



ICH Q2(R2): VALIDATION OF ANALYTICAL PROCEDURES

Training Module 3: Practical Applications of ICH Q2(R2)

Part A – ICH Q2(R2) Annex 1 and 2

Part B – Other Validation Topics

Platform Analytical Procedures

Use of Confidence Intervals

Use of Replicates

Use of Development Data

Single Point Calibration

Extrapolation of Validation Range

Quantitative Test vs Limit Test

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International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

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Disclaimer

The materials presented in this ICH Q2(R2) / Q14 module are example approaches relating to selected aspects of analytical procedure development, validation and lifecycle. The approaches presented have been constructed to illustrate potential applications of the principles contained within the ICH Q2(R2) / Q14 guidelines and are not considered to be exhaustive. The examples are not intended to be mandatory, and alternative approaches (fulfilling the intent of the guidelines) may also be acceptable.

In some cases, additional elucidation of specific approaches is provided to aid in general understanding of a concept. This is not intended to be a promotion of the elucidated approach, nor indicate a preference for a specific approach.

Provision of acceptance criteria has been deliberately limited within this training material.

In practice, scientific rigor must be applied on a case-by-case basis when determining an appropriate approach or criterion.

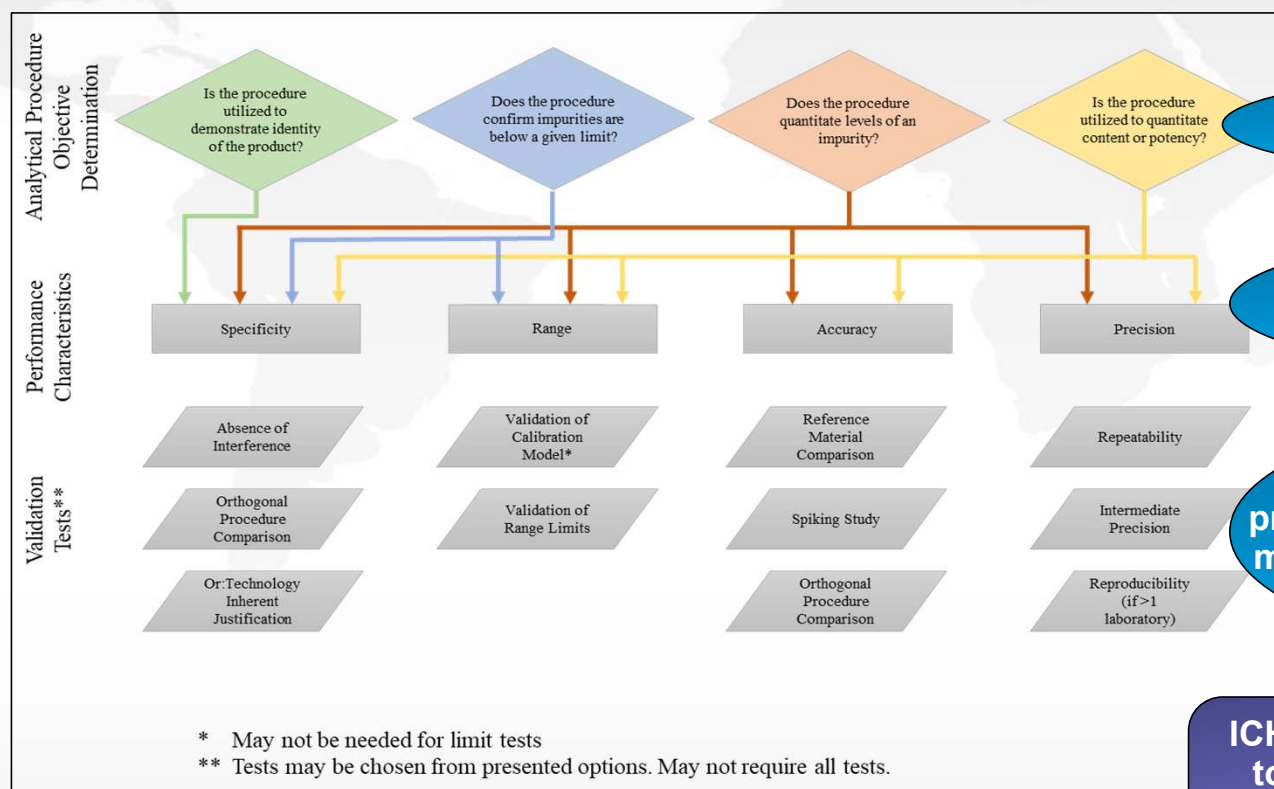
Module 3 – Practical Applications of ICH Q2(R2)

Part A:

ICH Q2(R2) Annex 1 and 2

ICH Q2(R2) Framework

ICH Q2(R2) Annex 1 Selection of Validation Tests



Objectives of the analytical procedure are determined

Relevant performance characteristics are selected based upon the intended use of the analytical procedure

Suitable validation test(s) are chosen based on specific procedure and product considerations, e.g., available reference materials, inherent properties of the technology used, requirements of the product specification.

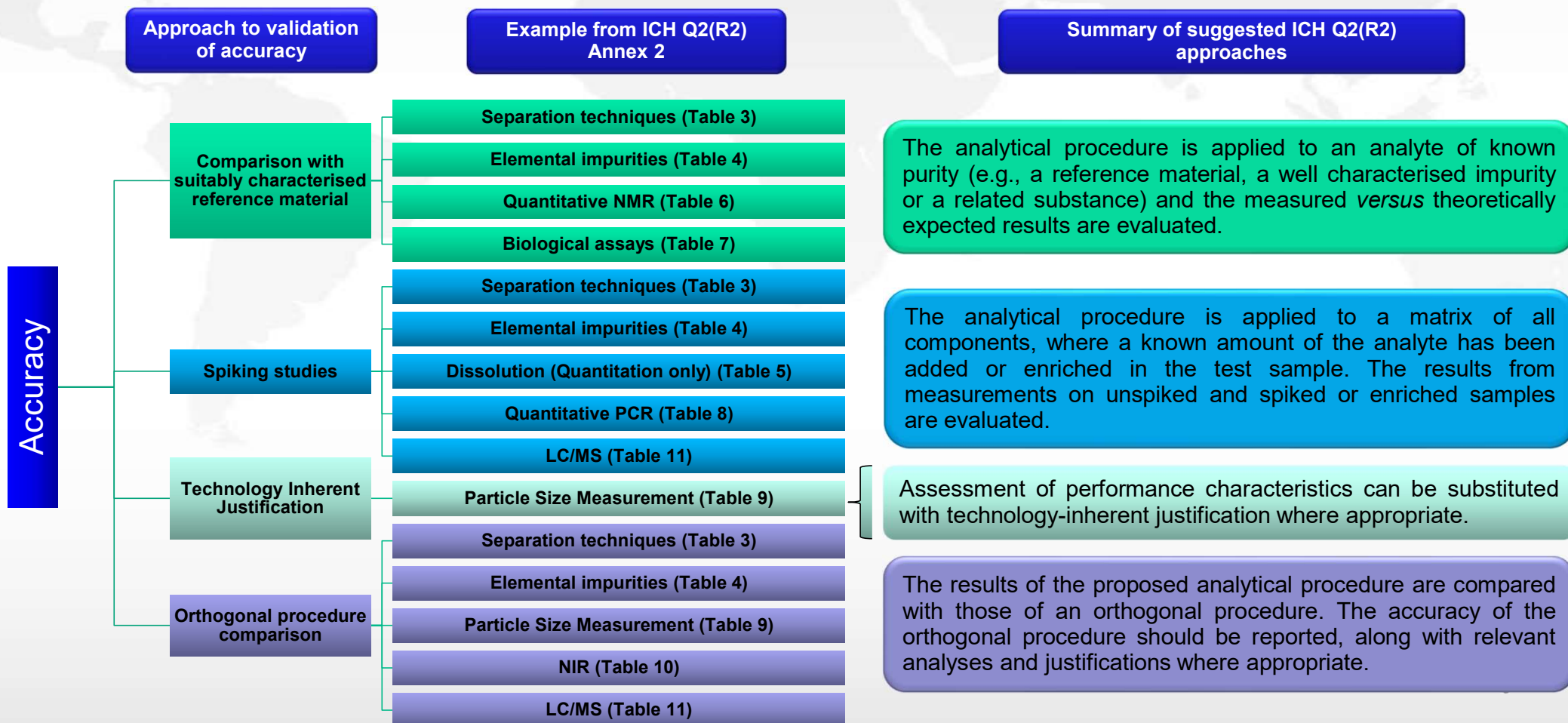
ICH Q2(R2) provides a framework for the approach to analytical procedure validation, which can be applied irrespective of the measured quality attribute or the technology used.

ICH Q2(R2) Figure 2: Examples of relevant validation tests based on the objective of the analytical procedure

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Framework Application

- The framework provided by ICH Q2(R2) can be applied across a wide variety of techniques, as exemplified here for 'Accuracy'. References to the relevant tables in ICH Q2(R2) Annex 2 are provided.



ICH Q2(R2) Annex 2 Examples

- The tables presented in ICH Q2(R2) Annex 2 provide example approaches for analytical procedure validations.
 - The technologies and approaches presented were constructed to illustrate potential applications of the principles contained within the guideline and are not exhaustive.
 - The examples in Annex 2 are not intended to be mandatory, and alternative approaches (fulfilling the intent of the guideline) may also be acceptable.
- Examples have been elucidated for four of the technologies contained in the tables in Annex 2.
 - These examples provide an additional layer of information beyond that in Annex 2, and exemplify the data which may be collected during analytical procedure validation.
 - This additional information is not intended to be mandatory, and alternative approaches (fulfilling the intent of the guideline) may also be acceptable.
- The following slides present example validation data relating to:
 - ICH Q2(R2) Annex 2, Table 3:
 - Separation techniques with relative area quantitation (e.g., product-related substances such as charge variants).
 - ICH Q2(R2) Annex 2, Table 5:
 - Dissolution with high-performance liquid chromatography (HPLC) as product performance test for an immediate release dosage form.
 - ICH Q2(R2) Annex 2, Table 8:
 - Quantitative polymerase chain reaction (qPCR) (quantitative analysis of impurities in drug substances or products).
 - ICH Q2(R2) Annex 2, Table 9:
 - Particle size measurement (dynamic light scattering; laser diffraction measurement) as a property test.

ICH Q2(R2) Annex 2, Table 3

**Separation techniques with relative area quantitation
(e.g., product-related substances such as charge variants)**

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Example Validation Data for Separation Techniques with Relative Area Quantitation (Annex 2, Table 3)

Technique	Separation techniques with relative area quantitation, (e.g., product-related substances such as charge variants)
Performance characteristic	Validation study methodology
Specificity / Selectivity	<p>Absence of relevant interference: 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>
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>
Accuracy	<p>Comparison with an orthogonal procedure and/or suitably characterised material (e.g., 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>
Reportable Range	<p><u>Validation of calibration model across the range:</u></p> <p><u>Linearity:</u> Between measured (observed) relative result <i>versus</i> theoretically expected relative result across specification range(s), e.g., by spiking or degrading material</p> <p><u>Validation of lower range limits:</u> QL (and DL) through a selected methodology (e.g., signal-to-noise determination)</p>
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<p><u>Deliberate variation of relevant parameters, e.g.,</u></p> <p>Sample preparation: extraction volume, extraction time, temperature</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 (e.g., a different specific UV absorbance), relative response factors should be calculated using the appropriate ratio of responses. This evaluation may be performed during validation or development, and should use the finalised analytical procedure conditions and be appropriately documented</p> <p>If the relative response factor is outside the range 0.8-1.2, then a correction factor should be applied. If an impurity/degradation product is overestimated, it may be acceptable not to use a correction factor</p>

Determination of Monoclonal Antibody Charge Variants by Ion Exchange Chromatography

Liquid Chromatography (LC) procedure

- Column: weak cation-exchange resin, 250 mm × 4.0 mm (10 µm)
- Gradient elution: mobile phase A (phosphate buffer),
mobile phase B (phosphate buffer, sodium chloride)
- Sample concentration: 1 mg/mL

Parameter	Set point
Flow rate	1.0 mL/min
Column temperature	40°C
Detection	Ultraviolet (UV) at 280 nm
Run time	80 min
Injection volume	50 µL

Acceptance criteria defined for % acidic peaks, % main peak, % basic peaks.

Specificity

Sample matrix interference

Analyse the separation and matrix component interference of the drug substance (DS).

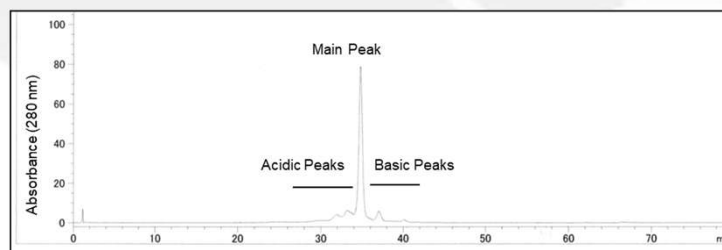
- DS shows clear separation order of acidic peaks, main peak and basic peaks.
- No significant interference from sample matrix components in the chromatographic region of interest.

Stability-indicating properties

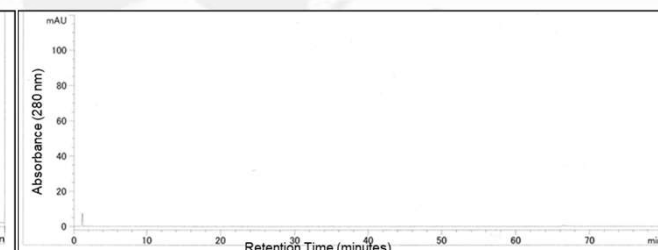
Comparison of chromatograms obtained with reference material and stressed sample.

- The chromatogram of the reference material should be distinguishable from that of stressed sample by visual comparison.

Chromatogram of the DS



Chromatogram for DS formulation buffer



Relative peak area (%)

	Acidic peaks			Main peak	Basic peaks	
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
Reference material	2.23	9.88	12.00	65.17	8.85	1.86
Stressed sample	3.55	11.00	11.66	46.82	11.54	3.35

Additional new peaks*: Relative peak area (%)

	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Sum
Stressed sample	1.78	1.03	3.56	2.63	2.98	12.08

*Table contains extra peaks labelled independently on whether the new peaks are acidic or basic peaks

Precision

Repeatability

- Assessed using 6 separate preparations of DS, 3 injections for each preparation.
- Report standard deviation relative standard deviation and the 95% confidence interval.

