Validation, ICH Q2

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INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1)

Current *Step 4* version
Parent Guideline dated 27 October 1994
(Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005)

Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;

- Quantitative tests for impurities' content;

- Limit tests for the control of impurities;

- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.

Typical validation characteristics:

-Accuracy
- Precision
= Repeatability
= Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Type of Analytical procedure	Identification	Testing for impurities		Assay – dissolution (measurement only)
Charateristics		Quant	Limit	- Content/potency
Accuracy	-	+	-	+
Precision				
-Repeatability	-	+	-	+
-Interm. Precision	-	+(1)	-	+(1)
Specificity(2)	+	+	+	+
Detection Limit	-	-(3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated
+ signifies that this characteristic is normally evaluated
(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed
(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
(3) may be needed in some cases

2. LINEARITY

"A linear relationship should be evaluated across the range (see section 3) of the analytical procedure"

"Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares".

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

For the establishment of linearity, **a minimum of 5 concentrations** is recommended.

Other approaches should be justified.

3. RANGE

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and or at precision.

- if assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities 1 to 120% of the assay specification;

4. ACCURACY

Accuracy should be established across the specified range of the analytical procedure.

4.1. Assay

4.1.1 Drug Substance

Several methods of determining accuracy are available:

a) application of an analytical procedure to an analyte of **known purity** (e.g. reference material);

b) comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure, see 1.2.);

c) **accuracy may be inferred once precision, linearity and specificity have been established.**

4.1.2 Drug Product

Several methods for determining accuracy are available:

a) application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;

b) in cases where it is impossible to obtain samples of all drug product components , it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well characterized procedure, the accuracy of which is stated and/or defined (independent procedure, see 1.2.).

c) accuracy may be inferred once precision, linearity and specificity have been established.

4.3. Recommended Data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/3 replicates each of the total analytical procedure).

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

5. PRECISION

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

5.1. Repeatability

Repeatability should be assessed using:

a) a minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/3 replicates each)

or

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

5.2. Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure.

Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

5.3. Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

5.4. Recommended Data

The standard deviation, relative standard deviation (coefficient of variation)

6. DETECTION LIMIT

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

6.1. Based on Visual Evaluation

...

6.2. Based on Signal-to-Noise

...

6.3 Based on the Standard Deviation of the Response and the Slope

6.3 Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

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

where σ= the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:

6.3.1 Based on the Standard Deviation of the Blank

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

6.3.2 Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

6.4 Recommended Data

The detection limit and the method used for determining the detection limit should bepresented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable forjustification.

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

7. QUANTITATION LIMIT

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

7.1. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

7.2. Based on Signal-to-Noise Approach

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7.3. Based on the Standard Deviation of the Response and the Slope

…

7.3. Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

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

where σ= the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways for example:

7.3.1 Based on Standard Deviation of the Blank

…

7.3.2 Based on the Calibration Curve

…

The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

7.4 Recommended Data

The quantitation limit and the method used for determining the quantitation limit should be presented.

The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

8. ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

…

9. SYSTEM SUITABILITY TESTING

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

…

Typical validation characteristics:

-Accuracy
- Precision
= Repeatability
= Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Summing up ICH Q2

At least 3 levels, 3 replicates each

At least 5 levels for linearity

Limits

$$LOQ = \frac{a + 10 \cdot S_{Y/X} - a}{b} = \frac{10 \cdot S_{Y/X}}{b}$$

$$LOD = \frac{a + 3 \cdot S_{Y/X} - a}{b} = \frac{3 \cdot S_{Y/X}}{b}$$

$S_{Y/X}$ = s fom table 1

Linear Regression

$$\hat{Y} = a_1 \cdot X + a_0$$

$$S = \sum_{i=1}^{n} error_i^2 = \sum_{i=1}^{n} \left(Y_i - a_0 - a_1 X_i\right)^2$$

$$a_1 = \frac{\sum \left(X_i - \overline{X}\right) \cdot \left(Y_i - \overline{Y}\right)}{\sum \left(X_i - \overline{X}\right)^2}$$

$$a_0 = \overline{Y} - a_1 \cdot \overline{X}$$

Linear Regression

(14) $$S_{XY} = \sum X_i Y_i - n\overline{X}\,\overline{Y}$$

(15) $$S_{XX} = \sum X_i^2 - n\overline{X}^2 = \sum \left(X_i - \overline{X} \right)^2$$

