient (requirement $R \geq 0.99$) and recovery (requirement 80 - 120% for levels $\leq 0.10\%$; 90 - 110% for levels $> 0.10\%$); a change within the MODR does not require revalidation
		gradient slope	1.0 - 10.0% eluent B/min	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
Accuracy	bias $\leq 10\%$ for impurity A	column temperature	20 - 60°C	32 - 60°C	32 - 45°C	accuracy ensured by - proven selectivity - proven linearity - proven precision - instrument qualification	validation requirements covered by DoE results demonstrated in the MODR and linearity/precision validation (no matrix effects for drug substances)
		gradient slope	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	85 : 15 - 95 : 5	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
	bias $\leq 3\%$ for active substance	column temperature	20 - 60°C	20 - 60°C	32 - 45°C	accuracy ensured by - proven selectivity - proven linearity - proven precision - instrument qualification	validation requirements covered by DoE results demonstrated in the MODR and linearity/precision validation (no matrix effects for drug substances)
		gradient slope	1.0 - 10.0% eluent B/min	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
Precision	RSD $\leq 10\%$ for impurity A	column temperature	20 - 60°C	32 - 60°C	32 - 45°C	- analytical procedure validation - instrument qualification - SST: RSD of reference solution (impurities) $\leq 2.5\%$	validation of precision: - repeatability (n = 6): RSD $\leq 5\%$ - intermediate precision (n = 6): RSD $\leq 5\%$ - intermediate precision (n = 12): RSD $\leq 7.5\%$ - intermediate precision: Δ vs. repeatability $\leq 5\%$
		gradient slope	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	85 : 15 - 95 : 5	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
		injection volume	1 - 20 µl	3 - 20 µl	3 - 8 µl		
	RSD $\leq 3\%$ for active substance	column temperature	20 - 60°C	20 - 60°C	32 - 45°C	- analytical procedure validation - instrument qualification - SST: RSD of reference solution (assay) $\leq 1.0\%$	validation of precision: - repeatability (n = 6): RSD $\leq 1.0\%$ - intermediate precision (n = 6): RSD $\leq 1.0\%$ - intermediate precision (n = 12): RSD $\leq 1.5\%$ - intermediate precision: Δ vs. repeatability $\leq 1.0\%$
		gradient slope	1.0 - 10.0% eluent B/min	1.0 - 10.0% eluent B/min	2.5 - 5.0% eluent B/min		
		gradient: starting conditions, ratio eluent A : eluent B	75 : 25 - 100 : 0	75 : 25 - 100 : 0	85 : 15 - 95 : 5		
		flow rate	0.5 - 1.5 ml/min	0.5 - 1.5 ml/min	0.7 - 1.3 ml/min		
		injection volume	1 - 20 µl	1 - 12 µl	3 - 8 µl		

¹⁾ The MODR is the intersection of the ranges found in column 5.

²⁾ An MODR is based on DoE studies which allow conclusions regarding the analytical procedure robustness, e.g., selectivity, sensitivity.

Interconnection of ICH Q2(R2) and ICH Q14

Figure 1 of ICH Q2(R2) describes how elements of ICH Q14 can be used to fulfil ICH Q2(R2) requirements, *i.e.*, validation protocol and validation report. Based on this, Table 3 provides examples how the ICH Q14 concepts (ATP, risk assessment, univariate and multivariate development data, analytical procedure control strategy and the analytical procedure validation strategy) can facilitate the establishment of the validation protocol. The ATP and the risk assessment facilitate the derivation of performance characteristics and associated acceptance criteria. The analytical procedure validation strategy is a documented way, *e.g.*, in the analytical procedure validation protocol, to define which performance characteristics and validation elements are already covered by development data and which still need validation tests to fulfil ICH Q2(R2) requirements. The analytical procedure validation strategy could also provide guidance on required validation tests in case of future changes.

Table 4 shows how development data can be used to fulfill ICH Q2(R2) requirements. A justification can be provided in the analytical procedure validation strategy. Specificity / selectivity as well as lower limit ranges and robustness may be covered by DoE study results, *e.g.*, generated for MODR establishment, whereas linearity and precision may need to be proven by validation tests. Accuracy may be concluded from specificity / selectivity, linearity, and precision.