Acidic peaks

Repeats	Test Results (Peak Area%)					
	Prep 1	Prep 2	Prep 3	Prep 4	Prep 5	Prep 6
1	24.2	23.7	23.5	23.9	23.7	23.5
2	24.4	23.6	23.5	23.7	23.6	23.6
3	25.1	23.7	23.6	23.8	23.6	23.6
Mean (<i>n</i> = 3) [%]	24.6	23.7	23.5	23.8	23.6	23.6
SD [%]	0.47	0.06	0.06	0.10	0.06	0.06
RSD [%]	1.92	0.24	0.25	0.42	0.24	0.24

Pooled statistics (12 degrees of freedom (DF))

Overall mean [%] **23.8**
 Pooled SD [%] **0.20** (upper 95% CL: 0.31)
 Pooled RSD [%] **0.85** (upper 95% CL: 1.29)

Main peak

Repeats	Test Results (Peak Area%)					
	Prep 1	Prep 2	Prep 3	Prep 4	Prep 5	Prep 6
1	65.2	65.3	63.4	62.7	65.7	63.4
2	65.4	65.4	63.4	62.8	65.5	63.8
3	65.1	65.1	63.3	62.9	65.6	63.6
Mean (<i>n</i> = 3) [%]	65.2	65.3	63.4	62.8	65.6	63.6
SD [%]	0.15	0.15	0.06	0.10	0.10	0.20
RSD [%]	0.23	0.23	0.09	0.16	0.15	0.31

Pooled statistics (12 degrees of freedom (DF))

Overall mean [%] **64.3**
 Pooled SD [%] **0.14** (upper 95% CL: 0.21)
 Pooled RSD [%] **0.21** (upper 95% CL: 0.32)

Basic peaks

Repeats	Test Results (Peak Area%)					
	Prep 1	Prep 2	Prep 3	Prep 4	Prep 5	Prep 6
1	10.1	9.8	10.9	11.8	9.5	11.8
2	9.8	9.8	10.9	11.8	9.3	11.6
3	10.0	9.9	10.8	11.7	9.6	12.1
Mean (<i>n</i> = 3) [%]	10.0	9.8	10.9	11.8	9.5	11.9
SD [%]	0.17	0.02	0.07	0.07	0.13	0.23
RSD [%]	1.73	0.16	0.60	0.59	1.32	1.95

Pooled statistics (12 degrees of freedom (DF))

Overall mean [%] **10.6**
 Pooled SD [%] **0.13** (upper 95% CL: 0.20)
 Pooled RSD [%] **1.26** (upper 95% CL: 1.91)

Precision

Intermediate precision

- Assessed by 6 individual assays, using 3 injections of 1 preparation of DS for each test, with consideration of analyst, instrument and column as variation factors (data collected by varying 4 events over the course of 6 separate tests):

- Two analysts
- Two instruments
- Two columns
- Three days

Acidic peaks

Repeats	Test Results (Peak Area%)					
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
1	23.6	23.7	23.7	24.4	23.6	23.5
2	23.6	23.8	23.6	24.1	23.7	23.5
3	23.7	23.9	23.8	24.3	23.8	23.6

Considering 18 results as one group

Mean [%] 23.8 (95% CI: 23.6, 23.9)
SD [%] 0.26 (upper 95% CL: 0.36)
RSD [%] 1.08 (upper 95% CL: 1.51)

Test 1: Analyst 1, Instrument A, Column X, Day 1
Test 2: Analyst 1, Instrument B, Column Y, Day 2
Test 3: Analyst 1, Instrument A, Column Y, Day 3

Main peak

Repeats	Test Results (Peak Area%)					
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
1	66.2	66.3	64.4	63.7	66.7	64.4
2	66.8	66.4	64.8	63.8	66.9	64.8
3	66.1	66.1	64.3	63.9	66.6	64.6

Considering 18 results as one group

Mean [%] 65.4 (95% CI: 64.8, 66.0)
SD [%] 1.16 (upper 95% CL: 1.63)
RSD [%] 1.78 (upper 95% CL: 2.49)

Basic peaks

Repeats	Test Results (Peak Area%)					
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
1	11.85	11.03	10.68	10.93	10.21	11.93
2	11.62	11.32	10.82	10.54	9.72	11.95
3	11.79	10.80	10.76	10.86	10.06	11.82

Considering 18 results as one group

Mean [%] 11.0 (95% CI: 10.7, 11.4)
SD [%] 0.68 (upper 95% CL: 0.95)
RSD [%] 6.16 (upper 95% CL: 8.63)

Test 4: Analyst 2, Instrument A, Column Y, Day 1
Test 5: Analyst 2, Instrument B, Column X, Day 2
Test 6: Analyst 2, Instrument B, Column Y, Day 3

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Accuracy

- Assessment of reference material, stressed DS sample, and of their 1:1 mixtures (10, 100 and 200% of routine sample concentration of 1 mg/mL).
- Accuracy demonstrated by comparing the observed area of each species in the mixture with the mean area of the respective species in the individual injections of the reference material and stressed sample, and calculating recovery rate.
- Report mean percent recovery together with the 95% confidence interval.

Assay 1 (10%)

Assay 1 (10%)		Peak Area%											
		Acidic peak region						Main peak		Basic peak region			
	Repeats	Peak 1	Mean	Peak 2	Mean	Peak 3	Mean	Peak 4	Mean	Peak 5	Mean	Peak 6	Mean
Reference material	1	2.321	2.286	9.843	9.896	12.123	12.147	65.023	64.978	8.790	8.668	1.900	2.024
	2	2.054		9.878		12.108		64.983		8.534		2.443	
	3	2.484		9.967		12.211		64.928		8.681		1.729	
Stressed sample	1	5.682	5.602	12.453	12.449	15.233	15.276	46.830	46.812	13.215	13.204	6.587	6.657
	2	5.393		12.464		15.316		46.794		13.134		6.899	
	3	5.731		12.431		15.278		46.813		13.262		6.485	
Mixture	1	3.683	3.685	11.385	11.334	13.750	13.787	55.412	55.645	10.869	10.820	4.901	4.729
	2	3.675		11.312		13.864		55.653		10.712		4.784	
	3	3.696		11.306		13.746		55.871		10.878		4.503	
	Expected area		3.944		11.173		13.712		55.895		10.936		4.341
	Recovery [%]		93		101		101		100		99		109

Assay 2 (100%) and Assay 3 (200%)

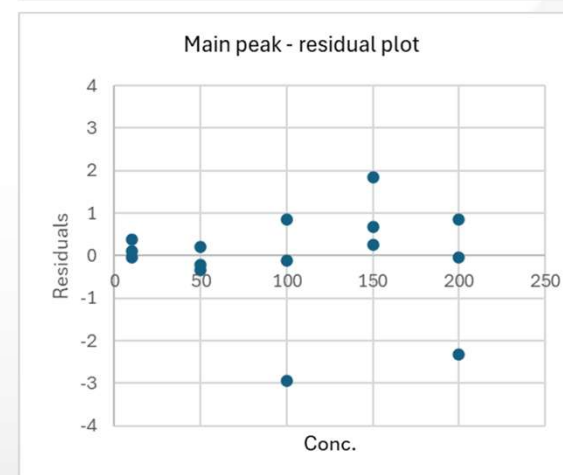
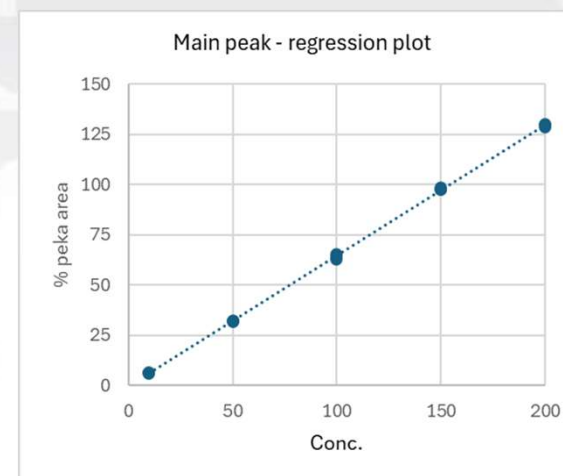
	Recovery [%]							
	Assay 1	Assay 2	Assay 3	Mean	LCL	UCL	SD	RSD [%]
Peak 1	93.42	95.72	88.53	92.56	83.44	101.68	3.7	3.97
Peak 2	101.45	99.84	102.84	101.38	97.65	105.11	1.5	1.48
Peak 3	100.55	101.42	93.52	98.50	87.74	109.26	4.3	4.40
Peak 4	99.55	99.86	98.47	99.29	97.48	101.11	0.7	0.74
Peak 5	98.94	97.25	96.60	97.60	94.60	100.59	1.2	1.24
Peak 6	108.96	107.23	94.42	103.54	83.81	123.26	7.9	7.67

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Reportable Range - Response

- Evaluate a linear relationship between analyte concentration and response across the range, using 5 different sample concentrations (10, 50, 100, 150 and 200% of the routine sample concentration of 1 mg/mL), and triplicate injections for each concentration.
- Report a plot of data, the coefficient of determination, y-intercept and slope of the regression line.

Parameters	Main peak	Acidic peaks	Basic peaks
Coefficient of determination	0.9998	0.9969	0.9925
y-axis intercept	-0.374	-0.428	0.802
95%CI of y-axis intercept	-1.123, 0.375	-1.436, 0.580	0.137, 1.467
Slope	0.651	0.245	0.104
95%CI of slope	0.644, 0.657	0.237, 0.253	0.099, 0.110



For the purpose of this example, only plots corresponding to the main peak are shown.

No particular trend observed in residuals

Lower range

- Estimated by 5 different sample concentrations (1.0, 2.5, 5.0, 7.5, 10% of the routine sample concentration of 1 mg/mL), triplicate injections for each concentration.
- Report a plot of data, the coefficient of determination, y-intercept and slope of the regression line.

Parameters	Main peak
Coefficient of determination	0.9648
y-axis intercept	-0.631
95%CI of y-axis intercept	-0.644, -0.619
Slope	0.692
95%CI of slope	0.684, 0.700

parameters calculated for $\ln(y)$ vs $\ln(\text{conc.})$ linear regressions

Validation of lower range limits based on signal-to-noise:

- Evaluation of signal of basic peak 6:
 - The level showing a peak with a signal-to-noise ratio $\geq 10/1$ and acceptable residuals of $\leq 20\%$:
 - 1.25 μg (2.5% of routine sample concentration).
 - QL confirmed by 6 injections: RSD% of 10%.
- QL (relative area percent): 0.05%, obtained by dividing the mean peak area in the 6 injections by the mean total peak area from the reference material injections.

Robustness

- **Mobile phase pH variation:**

- Evaluate 2 mobile phase buffers, different in pH.
- Report RSD% of sample results.

- **Mobile phase stability:**

- Evaluate for 5 days, using the same sample.
- Report RSD% of sample results.

- **Column lot variation:**

- Evaluate lot-to-lot variation by using 2 columns.
- Report RSD% of sample results.

- **Sample solution stability:**

- Analyse same sample solutions on the day of preparation and 24h later in autosampler set at 10 °C.
- Report RSD% of sample results.

ICH Q2(R2) Annex 2, Table 5

**Dissolution with HPLC as product performance test for
an immediate release dosage form**

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Example Validation Data for Dissolution with HPLC for an Immediate Release Dosage Form (Annex 2, Table 5)

Technique	Dissolution with HPLC as product performance test for an immediate release dosage form	
Performance characteristic	Demonstration of performance of dissolution step <i>Typically demonstrated with development data</i>	Validation testing methodology <i>Typically demonstrated with final procedure</i>
Specificity/ Selectivity	<u>Discriminatory power:</u> Demonstration of the discriminatory power to differentiate between batches manufactured with different critical process parameters and/or critical material attributes which may have an impact on the bioavailability (performed as part of development of dissolution step)	<u>Absence of interference:</u> Demonstration of non-interference with excipients and dissolution media likely to impact the quantitation of the main analyte
Precision	<u>Repeatability and intermediate precision:</u> Understanding of variability by performing, e.g., vessel-to-vessel repeatability studies or intermediate precision studies (operators, equipment) <i>Note: The study provides a combined assessment of variability of product quality and product dissolution performance in addition to the variability of the quantitative procedure</i>	<u>Repeatability and intermediate precision:</u> Demonstration with an homogeneous sample from one dissolved tablet, e.g., several samples drawn from the same vessel, after analyte in sample has been fully dissolved
Accuracy	(Not applicable for dissolution step)	<u>Spiking study:</u> Add known amounts of the reference material to the dissolution vessel containing excipient mixture in dissolution media and calculate recovery within defined working range
Reportable Range	(Not applicable for dissolution step)	Validation of calibration model across the range <u>Linearity:</u> Demonstrate linearity from sample concentrations (as presented to quantitative measurement) in the range of Q – 45% of the lowest strength up to 130% of the highest strength, for one point specification, and in the range of QL up to 130% of the highest strength, for multiple point specification <i>If lower concentration ranges are expected to be close to QL:</i> Validation of lower range limits, see separation techniques
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	Justification of the selection of the dissolution procedure parameters, e.g., medium buffer composition, surfactant concentration, use of sinkers, pH, deaeration, volume, agitation rate, sampling time	<u>Deliberate variation of parameters of the quantitative procedure</u> , see separation technique

Dissolution Test of 2.5 mg Immediate Release Tablets with Analysis by Liquid Chromatography (LC) (UV detection)

Dissolution test

- Apparatus: Paddle apparatus
- Dissolution medium: Acetate buffer pH 4.5
- Medium deaeration: without deaeration
- Volume: 900 mL
- Rotation speed: 75 rpm
- Testing time: 30 min

LC procedure

- Column: RP18, 60 mm × 4.0 mm (3 µm)
- Column temperature: 40°C
- Mobile phase: acetonitrile/water (40/60)
- Flow rate: 1.0 mL/min
- Detection: UV at 250 nm
- Injection volume: 20 µL
- Run time: twice the retention time of the main analyte (RT = about 1 min)

Acceptance criterion: Q = 80% at 30 min

Development: Discriminatory Power of the Dissolution Step

- Demonstrate the ability of the dissolution step to differentiate between batches manufactured with different critical process parameters and/or critical material attributes.
- Variant batches were determined based on risk analysis driven by understanding of drug substance properties, formulation and process understanding, biopharmaceutics, as well as product control strategy.
- Examples of variant batches would consider:
 - the influence of drug substance attributes (e.g., drug substance particle size),
 - the influence of a formulation component (e.g., disintegrant level),
 - the influence of a process parameter (e.g., compression force).

A case study for development of a dissolution procedure is illustrated in Module 5, Part E.

Development: Robustness of the Dissolution Step

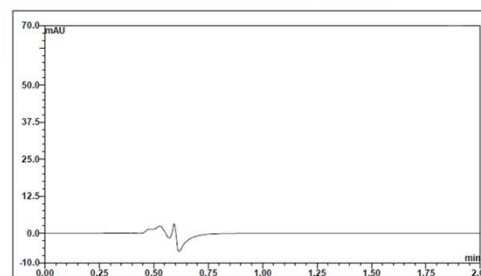
Evaluate effect of small deliberate changes of dissolution parameters on dissolution profiles – example:

- **Effect of temperature:** dissolution testing below and above the target temperature of 37.0 °C (± 0.5 °C).
- **Effect of agitation (or stirring) speed:** dissolution testing using agitation speed in the range of 75 rpm \pm 3 rpm.
- **Effect of pH-changes within a small range:** dissolution testing below and above the target pH of 4.5 of the dissolution medium (± 0.05 pH units).
- **Effect of deaeration:** air bubbles on the surface of the tablets could slow down dissolution; perform comparative study using degassed and non-degassed medium.

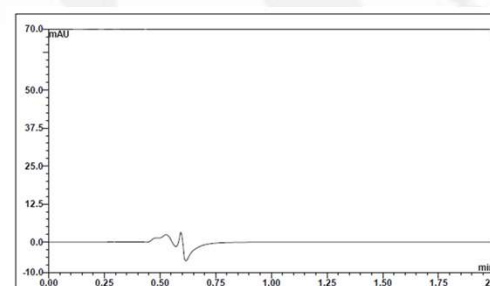
Validation of LC Procedure for Dissolution: Specificity

- Evaluate the interference of the peaks in the dissolution medium, placebo and spiked sample solutions by comparing the peak areas of the main analyte in these solutions with that in a standard solution.
- Show the absence of interference by demonstrating that the quantitation of the main analyte is not impacted.
- Difference in the peak area of the main analyte obtained in the chromatogram of the standard solution and the spiked sample solution is within predefined acceptance criterion.

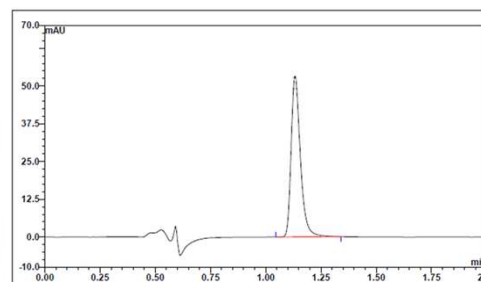
Chromatogram of the dissolution medium



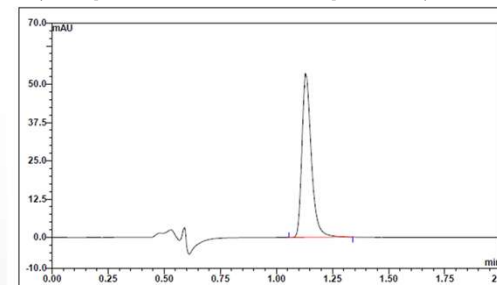
Chromatogram of the placebo



Chromatogram of the standard solution



Chromatogram of the spiked sample solution (sample solution with impurities)



Validation of LC Procedure for Dissolution: Repeatability

Precision

- Six replicate samples withdrawn from a single dissolution vessel.
- Determination at 100% concentration level of DS.
- Evaluation using spiked solutions.
- Report standard deviation, relative standard deviation and the 95% confidence interval.

Sample solution	%DS dissolved at 30 min
1	100.6
2	100.3
3	100.5
4	100.1
5	101.0
6	100.3
Mean (n = 6)	100.5
SD	0.3
UCL	0.7
RSD [%]	0.3

Intermediate precision

- Establish the effects of random events on the precision of the analytical procedure (100% concentration level):
 - **Two analysts**
 - **Two dissolution systems**
 - **Two days**

Analyst 1	% DS dissolved at 30 min		Analyst 2	% DS dissolved at 30 min		Overall	
Sample solution	System 1/ Day 1	System 2/ Day 2	Sample solution	System 1/ Day 1	System 2/ Day 2		
1	100.9	99.9	1	100.9	99.4	Mean (n = 24)	100.7
2	100.6	100.7	2	100.8	101.2		
3	100.4	100.3	3	101.1	100.9		
4	100.8	100.7	4	100.5	101.3		
5	100.7	100.9	5	101.4	100.3		
6	100.5	101	6	101.0	100.7		
Mean (n = 6)	100.7	100.6	Mean (n = 6)	101.0	100.6	SD	0.4
SD	0.2	0.4	SD	0.3	0.7	UCL	0.6
UCL	0.4	0.9	UCL	0.6	1.5	RSD [%]	0.4
RSD [%]	0.2	0.4	RSD [%]	0.3	0.7		

95% CIs are reported

Validation of LC Procedure for Dissolution: Accuracy

- Established across the reportable range of 35% - 130% of label claim.
- Spiking study: drug substance is added to a matrix of all placebo components.
- Assessment at 3 concentration levels and 3 replicates each.
- Report mean percent recovery together with the 95% confidence interval.

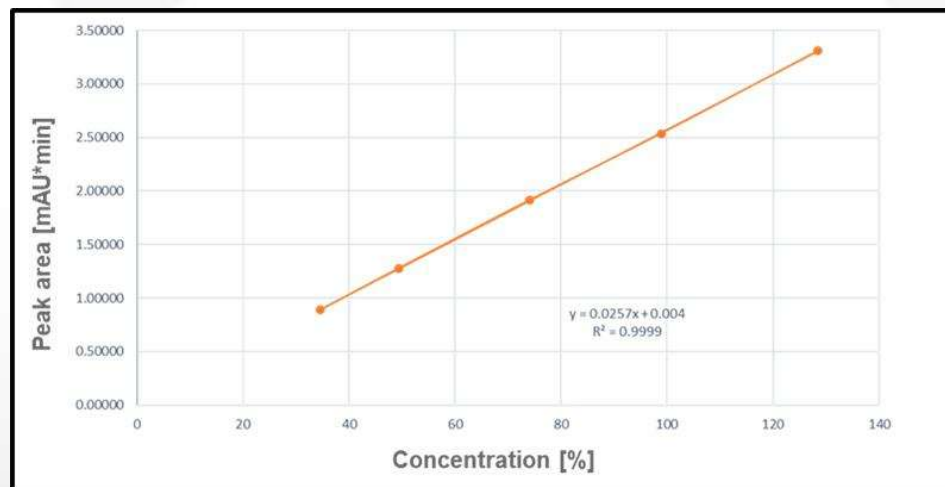
% of strength (Level)	Added amount [mg]			Calculated amount [mg]			Recovery (%)			Recovery [%]				
	replicate			replicate			replicate							
	1	2	3	1	2	3	1	2	3	Mean (<i>n</i> = 3)	LCL	UCL	SD	RSD [%]
A1 (35%)	0.9114	0.8967	0.9111	0.9079	0.8936	0.9198	99.6	99.7	101.0	100.1	98.2	102.0	0.8	0.8
A2 (100%)	2.4711	2.4474	2.4486	2.4691	2.456	2.4773	99.9	100.4	101.2	100.5	98.9	102.1	0.6	0.6
A3 (130%)	3.2371	3.2304	3.2348	3.2511	3.2312	3.2806	100.4	100.0	101.4	100.6	98.8	102.4	0.7	0.7
Overall										Mean (<i>n</i> = 9)	LCL	UCL	SD	RSD [%]
										100.4	99.9	100.9	0.7	0.7

95% CIs are reported

Validation of LC procedure for dissolution: Reportable Range - Response

- Evaluate a linear relationship between analyte concentration and response across the range, using spiked samples.
- Analyse five concentration levels appropriately distributed across the range.
- Report a plot of data, the coefficient of determination, y-intercept and slope of the regression line.

Sample solution	% of strength (Level)	Concentration [%]	Peak area (n =2)
1	35	34.593	0.88816
2	50	49.418	1.27837
3	75	74.127	1.91606
4	100	98.836	2.53332
5	130	128.490	3.30900



LC Procedure for Dissolution: Robustness

• Methodology:

- At the 100 % level based on a tablet containing 2.5 mg of DS for 2 h at 40 °C (sample solution 1) simulating the temperature stress during the dissolution test.
- Additionally sample solution stored for 48 h at RT (sample solution 2) simulating temperature stress during analytical procedure.
- Stability of reference material preparations should be assessed (not shown in this example).

• Evaluation:

- Stability acceptable if the mean recovery of the two sample solutions after storage is within predefined criteria, as compared with the initial analysis (before storage).

Solution stability

Example: 2h @ 40 °C; 48 h @ RT

Sample solution	Injection	Conc. before storage [%]	Conc. after storage [%]	Recovery [%]
1	1	98.15	97.71	99.55
1	2	99.28	97.65	98.36
1	3	97.72	97.79	100.07
2	1	98.51	98.07	99.55
2	2	99.74	97.72	97.97
2	3	98.06	97.38	99.30
Mean				99.13
SD				0.80
RSD [%]				0.81

LC Procedure for Dissolution: Robustness

Variation of chromatographic conditions*

Parameter	Variation	Area mean	Area RSD [%]	Retention time mean	Retention time RSD [%]
Original conditions	N/A	2.49	0.61	1.12	0.04
Detection wavelength	245 nm	2.39	0.69	1.12	0.05
	255 nm	2.33	0.68	1.12	0.05
Flow rate	0.7 mL/min	3.56	0.52	1.59	0.11
	1.3 mL/min	1.95	0.64	0.87	0.10
Column temperature	35 °C	2.55	0.45	1.16	0.04
	45 °C	2.57	0.65	1.11	0.14
Injection volume	10 µL	1.25	0.97	1.10	0.11
	40 µL	5.13	0.12	1.15	0.07
Mobile phase composition (% ACN/water)	35 / 65	2.51	0.72	1.55	0.05
	45 / 55	2.54	0.52	0.92	0.19
Same column type from different vendor(s)	Second vendor	2.59	1.12	1.16	0.10

*selected parameters based on assessed risk to performance of the LC procedure

ICH Q2(R2) Annex 2, Table 8

Quantitative PCR (quantitative analysis of impurities in drug substances or products)

Example Validation Data for PCR (Annex 2, Table 8)

Technique	Quantitative PCR (quantitative analysis of impurities in drug substances or products)
Performance Characteristic	Validation Study Methodology
Specificity/Selectivity	<p><u>Orthogonal Procedure Comparison:</u> Test reaction specificity by gel electrophoresis, melting profile, or DNA sequencing</p> <p><u>Absence of interference:</u> Positive template, no-reverse transcription control for RT-qPCR and no template control. Test primer and probe target specificity against gene bank with sequence similarity search program (e.g., nucleotide BLAST). Evaluate the slope of standard curve for efficiency</p>
Precision	<p><u>Repeatability:</u> Independent preparations of 5 positive control levels evenly distributed along the standard curve and assayed in triplicate within a single assay assessment. The results can be compared using coefficient of variation (CV)</p> <p><u>Intermediate precision:</u> At least 3 replicates per run at each positive control level in at least 6 runs over 2 or more days</p>
Accuracy	<p><u>Spiking Study:</u> Test (e.g., n=6) replicates at 3 to 5 template spike levels from the standard curve concentrations</p> <p>Efficiency/consistency of RNA/DNA extraction method should be accounted for</p>
Reportable range	<p><u>Linearity:</u> Working range should cover at least 5 to 6 log to the base 10 concentration values. Correlation coefficients or standard deviations should be calculated through the entire dynamic range</p> <p><u>Validation of lower working range limits based on the calibration curve:</u> DL defined by template spiking in samples or from standard curves. DL is lowest point meeting the response curve parameters</p> <p>QL demonstrated through showing sufficient recovery and acceptable CVs from the accuracy experiment</p>
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<p><u>Deliberate variation of parameters</u>, e.g., Equipment, master mix composition (concentrations of salts, dNTPs, adjuvants), master mix lots, reaction volume, probe and primer concentrations, thermal cycling parameters</p>

Table 8: Example for Quantitative PCR

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

Residual Host Cell DNA Quantification in Protamine Sulfate Drug Product using qPCR

- **Protamine Sulfate (ProS)**

- ProS drug product is biologically derived from chum salmon sperm and therefore may be contaminated with residual salmon sperm DNA.
- ProS is an arginine-rich, highly positively charged polypeptide, and may strongly interfere with the DNA assay by binding to anionic DNA.

- **Quantitative PCR (qPCR)**

- **DNA extraction:**

- To release DNA from binding with ProS, ProS samples are digested and residual host cell DNA extracted per the commercial kit instructions.

- **qPCR method:**

- Salmon sperm DNA was protease-digested. The digested DNA stock solution was diluted to a serial of concentrations for standard curve generation.
- A commercial kit was prepared according to the supplier instructions.
- Twelve μL of a master mix was added to each well, followed by 10 μL of DNA standard, digested ProS sample (with or without spiked digested DNA) or TE buffer (no template control).
- Twenty μL was transferred for qPCR analysis.
- Thermal cycling conditions: Step 1: Polymerase activation for 10 min at 95 °C, Step 2 DNA denaturation at 95 °C for 15 s, and Step 3 Annealing and extension at 60 °C for 60 s for 40 cycles.

Specificity/Selectivity

Absence of Interference

- To maximise the specificity for salmon DNA quantification, a conserved region of the multicopy gene for 5S ribosomal RNA (rRNA) from salmon DNA sequences was chosen and a nucleotide blast of the target demonstrated broad species specificity.
- Both TE buffer (no template control) and ProS negative controls (no spike-in DNA) showed signal below that from the DNA spike-in control at the lowest concentration limit based on calibration curve, indicating the target specificity of the selected primers and probe and the lack of interference from matrix or the presence of drug product.
- DNA spike-in positive controls subjected to DNA extraction process showed comparable signals with DNA spike-in controls without extraction, indicating that quantitation is not impacted by extraction procedure.

Precision

Repeatability

- **Within a single assay: at least 5 DNA concentration levels covering the reportable range, at least 3 replicates each level.**

DNA Level in Spike-in Samples (pg/μL)	qPCR assay result (pg/μL)									
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean	% CV	SD	95% CI of SD (upper limit)
0.01	0.009	0.011	0.010	0.008	0.006	0.006	0.008	24.8	0.002	0.005
0.02	0.016	0.019	0.016	0.017	0.014	0.015	0.016	10.3	0.002	0.004
0.05	0.036	0.049	0.040	0.046	0.041	0.038	0.042	11.8	0.005	0.012
0.25	0.199	0.232	0.173	0.207	0.195	0.207	0.202	9.5	0.019	0.047
1.25	0.880	1.045	0.955	0.964	0.831	1.087	0.960	10.0	0.096	0.236

Precision

Intermediate Precision

- Establish the effects of random events on the precision.
- Within 2 or more days: at least 6 runs, at least 3 replicates each run.

DNA Level in Spike-in Samples (pg/μL)	qPCR assay result (pg/μL)																			
	Day 1								Day 2								Inter-Day			
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean	% CV	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean	% CV	Mean	% CV	SD	95% CI of SD (upper limit)
0.01	0.009	0.011	0.010	0.008	0.006	0.006	0.008	24.8	0.007	0.009	0.008	0.008	0.007	0.008	0.008	8.9	0.008	18.1	0.001	0.003
0.05	0.036	0.049	0.040	0.046	0.041	0.038	0.042	11.8	0.040	0.048	0.044	0.039	0.032	0.037	0.040	14.5	0.041	12.7	0.005	0.009
1.25	0.880	1.045	0.955	0.964	0.831	1.087	0.960	10.0	1.013	1.300	1.125	1.075	0.888	0.850	1.042	15.8	1.001	13.6	0.136	0.230

Spiking Study

- Demonstrate through comparison of the measured results with expected values (% Recovery) across the reportable range.
- At least 3 spike levels covering the reportable range, 6 replicates each level.

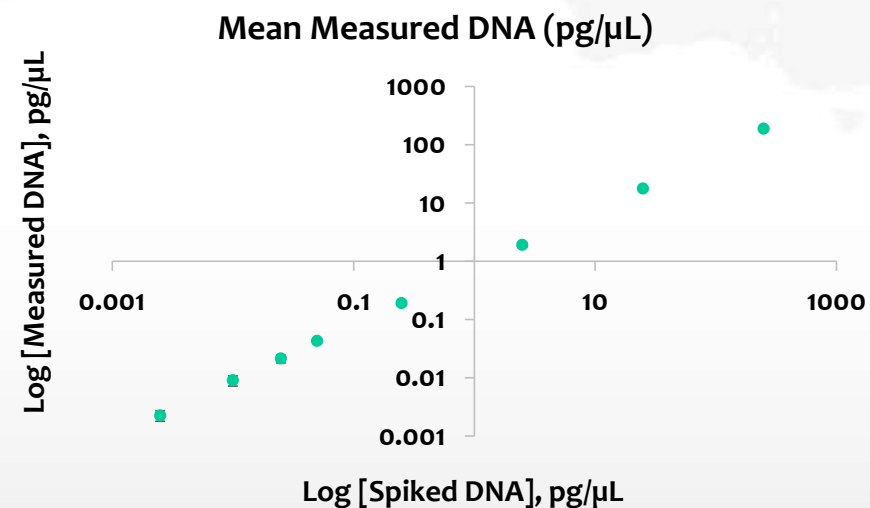
DNA Level in Spike-in Samples (pg/μL)	% Recovery									
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean	SD	% CV	95% CI of Mean Recovery
0.01	90.00	60.00	70.00	80.00	70.00	100.00	78.33	14.72	18.8	62.89-93.78
0.05	72.00	98.00	82.00	92.00	82.00	74.00	83.33	10.09	12.1	72.74-93.93
1.25	70.40	83.60	77.12	79.60	85.20	68.40	77.39	6.84	8.8	70.20-84.57

Reportable Range

Linearity

- At least 5 log to the base 10 concentration range.
- A minimal of 5 concentration levels across the entire range.
- Provide a plot of the data, the coefficient of determination, y-intercept, and slope of the regression line.

DNA Level in Spike-in Controls (pg/μL)	Mean Measured DNA (pg/μL)	SD	% CV	Regression	
0.0025	0.0020	0.0005	20.8	Coefficient of Determination (R^2)	0.9993
0.01	0.009	0.002	20.1		
0.025	0.021	0.003	15.6		
0.05	0.043	0.003	8.1	y-Intercept	0.0007
0.25	0.19	0.016	8.6		
2.5	1.90	0.15	7.8		
25	17.6	1.16	6.6	Slope	0.7537
250	187.5	10.5	5.6		



Validation of Lower Range Limits Based on the Calibration Curve

- Detection Limit (DL) is the lowest concentration meeting the response curve parameters.
 - DL = 0.0025 pg/μL, based on the calibration curve established for the concentration range of 0.0025 - 250 pg/μL.
- Quantitation Limit (QL) can be directly validated by accuracy and precision measurement at the lower range limit meeting acceptance criteria (% CV ≤ 25%, % Recovery within 70 – 130%).
 - QL = 0.01 pg/μL, based on the precision and accuracy measured for 0.01 pg/μL DNA spike-in samples :
 - Precision measured from 3 replicates at % CV of 20.8%;
 - Accuracy measured from 6 replicates at % Recovery of 78.33% with 95% CI at 84.55% - 115.45%.

Deliberate variation of parameters

- Robustness testing shows the reliability of the procedure in response to deliberate variations in procedure parameters and the stability of the samples and reagents for the duration of the procedure.
- Parameters to consider in qPCR residual DNA analysis in ProS samples:
 - DNA extraction parameters – e.g., protease digestion time and temperature, volume of TE buffer for elution of digested samples, storage period of digested samples at 4°C.
 - qPCR method parameters – e.g., master mix composition, master mix lots, probe and primer concentrations, reaction volumes, thermal cycling parameters.

DNA Level in Spike-in Samples (pg/μL)	Thermal cycling annealing temperature																
	60°C					59.5°C (60°C - 0.5°C)					60.5°C (60°C + 0.5°C)					Across all runs	
	Rep 1	Rep 2	Rep 3	Mean	% CV	Rep 1	Rep 2	Rep 3	Mean	% CV	Rep 1	Rep 2	Rep 3	Mean	% CV	Mean	% CV
0.01	0.009	0.006	0.007	0.007	20.8	0.008	0.007	0.010	0.008	18.3	0.008	0.011	0.010	0.008	18.3	0.008	19.7
0.05	0.036	0.049	0.041	0.042	12.7	0.046	0.041	0.051	0.046	8.9	0.037	0.038	0.042	0.039	5.5	0.042	12.5
1.25	0.880	1.045	0.964	0.963	8.6	0.995	1.065	0.855	0.972	11.0	1.065	1.087	0.960	0.972	11.0	0.991	8.4

ICH Q2(R2) Annex 2, Table 9

Particle size measurement

**(dynamic light scattering; laser diffraction
measurement) as a property test**

ICH Q2(R2) / Q14 Training Module 3

Example Validation Data for Particle Size Measurement

1. Dynamic Light Scattering (DLS)

- Intended purpose: measuring the particle size of liposomes in drug product for release test.
- Reportable value: mean particle diameter, Polydispersity Index (PDI).

2. Laser Diffraction (LD)

- Intended purpose: measuring the particle distribution of drug substance powders by dry dispersion.
- Reportable value: D10, D50, D90.