Table 3: Use of ICH Q14 concepts to fulfil ICH Q2(R2) requirements and to facilitate post-approval changes

ICH Q2(R2) Requirements	ICH Q14 Concepts	Illustrative Exemplification
Validation Protocol	ATP	<ul style="list-style-type: none"> Supports the identification of the performance characteristics and allows the justification of the associated acceptance criteria as shown in Table 2, columns 1 and 2 Reference document for post approval changes
	Risk Assessment	<ul style="list-style-type: none"> Guides the design of development studies, supports the establishment of analytical procedure control strategy and validation strategy (columns 3, 4, 7 and 8 of Table 2)
	Development Data (uni-/multivariate)	<ul style="list-style-type: none"> Use of development data as part of validation tests, <i>e.g.</i>, specificity, robustness (columns 7 and 8 of Table 2)
	Analytical Procedure Control Strategy	<ul style="list-style-type: none"> Elements of the control strategy which need to be covered by validation data (columns 7 and 8 of Table 2)
	Analytical Procedure Validation Strategy	<ul style="list-style-type: none"> Allows definition of which development data and prior knowledge can be used instead of validation testing (column 8 of Table 2) Allows definition of validation tests needed to fulfil the ICH Q2(R2) requirements Guides the identification of required validation tests for post-approval changes Allows predefinition of validation activities when moving within an MODR Allows definition of validation tests required for analytical procedure transfers, <i>e.g.</i>, as part of a co-validation
Validation Report	---	<ul style="list-style-type: none"> Describes the part of the MODR which is covered by validation data, <i>e.g.</i>, centre points

Table 4: Example how to use development data to fulfil ICH Q2(R2) validation requirements (related to the example in Table 2)

ICH Q2(R2) Performance Characteristics	Development Data	Explanation
Specificity / Selectivity	---	---
Absence of relevant interference	DoE studies leading to parameter ranges (<i>e.g.</i> , MODR) with acceptable performance (columns 5 and 6 of Table 2)	Results of DoE studies allow identification of parameter ranges which ensure specificity / selectivity
Precision	---	---
Repeatability	not applicable for this example	Needs validation tests
Intermediate precision	not applicable for this example	Needs validation tests
Accuracy	DoE studies leading to parameter ranges (<i>e.g.</i> , MODR) with acceptable specificity / selectivity as integral part (columns 5 and 6 of Table 2)	Results of DoE studies allow identification of parameter ranges which ensure specificity / selectivity In combination with linearity and precision shown in validation tests, accuracy can be concluded
Reportable Range	---	---
Linearity	not applicable for this example	Needs validation tests
Lower range limits	DoE studies leading to parameter ranges (<i>e.g.</i> , MODR) with adequate sensitivity (columns 5 and 6 of Table 2)	Results of DoE studies allow identification of parameter ranges which ensure sensitivity
Robustness	DoE studies leading to parameter ranges (<i>e.g.</i> , MODR) with acceptable overall performance regarding specificity / selectivity and sensitivity (columns 5 and 6 of Table 2)	Results of DoE studies allow to identify parameter ranges which ensure specificity / selectivity and sensitivity

Validation Strategies for MODRs

ICH Q2(R2) provides the concepts for analytical procedure validation. The analytical procedure parameters used need to be covered by validation data. The extent of validation activities and the respective operational flexibility associated have to be assessed and justified on a case-by-case basis. The two examples in Figure 2 represent validation approaches – the minimum variant and the variant based on a full factorial validation design. In-between solutions using a fractional factorial design are also feasible. After regulatory approval of the MODR the analytical procedure can be implemented for operational use.

Minimum validation extent:	For validation, at minimum, a single set of operating parameters of the MODR is selected (the intended operational conditions or the set point). For future changes of the parameters within the MODR an assessment regarding the need for additional validation activities has to be performed. This assessment refers to available knowledge and understanding gained during development and/or operational use. It could be useful to include specific validation requirements for certain future moves within the MODR in the analytical procedure validation strategy. After performing the additional validation tests the analytical procedure control strategy is updated and the modified analytical procedure can be used. This process can be handled within the company's PQS.
Optional validation extent:	
Full factorial extent:	Once the validation of the set point (<i>e.g.</i> , centre point) and additionally the extrema of the MODR is available, full operational flexibility within the MODR is allowed without demand for further validation activities.
Fractional factorial extent:	The freedom to change the analytical procedure parameters within the MODR is linked to the extent of the validation. The section of the MODR being covered by the validation needs to be defined and the validation activities should be justified.