ICH Q2(R2) / Q14 Training Module 3

Example Validation Data for Particle Size Measurement: DLS

Technique	Particle size measurement	Example 1: Particle size measurement of liposomal products by using dynamic light scattering
Performance Characteristic	Validation Study Methodology*	Results
Specificity/Selectivity	<u>Absence of interference:</u> <ul style="list-style-type: none"> Evaluate blank and sample to determine the appropriateness of the equipment settings and sample preparation. 	<ul style="list-style-type: none"> No interference from matrix component for measurements of one homogenised sample .
Precision	<u>Repeatability:</u> <ul style="list-style-type: none"> Test at least 6 replicates using established analytical procedure parameters at target range. <u>Intermediate precision:</u> <ul style="list-style-type: none"> Analysis performed on different days, environmental conditions, analysts, equipment setup. 	<u>Repeatability:</u> <ul style="list-style-type: none"> Demonstrated by 6 replicate measurements of one homogenised sample RSD of mean diameter: $\leq 5\%$ <u>Intermediate precision:</u> <ul style="list-style-type: none"> Demonstrated by 6 replicate measurements of one homogenised with 2 analysts at 3 days using different instruments. RSD of mean diameter : $\leq 5\%$
Accuracy	<u>Technology inherent justification:</u> <ul style="list-style-type: none"> Confirmed by an appropriate instrument qualification. or <u>Orthogonal procedure comparison:</u> <ul style="list-style-type: none"> Qualitative comparison using a different technique, like optical microscopy, to confirm results. 	<u>Technology inherent justification:</u> <ul style="list-style-type: none"> Confirmed by instrument qualification with the measurements of three different sizes of particle standard 50 nm, 100 nm and 150 nm.

*Copied from Table 9 in ICH Q2(R2) Annex 2

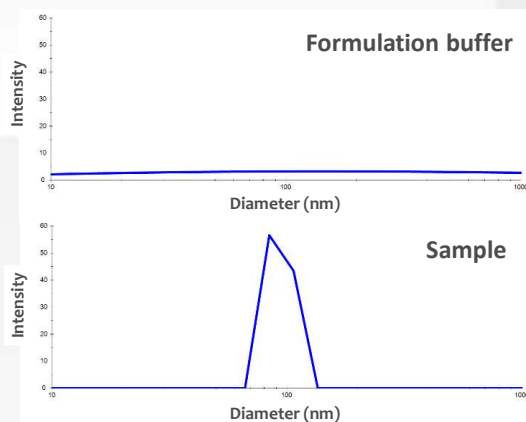
Example Validation Data for Particle Size Measurement: DLS (Cont.)

Technique	Particle size measurement	Example1: Particle size measurement of liposomal products by using dynamic light scattering
Performance Characteristic	Validation Study Methodology*	Results
Reportable range	<u>Technology specific justification</u> , e.g., particle size range covered	<u>Technology specific justification</u> , <ul style="list-style-type: none"> The dynamic light scattering instrument covers a few nm to about 1 µm per manufacturer stated range.
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<u>Deliberate variation of parameters, e.g.,</u> <ul style="list-style-type: none"> Evaluation of expected size ranges for the intended use of the analytical procedure. Dispersion stability for liquid dispersions (stability over potential analysis time, stir rate, dispersion energy equilibration or stir time before measurement). Dispersion stability for dry dispersions (sample amount, measurement time, air pressure and feed rate). Obscuration range (establish optimum percentage of laser obscuration) Ultrasound time/percentage for sample, if applicable. 	Dispersion stability for liquid dispersions <ul style="list-style-type: none"> Confirmed data quality of sample solution 4, 24, and 48 h after preparation.

*Copied from Table 9 in ICH Q2(R2) Annex 2

Example Validation Data for Particle Size Measurement: DLS

Absence of interference



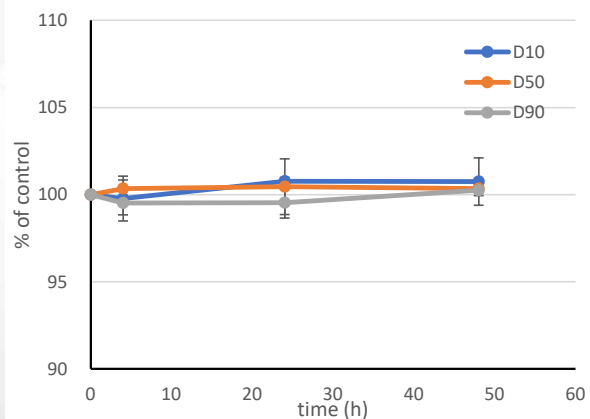
Intermediate precision

Mean diameter (nm)	Day	1	2	3	4	5	6
	Analyst	1	2	2	1	1	2
	Instrument	1	2	1	2	1	2
Replicates	1	84.37	88.34	85.11	86.36	84.77	85.69
	2	83.88	89.52	83.82	87.53	83.91	89.83
	3	85.37	86.39	85.47	88.71	85.48	87.21

Analysis of variance

	Sum of squares	Degree of freedom	Variance
Within Run	42.38	5	8.48
Between Run	20.43	12	1.70
Total	62.80	17	3.69

Dispersion stability for liquid dispersions



	SD	RSD(%)	upper CI (%)
Intermediate precision	1.9899	0.0231	0.0202
Repeatability	1.3046	0.0151	0.0086

ICH Q2(R2) / Q14 Training Module 3

Example Validation Data for Particle Size Measurement: LD

Technique	Particle size measurement	Example 2: Particle size measurement of drug substance powders by dry dispersion using laser diffraction
Performance Characteristic	Validation Study Methodology*	Results
Specificity/Selectivity	<u>Absence of interference:</u> <ul style="list-style-type: none"> Evaluate blank and sample to determine the appropriateness of the equipment settings and sample preparation. 	<u>Absence of interference:</u> <ul style="list-style-type: none"> Pass with background from signal channels was less than 5% using air as blank.
Precision	<u>Repeatability:</u> <ul style="list-style-type: none"> Test at least 6 replicates using established analytical procedure parameters at target range. <u>Intermediate precision:</u> <ul style="list-style-type: none"> Analysis performed on different days, environmental conditions, analysts, equipment setup. 	<u>Repeatability:</u> <ul style="list-style-type: none"> Demonstrated by 6 replicate measurements of one homogenised sample at the undersize values of 10%, 50%, and 90% (D10, D50, and D90, respectively) RSD % of D10, D50 and D90: 2.3%, 4.8%, 4.9% 95% upper one sided confidence interval: 4.9%, 10.1%, 10.1% for D10, D50 and D90, respectively <u>Intermediate precision:</u> <ul style="list-style-type: none"> Demonstrated by 6 replicate measurements of the particle sizes with 2nd analyst at 2nd day using the same instrument RSD% of D10, D50 and D90: 2.0%, 4.3%, 4.7% 95% upper one sided confidence interval: 4.2%, 9.0%, 9.9% for D10, D50 and D90, respectively Diff% of mean at 2nd day to that of 1st day: 2.6%, 6.7%, 7.7% for D10, D50 and D90, respectively RSD% of D10, D50 and D90 of the total 12 replicate measurements: 2.5%, 5.7%, 5.9% 95% upper one sided confidence interval: 3.9%, 8.8%, 9.2% for D10, D50 and D90, respectively

*Copied from Table 9 in ICH Q2(R2) Annex 2

ICH Q2(R2) / Q14 Training Module 3

Example Validation Data for Particle Size Measurement: LD (cont.)

Technique	Particle size measurement	Example 2: Particle size measurement of drug substance powders by dry dispersion using laser diffraction
Performance Characteristic	Validation Study Methodology*	Results
Accuracy	<u>Technology inherent justification:</u> <ul style="list-style-type: none"> Confirmed by an appropriate instrument qualification. or <u>Orthogonal procedure comparison:</u> <ul style="list-style-type: none"> Qualitative comparison using a different technique, like optical microscopy, to confirm results. 	<u>Technology inherent justification:</u> <ul style="list-style-type: none"> Confirmed by an appropriate instrument qualification <p>Note: according to the intended purpose, the analytical procedure of PSD in this example is for release testing of the product (drug substance powder). The product specification was established based on batches of PSD results using this procedure, therefore orthogonal procedure comparison with optical microscopy to verify the method accuracy was not needed.</p>
Reportable range	<u>Technology specific justification</u> , e.g., particle size range covered	<u>Technology specific justification,</u> <ul style="list-style-type: none"> According to the instrument qualification, it covers the range needed for the PSD testing.
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	<u>Deliberate variation of parameters, e.g.,</u> <ul style="list-style-type: none"> Evaluation of expected size ranges for the intended use of the analytical procedure. Dispersion stability for liquid dispersions (stability over potential analysis time, stir rate, dispersion energy equilibration or stir time before measurement). Dispersion stability for dry dispersions (sample amount, measurement time, air pressure and feed rate). Obscuration range (establish optimum percentage of laser obscuration) Ultrasound time/percentage for sample, if applicable. 	<ul style="list-style-type: none"> Max Diff% of D10, D50 and D90 at each varied condition to that at target operation condition is 4.4%, 7.1%, 7.7%.

*Copied from Table 9 in ICH Q2(R2) Annex 2

Example Validation Data for Particle Size Measurement: LD

Validation Results

Repeatability & Intermediate Precision

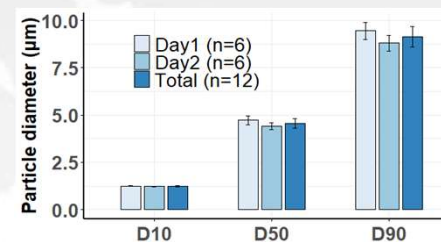
Performances (µm)		Replicates	D10	D50	D90
Intermediate Precision	Repeatability Day 1 Analyst 1	1	1.23	4.57	9.13
		2	1.21	4.41	8.86
		3	1.30	5.09	10.2
		4	1.26	4.77	9.52
		5	1.26	4.74	9.50
		6	1.25	4.74	9.49
	Repeatability Day 2 Analyst 2	1	1.24	4.58	9.18
		2	1.21	4.33	8.67
		3	1.21	4.32	8.63
		4	1.25	4.67	9.39
		5	1.19	4.15	8.25
		6	1.21	4.37	8.67

Robustness

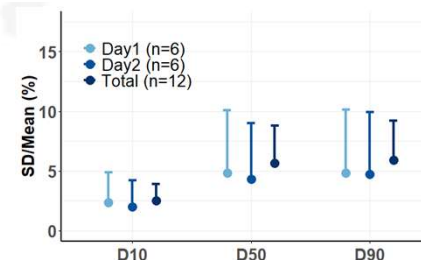
Robustness (µm)		D10	D50	D90
Dispersion Pressure	Nominal+0.5bar	1.19	4.14	8.21
	Nominal-0.5bar	1.22	4.41	8.81
Sample Amount	Nominal+20%	1.18	4.29	8.61
	Nominal-20%	1.34	5.06	9.82
Sample Feeding Rate	Nominal+5%	1.22	4.46	9.00
	Nominal-5%	1.27	4.82	9.72

Repeatability & Intermediate Precision

Data presentation of mean and SD of D10, D50 and D90

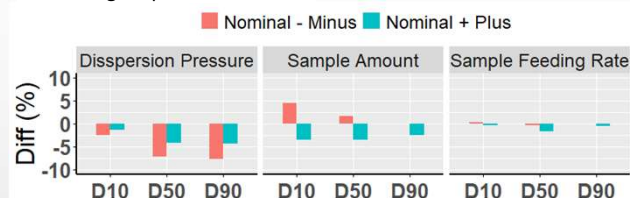


Data presentation of SD and 95% upper one sided confidence interval



Robustness

Diff% of D10, D50 and D90 at each varied condition to that at target operation condition



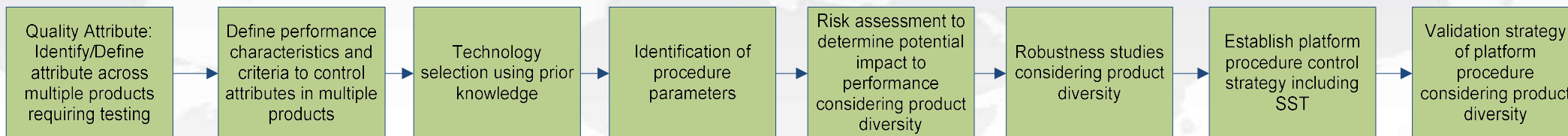
Module 3 – Practical Applications of ICH Q2(R2)

Part B: Other Validation Topics

Platform Analytical Procedures

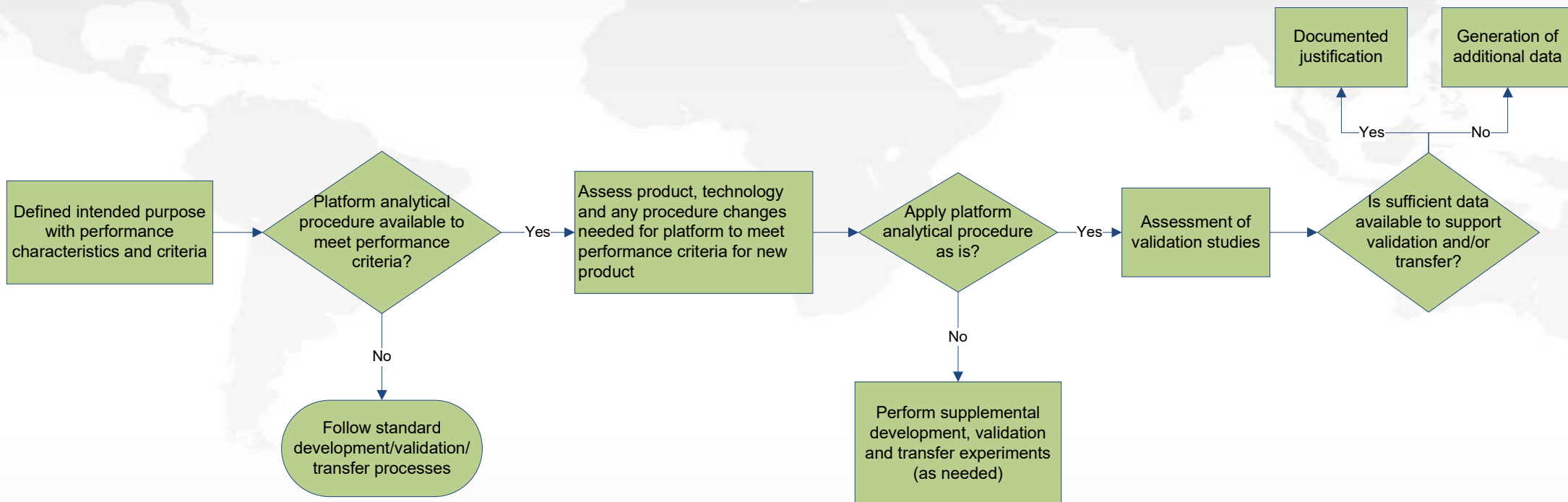
ICH Q2(R2) / Q14 Training Module 3

Development of a Platform Analytical Procedure



- Development of a platform analytical procedure follows a similar process to that of a product-specific analytical procedure, in that the product control strategy will define the specific quality attribute that needs to be measured.
- While not mandatory, an associated analytical target profile (ATP) can be defined that is product-agnostic, and suitable technology is selected, along with identification of the analytical procedure parameters.
- The establishment and utilisation of a platform analytical procedure is supported by a risk assessment that evaluates the potential impact of factors on the performance of the procedure from a multi-product perspective, e.g., 3 products in which the same quality attribute will be measured.
- Based on the risk assessment, robustness studies are designed and performed considering the product diversity.
- The platform analytical procedure control strategy, including system suitability test(s) (SST), is established. Based on the technology and the intended purpose, the SST may consist of platform specific tests and of additional product specific tests as appropriate.
- The validation strategy is developed based on an understanding of risk from the product diversity and executed.
- A suitable platform analytical procedure may also be established retrospectively based on data used for previously validated procedures from multiple products.

Platform Analytical Procedure Applied to a New Product



Platform Analytical Procedure Applied to a New Product

- The intended purpose of the analytical procedure (i.e. the attributes it will measure), as well as the prospective performance characteristics and associated criteria are defined (e.g. in an ATP or other documentation).
- If a platform analytical procedure is available that meets the performance criteria, it is assessed against the new product to see if any modifications are needed to the procedure to meet the performance criteria. If the platform analytical procedure does not meet the performance criteria, standard procedure development/validation/transfer processes are followed.
- If the platform analytical procedure requires modification, supplemental development, validation, and/or transfer experiments are performed as part of the validation strategy and documented accordingly.
- If the platform analytical procedure can be applied without modifications, an assessment of the validation studies is performed to determine if sufficient data is available to support the validation and/or transfer of the procedure for the new product:
 - If sufficient data is available to support the validation and/or transfer of the platform analytical procedure for the new product, documented justification should be provided.
 - If additional experiments are required to support validation and/or transfer, they are performed and documented appropriately as part of the validation strategy.

Use of Confidence Intervals

Use of Confidence Intervals in ICH Q2(R2)

- ICH Q2(R2) text regarding recommended data for accuracy (Section 3.3.1.4) and precision (Section 3.3.2.4) has been clarified in relation to the use of confidence intervals:

Accuracy:

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

Precision:

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

The following slides provide additional information on use of confidence intervals and potential approaches for consideration.

Note: $(1-\alpha)$ is the confidence coefficient. E.g., where $\alpha = 0.05$, $100(1-\alpha) = 95\%$ confidence interval; where $\alpha = 0.1$, $100(1-\alpha) = 90\%$ confidence interval.

- It is usually easiest, if possible, to work with normally distributed data.
- Non-normal data can often be transformed (e.g., log transformation for bioassay) to obtain approximate normality.
 - This may result in the need for alternative statistical approaches, e.g. use of geometric mean and geometric CV.
- In the following slides, we assume that the data being analysed is normally distributed.
- Suppose we have n observations: x_1, \dots, x_n
- The data are assumed to be sampled from a normal population with mean μ and standard deviation σ .

Precision: Confidence Interval for a Standard Deviation

- For a single standard deviation from independent observations of a normal distribution with unknown mean, a one- sided 100 (1- α)% upper confidence limit is calculated by:

$$\text{Upper Confidence limit} = s \sqrt{\frac{n-1}{\chi_{\alpha, n-1}^2}},$$

where $\chi_{\alpha, n-1}^2$ is the critical value of the chi-square distribution with $n - 1$ degrees of freedom, s is the sample standard deviation of the measurements x_1, \dots, x_n and \bar{x} is the sample mean of these measurements:

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \text{ and } \bar{x} = \frac{\sum_{i=1}^n x_i}{n}.$$

- Variance Component Analysis and according confidence intervals can be used for more complex designs.

Precision: Confidence Interval for a Relative Standard Deviation

- For normal distributions, an approximation of the upper limit of the confidence interval for the relative standard deviation (coefficient of variation) involves taking the upper limit of the confidence interval of the SD and simply dividing by the mean. Exact confidence bounds for the relative standard deviation are obtained using a non-central Student's t-distribution (1).
- If the target concentration is known, the upper limit of the confidence interval of the SD can simply be divided by the target concentration.

Accuracy: Confidence Interval for Mean

- For the mean of independent observations from a normal distribution with unknown standard deviation, two one-sided 100 (1- α)% confidence limits are calculated. Mathematically, the limits of the two one-sided confidence intervals are equivalent to the limits of the two-sided 100 (1-2 α)% confidence interval.

$$\left[\bar{x} - t_{1-\alpha, n-1} \frac{s}{\sqrt{n}}, \bar{x} + t_{1-\alpha, n-1} \frac{s}{\sqrt{n}} \right]$$

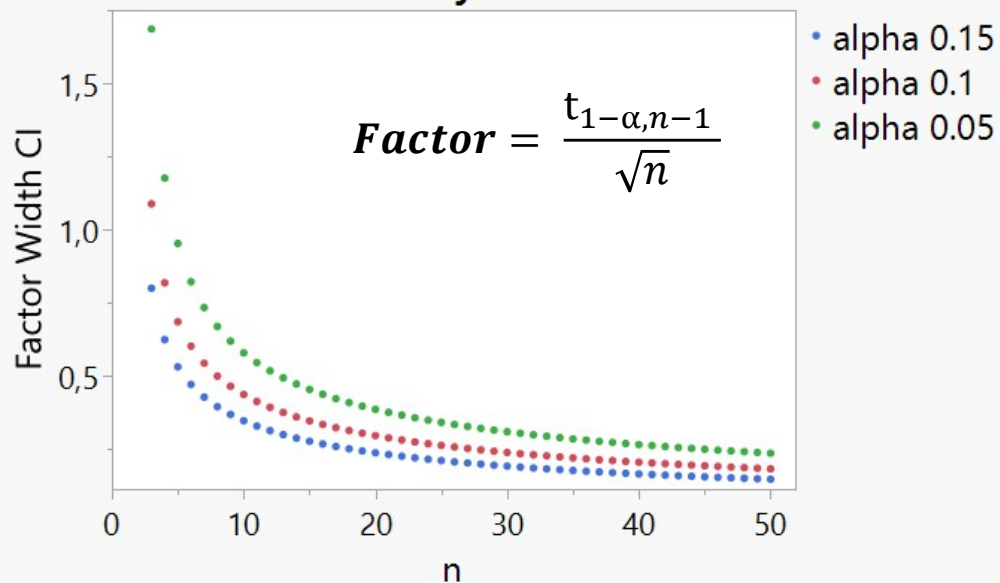
- Confidence interval for the mean can also be computed from more complex models, such as mixed effect models.

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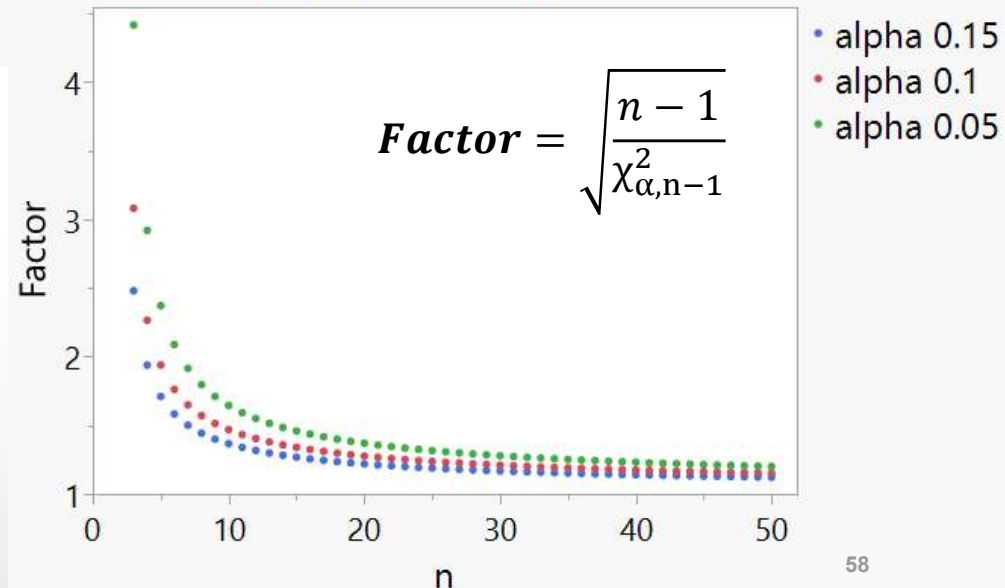
Confidence Interval Width

- Confidence interval width is determined by n and alpha (α).
- Larger n typically leads to smaller width.
- Larger alpha (α) leads to smaller width.

Factor for Width of two sided (1-2alpha) CI for Accuracy



Factor one sided (1-alpha) upper CL for standard deviation

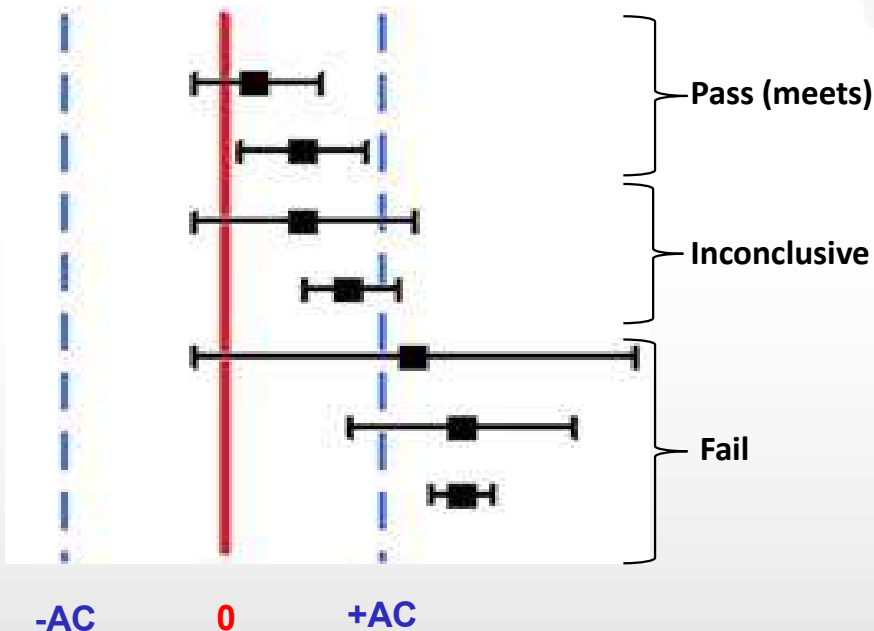


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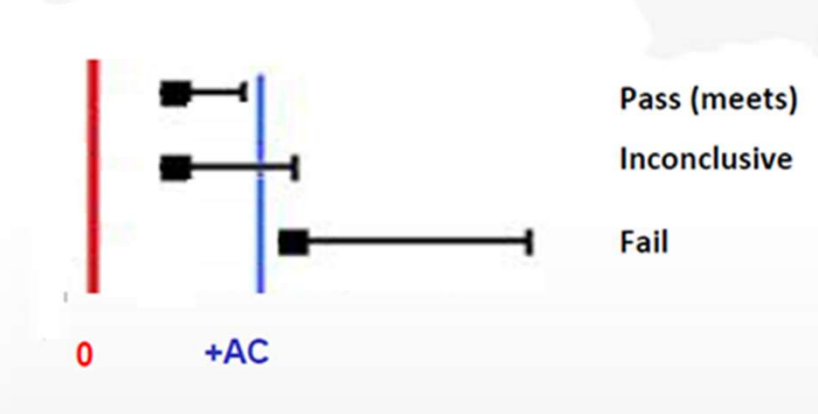
Interval Compatibility with Acceptance Criteria

- If the point estimate and the confidence interval are within the Acceptance Criteria (AC), validation of the performance characteristic is successful.
- If the point estimate is within the AC and the confidence interval exceeds the AC, the validation of the performance characteristic is inconclusive. The result may be considered compatible with the AC based upon additional assessments and / or actions (see slide 64).
- If the point estimate is outside of the AC, validation of the performance characteristic is unsuccessful.

Accuracy



Precision



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Bayesian Credible Interval

- A Bayesian credible interval may be used as an alternative to a frequentist confidence interval.
- In the Bayesian setting, the parameter of interest, θ , is considered to be a random variable. θ could be the mean (μ) or relative bias for accuracy, the standard deviation (σ) or RSD for precision, or a joint distribution of multiple parameters.
- A prior distribution for the parameter of interest must be chosen and justified (no matter the level of informativeness). It reflects the level of knowledge about the parameter of interest prior to the validation study.
- The prior distribution is combined with the validation data using Bayes' theorem to form the posterior distribution from which the credible interval is calculated.

$$\text{posterior distribution} \propto \text{validation data} \times \text{prior distribution}$$

- A $100(1 - \alpha)\%$ credible interval for θ is an interval, such that the probability that θ is contained in the interval is $1 - \alpha$.

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Bayesian Credible Intervals

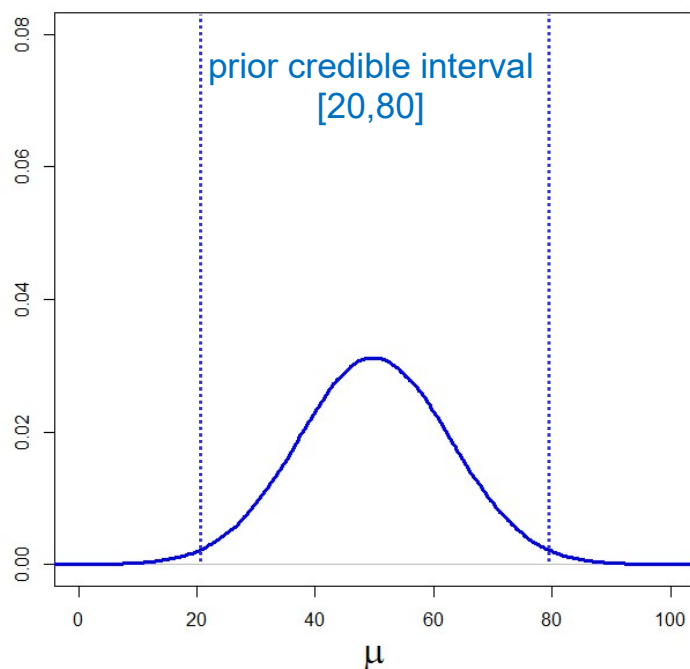
Prior distribution
(using prior knowledge)

+

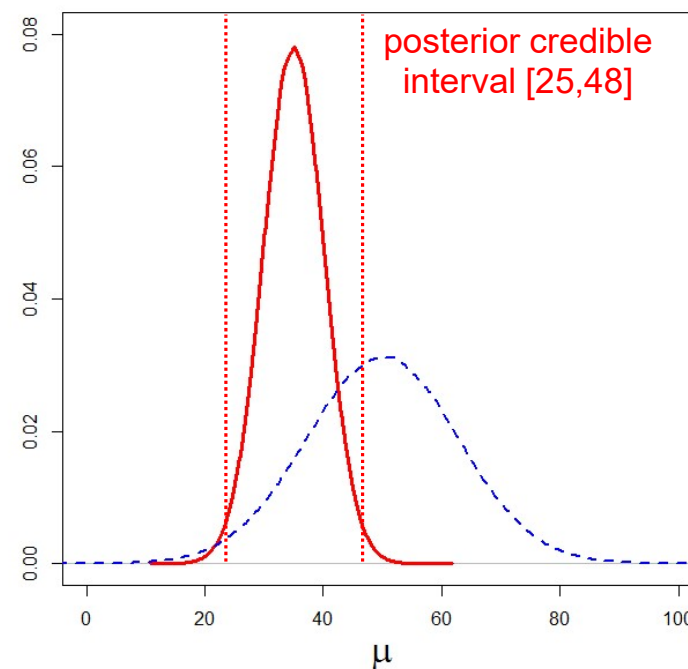
**validation
data**

⇒

Posterior distribution
(prior knowledge + validation data)



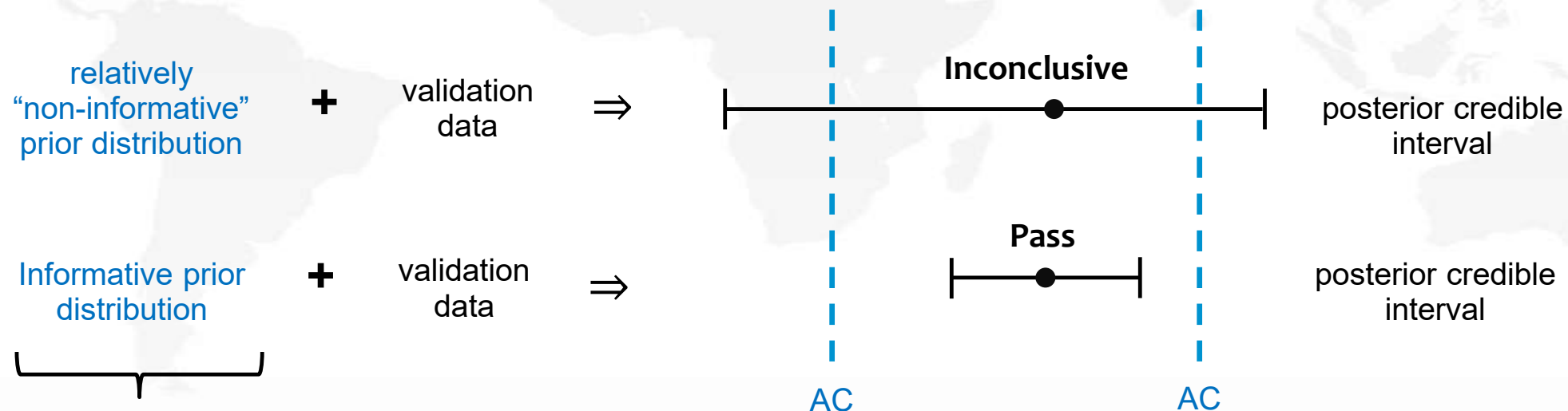
Prior is updated
with validation
data to form
posterior



The combination of prior distribution data and validation data can result in a reduction of uncertainties around the parameter of interest.

Interval Compatibility with Acceptance Criteria

- A prospective use of the Bayesian approach using informative prior distributions may improve the precision around the point estimate.