Table 5 illustrates how the analytical procedure validation tests for MODR validation could be configured for this HPLC example. The number of validation experiments/points if all extrema for all dimensions are to be covered can be calculated by 2^n+1 , where n represents the number of dimensions (numbers of analytical procedure parameters included into the MODR) and “1” represents the set-point. For the example with five dimensions this gives $32+1$ validation points. MODRs with fewer dimensions, *e.g.*, three dimensions, have correspondingly fewer $(8+1)$ validation points.

MODR validation should be included in the analytical procedure validation protocol and report.

Figure 2: analytical procedure lifecycle following different validation options (minimum validation extent vs. full factorial validation extent)

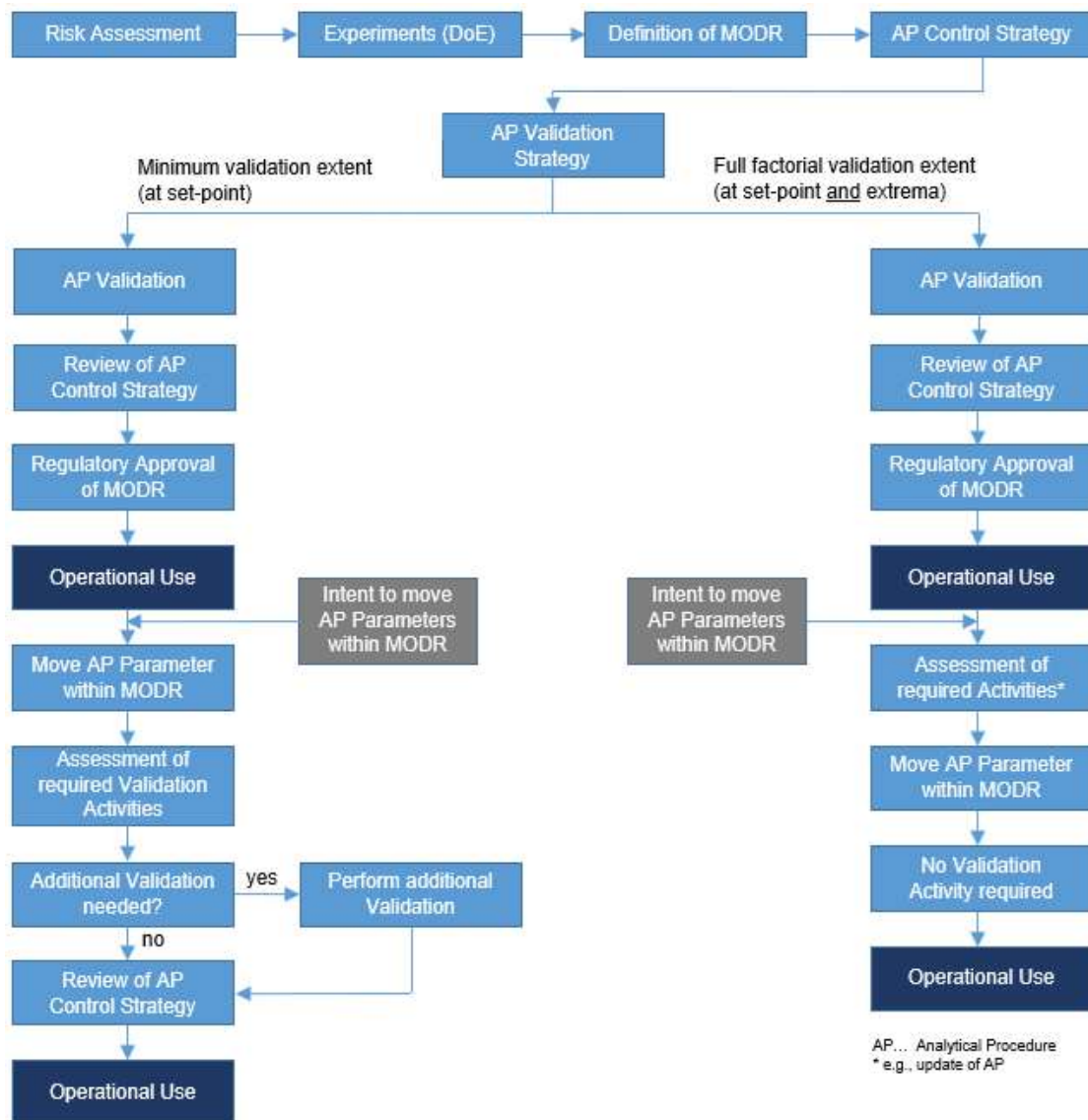


Table 5: HPLC example - MODR validation: Validation experiments for minimum validation (1 validation point) and for full factorial validation (32 + 1 validation points)

Analytical Procedure Parameter	Column temperature [°C]	Gradient slope [%/min]	Gradient - starting conditions (ratio eluents A:B)	Flow rate [mL/min]	Injection volume [µL]
Minimum validation extent (at set-point)	38.5	3.75	90:10	1.0	5.5
Full factorial validation extent, extremum 1	45.0	5.00	95:5	1.3	8.0
Full factorial validation extent, extremum 2	32.0	2.50	85:15	0.7	3.0
Full factorial validation extent, extremum 3	32.0	5.00	95:5	1.3	8.0
Full factorial validation extent, extremum 4	45.0	2.50	95:5	1.3	8.0
Full factorial validation extent, extremum 5	45.0	5.00	85:15	1.3	8.0
Full factorial validation extent, extremum 6	45.0	5.00	95:5	0.7	8.0
Full factorial validation extent, extremum 7	45.0	5.00	95:5	1.3	3.0