Qualification study

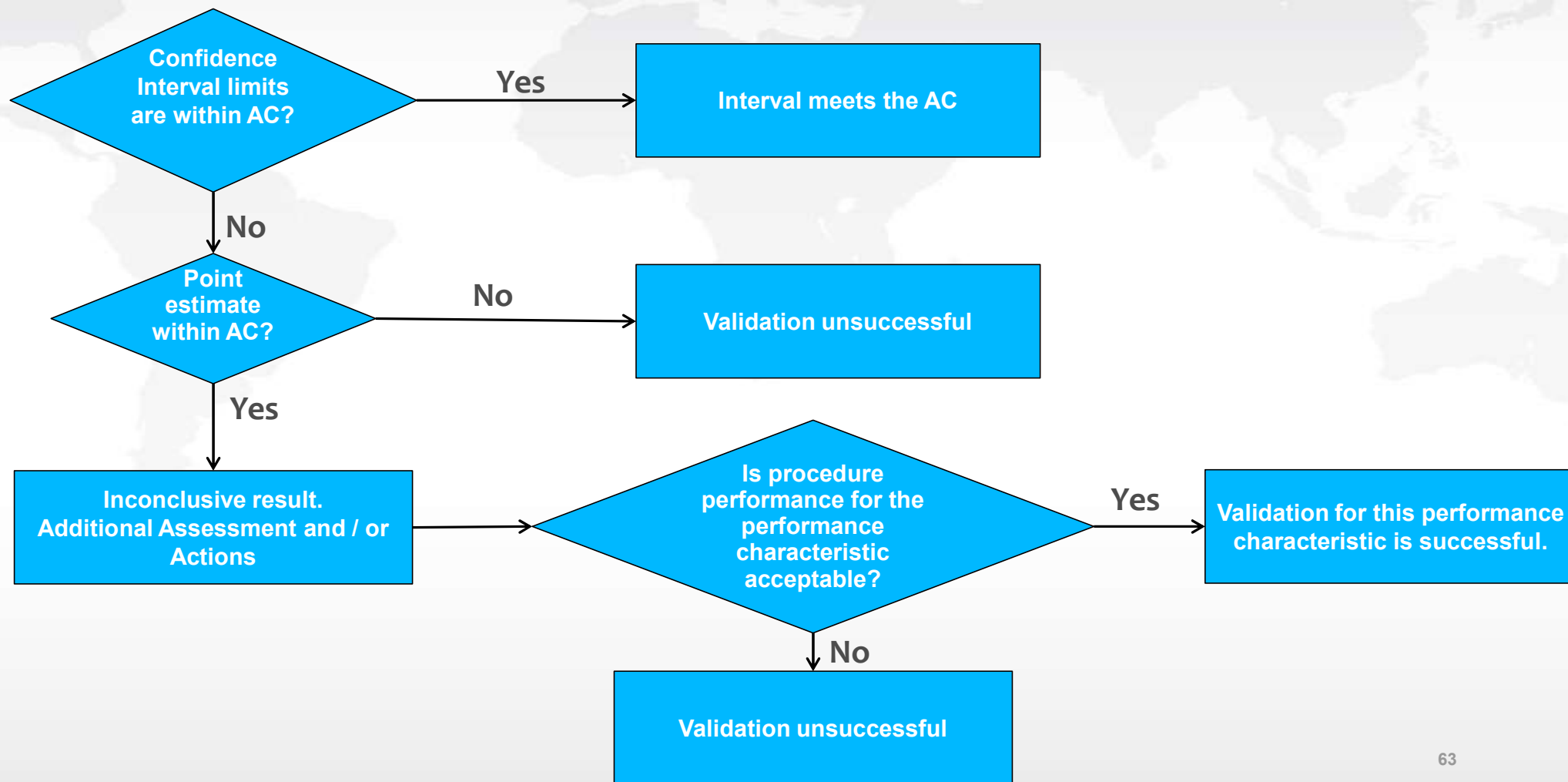
Analytical procedure development

Stability studies

Clinical batch release

Platform knowledge

Assessment of Confidence Interval Results



Additional Assessment and / or Actions

- Where the point estimate is within the acceptance criteria but the confidence interval exceeds the acceptance criteria, the following approaches may be considered:
 - Consider additional input from prior knowledge if not already included in the study.
 - Generate additional experimental data:
 - Re-assess the compatibility of the confidence interval with the acceptance criteria using the new data set.
 - The applicability and statistical validity of the approach taken should be documented and justified prior to the generation of additional data.
 - Consider adjustments to the number of replicates that may narrow confidence interval widths.
 - Consider the combined effect of accuracy and precision.
 - Justify acceptance of the risk.

Confidence Interval Example: Small Molecule

Separate Accuracy & Precision Acceptance Criteria

- An analytical procedure is developed to determine the amount of Drug Substance (DS) in the Drug Product (DP) final dosage form, e.g., tablet. The DP Assay is the measured mg amount in a lot of DP. The DP consists of the DS (900mg to 1100mg) as well as the presence of excipients and impurities.
- Acceptance Criteria (AC) - The DP procedure determines the DS amount over the range 900 to 1100 mg (90% to 110% of the 1000 mg label claim) and requires an Intermediate Precision (IP) SD no greater than 1.5 % label claim (15 mg) and bias that is no greater than 3.0 % label claim (30 mg).
- These acceptance criteria are informed by prior knowledge of similar products and procedures as well as the expected specification limits and acceptable Out-of-Specification rate (OOS) of this product when the process is in control with expected variation composition (lot-to-lot and analytical procedure).

- Sample size determination depends on the criteria, expected magnitude of the standard deviation, and desired type 1 and type 2 error rates.
- Example provided here is for the IP validation study, i.e., CI on variance.

Acceptance criteria

Smallest n such that

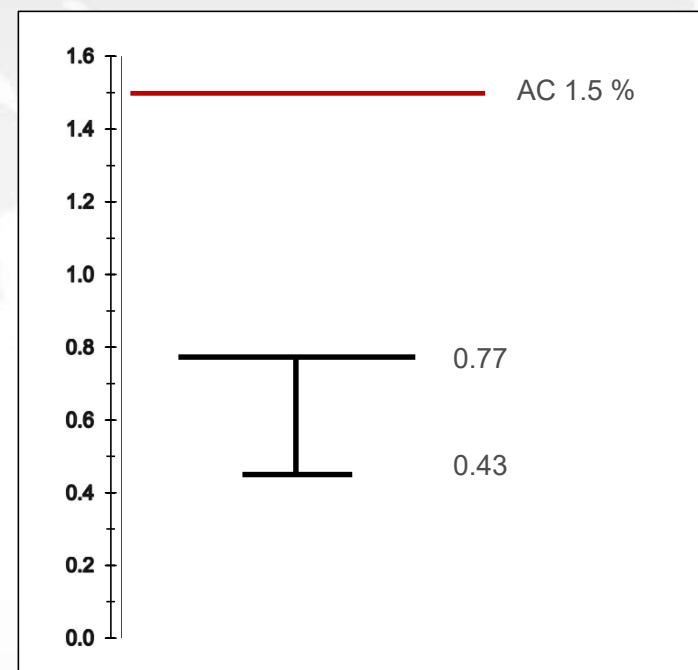
$$\frac{\text{Expected RR SD}}{\text{Required RR SD}} \leq \sqrt{\frac{\chi_{\alpha:n-1}^2}{\chi_{1-\beta:n-1}^2}}$$

Required IP SD ≤	1.5
Expected SD (prior knowledge)	0.7
type 1 error rate (α)	0.05
type 2 error rate (β)	0.2
Sample size (smallest n)	8

Type 1 error is falsely declaring compatibility with the criteria.
Type 2 error is failing to claim compatibility when such is the case.

Data Analysis – IP Study

Replicate	Reportable Result (%LC)
1	99.607
2	98.843
3	99.59
4	98.722
5	99.053
6	99.939
7	99.633
8	99.367
Standard Deviation (SD)	0.429
Sample size (n)	8
Chi-squared for 95% CI on SD	2.167
95% upper bound for SD	0.771



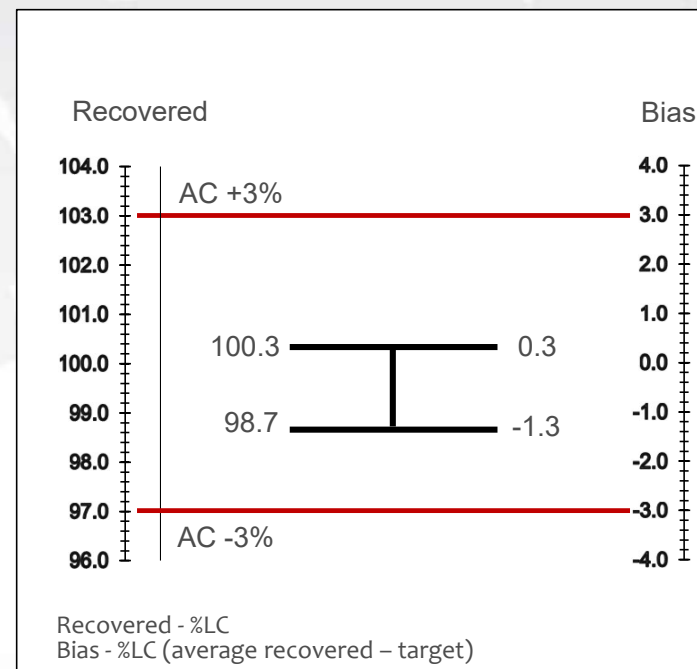
The one-sided 95% upper confidence bound of 0.77% LC is less than the AC of 1.5%. The procedure is compatible with the criterion.

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Data Analysis – Accuracy Study

Individual % LC	Average % LC	% RSD
98.5	99.30	1.395
100.9		
98.5		
100.8	99.83	1.003
98.8		
99.9		
98.3	99.37	0.951
99.7		
100.1		

		95% Confidence Limits (% LC)	
		Lower	Upper
Ave Recovery	99.5	98.7	100.3
Bias Estimate	-0.50	-1.3	0.3



The two-sided 95% confidence interval (-1.3 to 0.3) falls within AC range of (-3 to 3). This passes the required acceptance criterion for accuracy.

Confidence Interval Example: Validation of a Bioassay using Log Transformation

- Bioassay specifications, requirements, and data may require log transformation to satisfy the statistical assumptions associated with calculations.
 - Any base (\log_e , \log_{10} , \log_2) can be used for transformation and re-expression back to the potency scale.
 - Using: $\text{Trans}' = \log(\text{Value})$ to denote a transformed value.
- The specification range on relative potency (0.75,1.33) is asymmetric, with limits having a reciprocal relationship:
 - Lower limit = $1/\text{Upper Limit} = 1/1.33 = 0.75$.
- The \log_e specification range becomes:
 - $\log_e(0.75) = -0.29$ to $\log_e(1.33) = 0.29$ which is symmetric around $\log_e(1.00) = 0$.

Definitions of Accuracy and Precision

- Relative accuracy (USP<1033>):

$$\%Relative\ Bias(RB) = 100 \times \left(\frac{Measured\ potency}{Expected\ potency} - 1 \right) \%$$

$$\log_e \text{ scale: } RB' = \log_e \left(\frac{Measured\ potency}{Expected\ potency} \right) = \log_e \left(1 + \frac{\%RB}{100} \right)$$

- Intermediate Precision (IP) (USP<1033>):

$$\%IP = 100 \times \left(e^{\sqrt{\sigma^2}} - 1 \right) \%$$

$$\log_e \text{ scale: } IP' = \log_e \left(1 + \frac{\%IP}{100} \right) = \sqrt{\sigma^2}$$

where σ^2 is the variance of individual assay replicates, \log_e scale

- The % Geometric CV (%GCV) of the reportable result (RR): $\%GCV = 100 \times \left(e^{\sqrt{\sigma^2/n}} - 1 \right) \%$

Note: Alternative calculations exist for determination of %GCV.

Release Assay Format and Validation AC

- Release specification limits for Relative Potency (RP): 0.75 to 1.33.
- Prob (Initial OOS) $\leq 5\%$ based on procedure performance.
- Preliminary Reportable Result (RR) sample replication strategy is $n = 3$ independent replicates of the bioassay method.
- From all of the above, the validation acceptance criteria on the independent replicates are:
 - %RB is not more than 12%
 - Similar to the reciprocal relationship of the specification, the lower AC is
 $[(1/1.12) - 1] = -0.11$ (i.e., -11%)
 - %IP is not more than 20%

Note: Calculation of criteria performed as per USP <1033>. Alternative calculations may also be appropriate.

- 90% CI for %RB lies within (-11%, 12%).

90% CI for $RB' = \log_e \left(1 + \frac{\%RB}{100}\right)$ lies within $\left[\log_e \left(1 - \frac{11}{100}\right), \log_e \left(1 + \frac{12}{100}\right)\right] = [-0.1133, 0.1133]$

- Upper (1-sided) 95% Confidence Bound (CB) for %IP no more than 20%.

Upper (1-sided) 95% CL for $IP' = \log_e \left(1 + \frac{\%IP}{100}\right)$ no more than $\log_e \left(1 + \frac{20}{100}\right) = 0.1823$

Validation Study Design

- From prior knowledge:
 - Assumed %RB is 1%.
 - Assumed %IP is 10%.
- Dilutional linearity study at 5 levels:
 - 0.5, 0.71, 1, 1.41, 2
- For the purposes of this case study:
 - Point estimates and 90% CIs for %RB will be calculated separately per level.
 - Point estimate and upper 95% CI for average intermediate precision across levels in the reportable range will be calculated.
 - Consistency across levels to be verified during analysis.
- Refer to USP general chapter <1033>: Biological Assay Validation for details of more complex designs / calculations. Alternative calculations may also be appropriate.

Validation Study – Sample Size for Accuracy

- Success if 2-sided 90% CI for %RB lies within (-11%, 12%).

2-sided 90% CI for RB' lies within $\left[\log_e \left(1 - \frac{11}{100} \right), \log_e \left(1 + \frac{12}{100} \right) \right] = [-0.1133, 0.1133]$

- For 80% chance of study success (i.e., Power), assuming true %RB is 1%, true %IP is 10%:

$$n \geq \frac{(t_{0.95,(n-1)} + t_{0.90,(n-1)})^2 \times \left[\log_e \left(1 + \frac{\text{Assumed IP}}{100} \right) \right]^2}{\left[\log_e \left(1 + \frac{\text{Required RB}}{100} \right) - \log_e \left(1 + \frac{\text{Assumed RB}}{100} \right) \right]^2} = \frac{(t_{0.95,(n-1)} + t_{0.90,(n-1)})^2 \times \left[\log_e \left(1 + \frac{10}{100} \right) \right]^2}{\left[\log_e \left(1 + \frac{12}{100} \right) - \log_e \left(1 + \frac{1}{100} \right) \right]^2} = 9$$

→ $n \geq 9$ per level

Note: While 80% power is typical, this can be increased to improve the probability of success;
Similar to a t-test statistic, assumed IP is used in the calculation.

Validation Study – Sample Size for Precision

- Success if upper (1-sided) 95% CI for %IP no more than 20%

Upper (1-sided) 95% CL for IP' no more than $\log_e \left(1 + \frac{20}{100}\right) = 0.1823$

- For 80% chance of study success, assuming true %IP is 10%, data pooled across 5 levels:

$$\frac{\chi_{0.8, 5(n-1)}^2}{\chi_{(1-0.95), 5(n-1)}^2} \leq \frac{\left[\log_e \left(1 + \frac{\text{Required IP}}{100} \right) \right]^2}{\left[\log_e \left(1 + \frac{\text{Assumed IP}}{100} \right) \right]^2} = \frac{\left[\log_e \left(1 + \frac{20}{100} \right) \right]^2}{\left[\log_e \left(1 + \frac{10}{100} \right) \right]^2}$$

→ $n \geq 3$ per level

Note: While 80% power is typical, this can be increased to improve the probability of success.

Validation Study - Results: 9 Replicates per Level

- 9 runs per dilution level chosen to ensure power for both %RB per level and %IP averaged across levels.
- Study designed as a 3x3 factorial with 3-levels per factor each of Analysts and Media Lots (n = 9).
 - Factorial used to balance replicates of study factors.

Validation study results			Level									
			0.5		0.71		1		1.41		2	
Run	Analyst	Media lot	RP	Log _e	RP	Log _e	RP	Log _e	RP	Log _e	RP	Log _e
1	1	1	0.5155	-0.6626	0.7333	-0.3102	1.0158	0.0157	1.6274	0.4870	2.3639	0.8603
2	1	2	0.5278	-0.6390	0.5978	-0.5145	0.9636	-0.0371	1.2909	0.2553	1.9871	0.6867
3	1	3	0.4916	-0.7101	0.7492	-0.2887	0.9102	-0.0941	1.6561	0.5045	2.1277	0.7550
4	2	1	0.4736	-0.7474	0.8828	-0.1247	1.0552	0.0537	1.3788	0.3212	1.9382	0.6618
5	2	2	0.5722	-0.5583	0.6446	-0.4391	1.0462	0.0452	1.6243	0.4851	2.0581	0.7218
6	2	3	0.4868	-0.7199	0.7758	-0.2539	0.9903	-0.0097	1.3769	0.3198	1.9953	0.6908
7	3	1	0.5470	-0.6033	0.6501	-0.4306	0.9157	-0.0881	1.4761	0.3894	1.9073	0.6457
8	3	2	0.4187	-0.8706	0.6485	-0.4331	0.9949	-0.0051	1.4372	0.3627	1.9786	0.6824
9	3	3	0.4962	-0.7008	0.6336	-0.4563	0.9720	-0.0284	1.3779	0.3206	1.7913	0.5829
Average			n = 9	-0.6902	n = 9	-0.3612	n = 9	-0.0164	n = 9	0.3828	n = 9	0.6986
Standard Deviation				0.0905		0.1245		0.0520		0.0898		0.0774
Geometric Mean				0.5015		0.6968		0.9837		1.4664		2.0109
%Relative Bias				0.3%		-1.9%		-1.6%		4.0%		0.5%
%GCV				9.5%		13.3%		5.3%		9.4%		8.0%

Validation Study - Results: 9 Replicates per Level

- Calculation of confidence intervals

			Level									
			0.5		0.71		1		1.41		2	
			<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>
Average			<i>n</i> = 9	-0.6902	<i>n</i> = 9	-0.3612	<i>n</i> = 9	-0.0164	<i>n</i> = 9	0.3828	<i>n</i> = 9	0.6986
Standard Deviation				0.0905		0.1245		0.0520		0.0898		0.0774
Geometric Mean				0.5015		0.6968		0.9837		1.4664		2.0109
%Relative Bias				0.3%		-1.9%		-1.6%		4.0%		0.5%

- Accuracy at Level 0.5 - $\%RB = 100 \cdot [(e^{-0.6902}/0.5) - 1] = 0.3\%$
- Ln RP: $90\% CI = Avg \pm t_{0.95,9-1} \cdot SD/\sqrt{9} = -0.6902 \pm 1.8595 \cdot 0.0905/\sqrt{9} = (-0.746, -0.634)$
- $\%RB$: Lower CL = $100 \cdot [(e^{-0.746}/0.5) - 1] = -5.2\%$

$$Upper CL = 100 \cdot [(e^{-0.63} / 0.5) - 1] = 6.1\%$$

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Validation Study - Results: 9 Runs per Level

- Calculation of confidence intervals

			Level									
			0.5		0.71		1		1.41		2	
			<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>	<i>RP</i>	<i>Log_e</i>
Standard Deviation				0.0905		0.1245		0.0520		0.0898		0.0774
%GCV				9.5%		13.3%		5.3%		9.4%		8.0%

- Average IP across levels

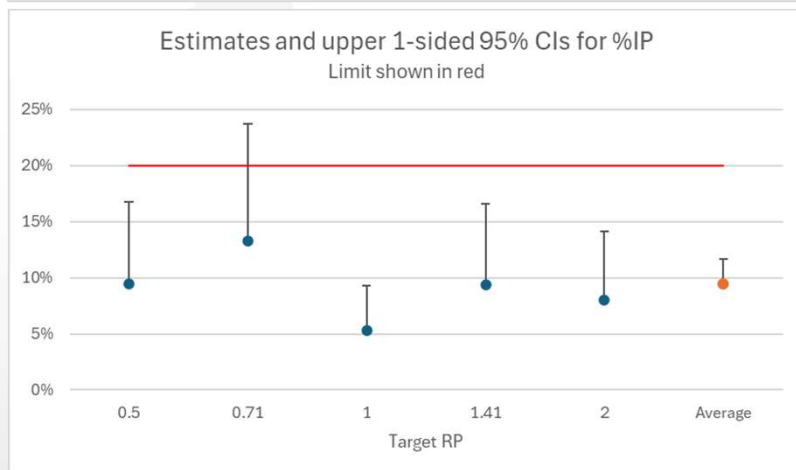
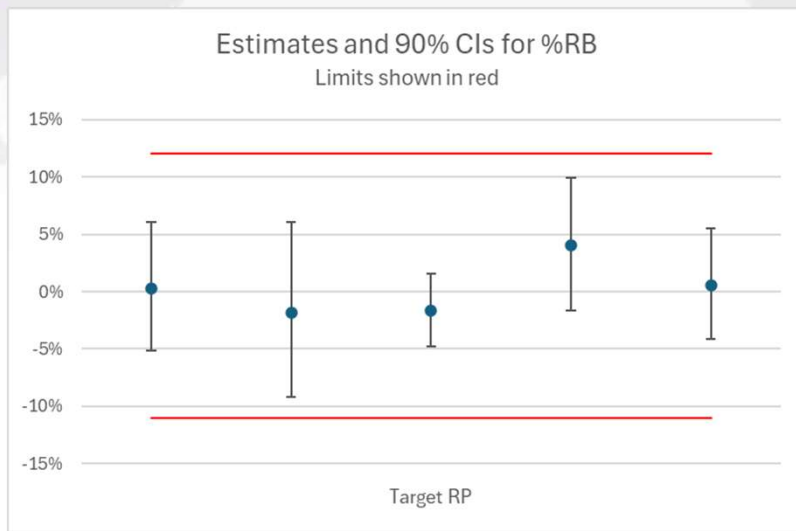
- Average SD (ln): $s_{Average} = \sqrt{[\sum_{i=1}^5 (n_i - 1) \cdot s_i^2] / [5 \cdot (n_i - 1)]}$, $n_i - 1 = 8$ at all levels

$$= \sqrt{(8 \cdot 0.0905^2 + 8 \cdot 0.1245^2 + 8 \cdot 0.0520^2 + 8 \cdot 0.0898^2 + 8 \cdot 0.0774^2) / (5 \cdot 8)} = 0.090$$

- IP (ln): Upper CL = $s_{Average} \cdot \sqrt{[5 \cdot (n_i - 1)] / \chi_{0.05, 5 \cdot (n_i - 1)}^2} = 0.090 \cdot \sqrt{(5 \cdot 8) / 26.509} = 0.110$

- IP: %GCV = $100 \cdot (e^{0.090} - 1) = 9.4\%$, Upper CL = $100 \cdot (e^{0.110} - 1) = 11.7\%$,

Validation Study - Results: 9 Replicates per Level



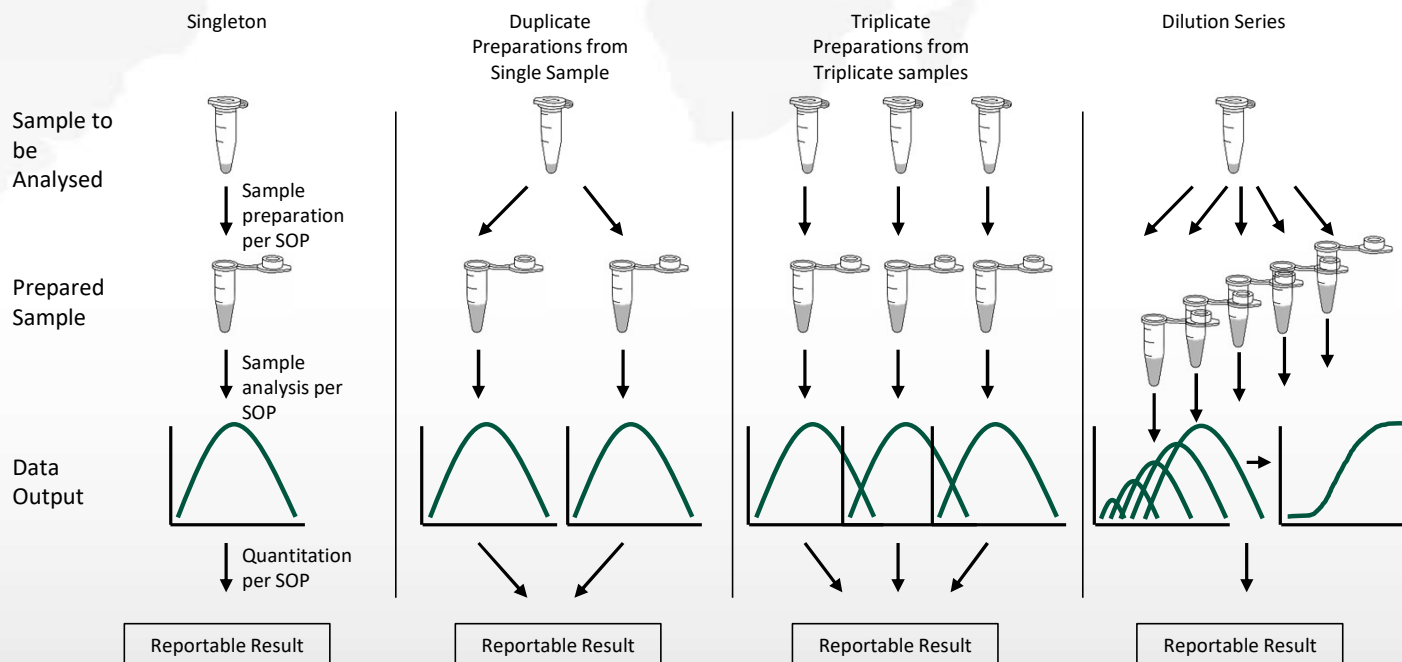
- 90% CIs for individual levels fall within the %RB acceptance limits (-11%, 12%).
 - The assay **passes** the requirement for relative accuracy.
- The upper (one-sided) 95% CI for the average %IP across levels falls below the acceptance limits ($\leq 20\%$).
 - No apparent pattern in %IP across levels.
 - Some CI's at individual levels exceed the criterion.
 - Consistency across levels was verified during analysis.
 - Confidence interval considering data across all levels = 11.7%.
 - The assay **passes** the requirement for intermediate precision.

Use of Replicates

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Replicates - Routine Analysis

- Replication is an element of the analytical procedure control strategy. Appropriate replication strategy:
 - Can vary widely depending on the analytical procedure (from $n=1$ to $n=6$ or more).
 - Is established during analytical procedure development.
 - Is documented in the analytical procedure.
- Examples:



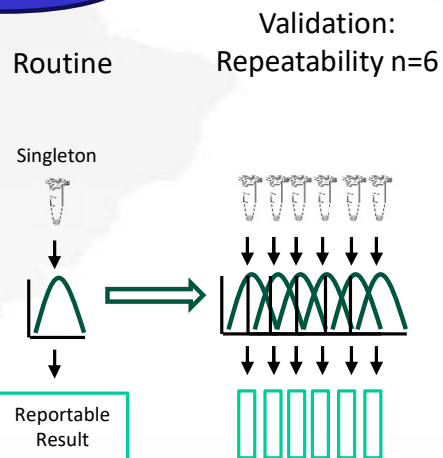
The reportable result is compared to specification limit(s)

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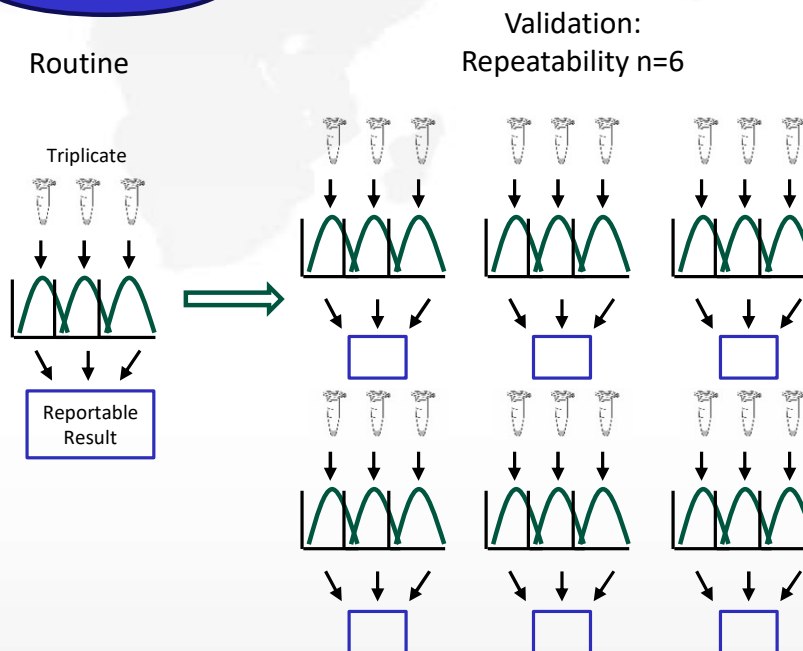
Replicates - in Validation Studies

ICH Q2(R2) guidance: “The experimental design of the validation study **should reflect the number of replicates used in routine analysis** to generate a reportable result.” (Section 2.1)

Example 1



Example 2



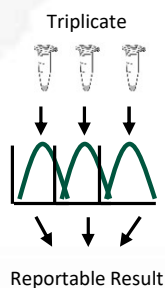
N= 6
reportable
results

ICH Q2(R2) / Q14 Training Module 3

Replicates - in Validation Studies

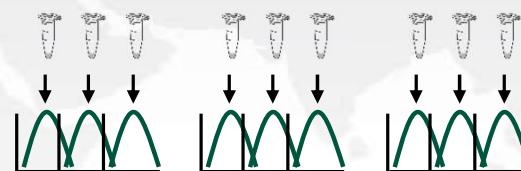
Example 3

Routine

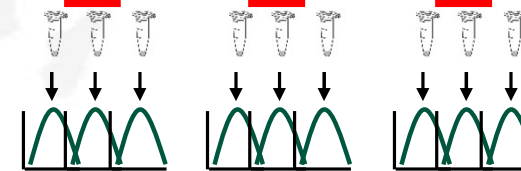


Validation:
Repeatability across
the range of the
method
(n=3 at 3 levels)

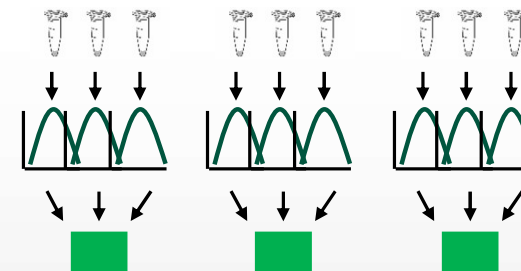
150% Level: n=3
reportable
results



100% Level: n=3
reportable
results



50% Level: n=3
reportable
results



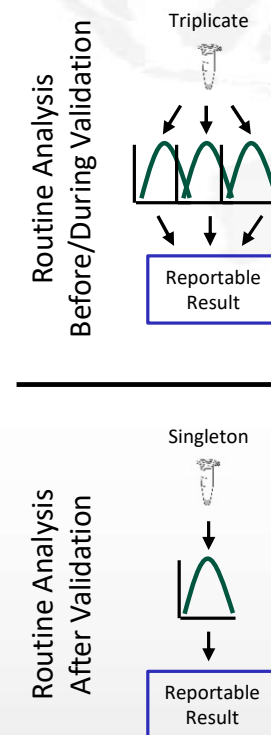
ICH Q2(R2) / Q14 Training Module 3

Replicates - in Routine Analysis

ICH Q2(R2) guidance: “If justified, it may be acceptable to perform some validation tests using a different number of replicates or to adjust the number of replicates in the analytical procedure based on data generated during validation.” (Section 2.1)

Example:

- HPLC analysis for content:
 - For routine analysis, each reportable result is generated from triplicate injections.
 - The repeatability requirement for method validation is that the RSD for reportable results $\leq 3\%$.
- Analytical Procedure Validation findings:
 - Validation examined $n=6$ reportable results.
 - All reportable results generated from triplicate injections, as required for routine analysis.
 - RSD for repeatability ($n=6$ reportable results) = 0.5% .
- Routine analysis changes based on validation study results:
 - Additional statistical assessment of the validation data indicates that repeatability would meet the validation requirements with singleton replication.
 - RSD for repeatability ($n=18$ injections) = 2.5% .
 - The risk of imprecision within replicates resulting in inaccurate results was sufficiently low that the decision was made to execute the analytical procedure as singleton injections, significantly increasing analytical procedure throughput and reducing resource consumption.



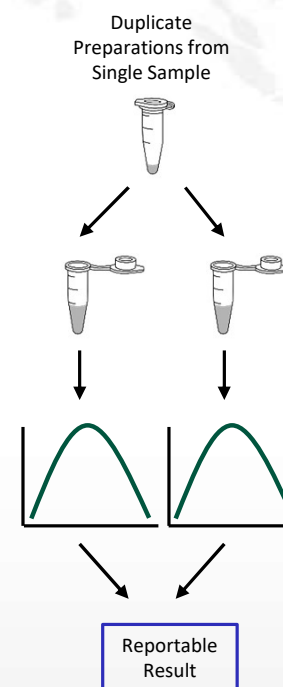
ICH Q2(R2) / Q14 Training Module 3

Replicates - in Validation Studies

ICH Q2(R2) guidance: “If justified, it may be acceptable to perform some validation tests using a different number of replicates or to adjust the number of replicates in the analytical procedure based on data generated during validation.” (Section 2.1)

Example:

- Peptide map analysis for percent oxidation at a specific Methionine residue:
 - Analytical procedure development studies demonstrated that the sample preparation process may increase oxidation in the sample – so for routine analysis, each reportable result is generated from duplicate preparations, with one injection per preparation.
 - Run time 60 minutes per injection.
 - Samples must be injected within 6 hours of preparation.
- Analytical procedure validation study design:
 - Repeatability precision was investigated as six preparations of a single sample:
 - Analysis of six samples with duplicate preparations would have resulted in half of the injections exceeding the sample stability limit of 6 hours.