Full factorial validation extent, extremum 8	45.0	2.50	85:15	0.7	3.0
Full factorial validation extent, extremum 9	32.0	5.00	85:15	0.7	3.0
Full factorial validation extent, extremum 10	32.0	2.50	95:5	0.7	3.0
Full factorial validation extent, extremum 11	32.0	2.50	85:15	1.3	3.0
Full factorial validation extent, extremum 12	32.0	2.50	85:15	0.7	8.0
Full factorial validation extent, extremum 13	32.0	2.50	95:5	1.3	8.0
Full factorial validation extent, extremum 14	32.0	5.00	85:15	1.3	8.0
Full factorial validation extent, extremum 15	32.0	5.00	95:5	0.7	8.0
Full factorial validation extent, extremum 16	32.0	5.00	95:5	1.3	3.0
Full factorial validation extent, extremum 17	45.0	2.50	85:15	1.3	8.0
Full factorial validation extent, extremum 18	45.0	2.50	95:5	0.7	8.0
Full factorial validation extent, extremum 19	45.0	2.50	95:5	1.3	3.0
Full factorial validation extent, extremum 20	45.0	5.00	85:15	0.7	8.0
Full factorial validation extent, extremum 21	45.0	5.00	85:15	1.3	3.0
Full factorial validation extent, extremum 22	45.0	5.00	95:5	0.7	3.0
Full factorial validation extent, extremum 23	32.0	2.50	85:15	1.3	8.0
Full factorial validation extent, extremum 24	32.0	2.50	95:5	0.7	8.0
Full factorial validation extent, extremum 25	32.0	2.50	95:5	1.3	3.0
Full factorial validation extent, extremum 26	45.0	2.50	85:15	0.7	8.0
Full factorial validation extent, extremum 27	45.0	2.50	85:15	1.3	3.0
Full factorial validation extent, extremum 28	45.0	5.00	85:15	0.7	3.0
Full factorial validation extent, extremum 29	45.0	2.50	95:5	0.7	3.0
Full factorial validation extent, extremum 30	32.0	5.00	85:15	1.3	3.0
Full factorial validation extent, extremum 31	32.0	5.00	85:15	0.7	8.0
Full factorial validation extent, extremum 32	32.0	5.00	95:5	0.7	3.0