Use of Development Data

Use of Development Data as part of Validation Data

The ICH Q2(R2) defines the following:

From Chapter 2 “General Considerations for Analytical Procedure Validation”: “Suitable data derived from development studies (see ICH Q14) can be used as part of validation data”.

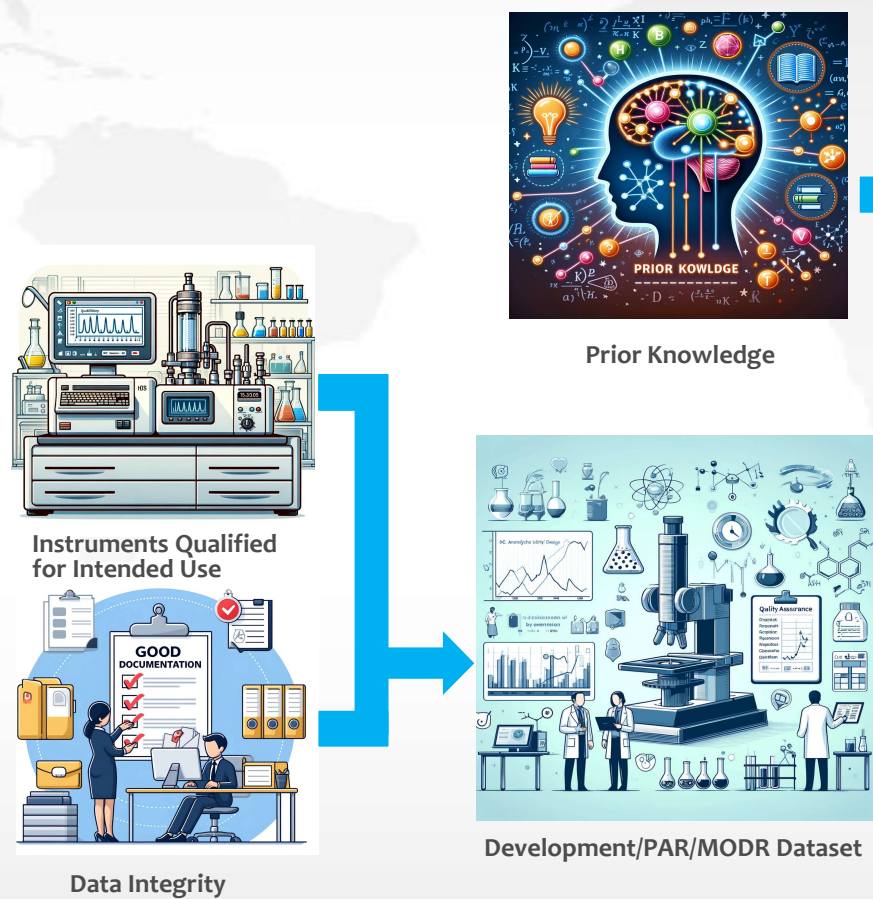
From Chapter 2.1 “Analytical Procedure Validation Study”: “In cases where prior knowledge is used (e.g., from development or from previous studies), appropriate justification should be provided”.

Prior knowledge and/or data generated during development, e.g., while establishing proven acceptable ranges (PAR) or method operable design regions (MODR), can be used as part of the validation study.

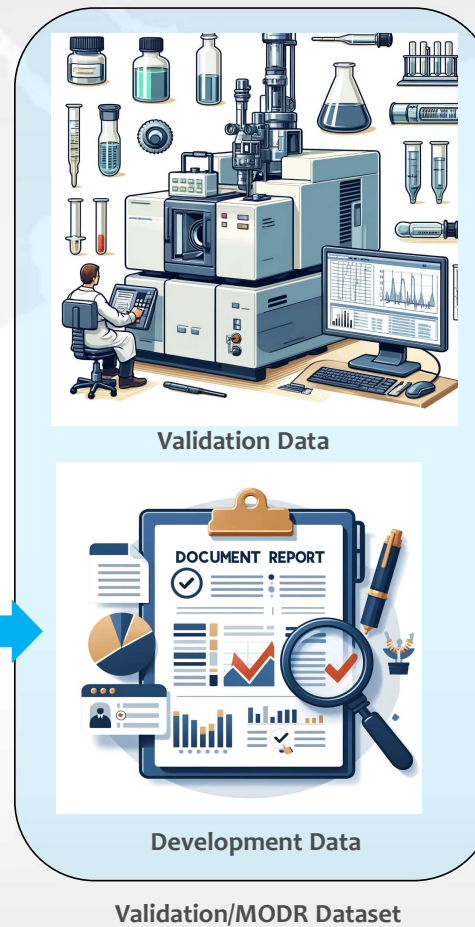
The broader the prior knowledge and the knowledge about the relationship between analytical parameters and analytical performance characteristics, the higher the chances to be able to use these data instead of generating them again during the validation study.

Use of Development Data as part of Validation Data

Development Study



Validation Study



Use of Development Data as part of Validation Data

Some examples on usage of development data are following:

- **Selectivity and Specificity:** Using specificity data generated during analytical procedure development as validation evidence.
- **Linearity:** Incorporation of linearity data from the development phase to demonstrate the range of the analytical procedure during validation.
- **Lower Range Limit (DL/QL):** Adapting sensitivity evaluations from development for validation, showing the ability of the analytical procedure to detect and quantify low amounts of analytes.
- **Robustness:** Applying robustness testing data from the analytical procedure development stage to demonstrate analytical procedure reliability under varied conditions.
- **Relative Response Factors:** This evaluation may be performed during validation or development, should use the finalised analytical procedure conditions and should be appropriately documented.
- **System Suitability Testing (SST):** Using SST data from development experiments to confirm system performance during analytical runs.
- **Sample Stability:** Utilising stability data from development studies to demonstrate stability during sample handling and execution of the analytical procedure.

Use of Development Data as part of Validation Data

The usage of these development data needs to be justified (e.g. in the validation protocol), in order to describe how the data have been generated and why their documentation is deemed acceptable.

Here are some example of justifications that can be provided:

- The analytical procedure parameters are the same as the one used for validation.
- The instrument used is qualified and calibrated for the intended use.
- The documentation ensures traceability and data integrity.
- Data selection criteria (e.g. development data).

The acceptance of development data as part of validation from regulatory bodies is linked to the quality and integrity of the dataset submitted, hence a well documented justification is important.

Single Point Calibration

ICH Q2(R2) / Q14 Training Module 3

Single Point Calibration Assays

- **In some cases, it is acceptable not to set up a standard curve when performing an analysis, but to use only one standard:**
 - Analytical procedure development data (prior knowledge) or analytical procedure validation results indicate that a single calibration point (combined with a zero-intercept on the x and y axes) provides sufficient accuracy and precision to be fit for the intended purpose.

$$\text{Concentration (sample)} = \frac{\text{Response (sample)} \times \text{Concentration (standard)}}{\text{Response (standard)}}$$

- **Analytical procedure validation is performed in the same way as if the analytical procedure were to be used with a standard curve.**
 - Range should be demonstrated across multiple levels, as per ICH Q2(R2).
 - Validation data (over the range of the analytical procedure) should be used to justify the appropriateness of the single-point calibration approach.
 - Standard curves should demonstrate that the y-intercept is not significantly different from zero.
- **The standard concentration should ideally be close to the expected sample concentration.**
 - Reportable results may be generated above or below the standard concentration (within range).
 - Reportable results must not exceed the range determined during validation.

Extrapolation of Validated Range

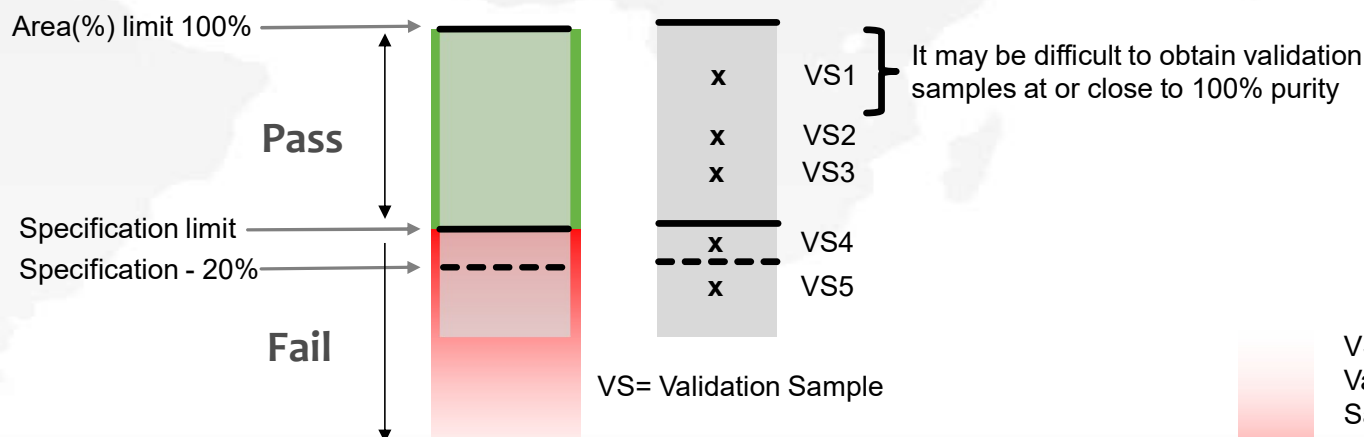
Justification for Extrapolation of Validated Range (Relative Area % Techniques)

- Relative area % analytical procedures (i.e., normalisation) quantitate purity or impurity as a % of total response.
 - A sample with no impurities would be '100%'.
 - 100% cannot be exceeded.
 - If an impurity is present, 100% cannot be achieved.
- Problem statement:
 - A sample at a purity of 100% or at an impurity content of 0% might not be physically available in order to use it into the validation.
 - Some routine samples might need to be tested that will have a purity > or a content < the highest/lowest sample available at the time of the validation.
 - However, those samples may have to be considered as pass despite falling outside the range where precision/accuracy/linearity has been demonstrated during validation.

Justification for Extrapolation of Validated Range

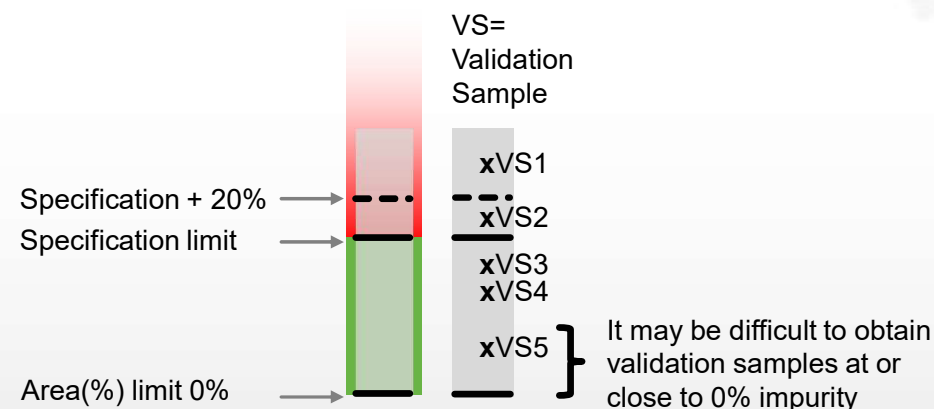
- Approach proposed in the case of a relative area(%) analytical procedure:**

- Purity: Unidirectional specification, “not less than”.
- Lower limit of range should cover “specification - 20%”.
- Upper limit of range for an area(%) procedure will be 100%.

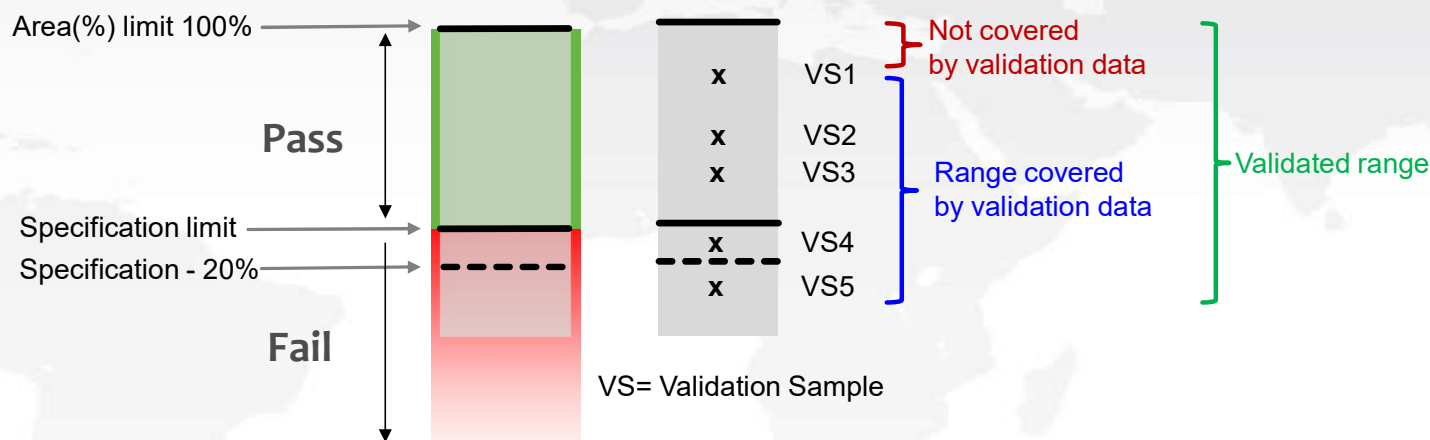


- Similar issues occur for impurity analytical procedures:**

- Unidirectional specification, “not more than”.
- Upper limit of range should cover “specification + 20%”.
- Lower limit of range for an area(%) procedure will be 0%.



Justification for Extrapolation of Validated Range



- Same concerns / solutions might apply in both purity and impurity situations:

- When it is physically impossible to reach the (100% / 0%) limit of the range, the experimental validation data do not cover the extreme of the range.
- Justify in validation protocol that, if the desired procedure performances are obtained for all validation samples, the range located between the highest validation standard and the “absolute limit” will be considered as part of the validated range.
- State that this will be the validated range where valid results can be reported.
- This is justifiable, once it can be assured that these extrapolated levels are within the specification limit, even if the performance of the procedure could not be demonstrated at these levels.

Quantitative Test vs. Limit Test

Quantitative Test vs Limit Test for Impurities (Purity) as per Table 1

	Quantitative	Limit
Expectation	<p>Establishment of a range with suitable level of precision, accuracy and response is required.</p> <p>The level of impurity and compliance with product specification can only be determined following calculation or processing.</p>	<p>Establishment of a range with suitable level of precision, accuracy and response is not required.</p> <p>The compliance with product specification is confirmed by direct comparison with a reference material without calculation or processing of data derived from analytical run.</p>
Examples	<p>Specification: Impurity B is not more than 1.5%</p> <p>The HPLC peak area of impurity B is not more than 1.5% of the sum of all peaks of the sample analysis.</p>	<p>Sulphate test (precipitation test).</p> <p>The turbidity produced by the sample is less than that produced by the reference material.</p>

Quantitative Test vs Limit Test for Impurities (Purity) as per Table 1

For the same specification, depending on the analytical procedure design, it could be a limit or quantitative test as illustrated by the example below:

For an impurity specification: Impurity X is not more than 0.10%

- **Analytical procedure 1:**

- The level of impurity X is calculated by using the peak area of impurity X over the sum of all peaks in the sample.
- To justify the use of this approach, establishment of a range with suitable level of precision, accuracy and linear response is required.
- The level of impurity and compliance with product specification can only be determined following calculation or processing.

>>> Quantitative Test

- **Analytical procedure 2:**

- Area of impurity X obtained with sample solution (10 mg/mL) is not more than the area of the corresponding peak in the chromatogram obtained with reference solution with 0.01 mg/mL impurity X.
- Establishment of a range with suitable level of precision, accuracy and response is not required. The compliance with product specification is confirmed by direct comparison with reference standard without calculation or processing of data derived from analytical run.

>>> Limit Test

- **Analytical procedure 3:**

- The amount of impurity X is calculated by a comparison of responses from the sample solution (10 mg/mL) against a reference solution with 0.02 mg/mL impurity X.
- Establishment of a range with suitable level of precision, accuracy and linear response is required.
- The level of impurity X and compliance with product specification can only be determined following calculation or processing.

>>> Quantitative Test

Contact

- For any questions please contact the ICH Secretariat:

admin@ich.org