(16) $$S_{YY} = \sum Y_i^2 - n\overline{Y}^2 = \sum \left(Y_i - \overline{Y} \right)^2$$

(17) $$a_1 = \frac{S_{XY}}{S_{XX}}$$

Linear Regression

(19) $$R_i = Y_i - \hat{Y}_i$$

The following relation is valid for the residuals

(20) $$\sum_{i=1}^{n} R_i = \sum_{i=1}^{n} \left(Y_i - \hat{Y}_i \right) = 0$$

Linear Regression

$$\sum\left(Y_i - \overline{Y}\right)^2 = \sum\left(\hat{Y}_i - \overline{Y}\right)^2 + \sum\left(Y_i - \hat{Y}_i\right)^2$$

(Pythagoras…)

Sum of squares about the mean	=	Sum of squares due to regression line	+	Sum of squares about regression line

$$R^2 = \frac{SS\ due\ to\ regression\ line}{SS\ about\ the\ mean}$$

$$K = \left(Sign\ of\ slope\right) \cdot \sqrt{R^2}$$

Linear Regression

42a $$X_U = \hat{X}_0 + \frac{\left(\hat{X}_0 - \overline{X}\right)\cdot g + \left|\left(\frac{t\cdot s}{a_1}\right)\cdot\sqrt{\frac{\left(\hat{X}_0 - \overline{X}\right)^2}{S_{XX}} + \frac{(n+q)\cdot(1-g)}{n\cdot q}}\right|}{1-g}$$

42b $$X_L = \hat{X}_0 + \frac{\left(\hat{X}_0 - \overline{X}\right)\cdot g - \left|\left(\frac{t\cdot s}{a_1}\right)\cdot\sqrt{\frac{\left(\hat{X}_0 - \overline{X}\right)^2}{S_{XX}} + \frac{(n+q)(1-g)}{nq}}\right|}{1-g}$$

43 $$g = \frac{t\left(\nu, 1-\frac{1}{2}\alpha\right)^2\cdot s^2\cdot S_{XX}}{a_1^2}$$

Where q is number of replicates

Linear Regression

n-2	$SS = \sum_{i=1}^{n} \left(Y_i - \hat{Y}_i\right)^2$	$s^2 = \frac{SS}{n-2}$

(from table 1)

In order for the equations for the confidence limits to yield meaningfull results the calibration curve – linear regression - must be well determined, i.e. g should be smaller than approx. 0.20 (t should be about 2.236).

Design of the Calibration

Appendix 1. Measuring the calibration curve

When a calibration curve is to be established, a series of standard solutions should be prepared according to the following strategy:

	C_1 = 2*LOQ	C_2 = (C_1 + C_3)/2	C_3 = (C_1 + C_5)/2	C_4 = (C_3 + C_5)/2	Max. Konc*1.25
Replicates	6	2	6	2	6

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Appendix 1. Measuring the calibra

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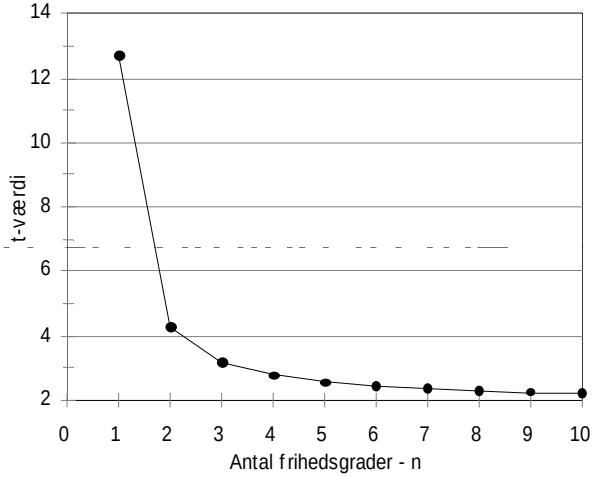

	$C_1 = 2*LOQ$	$C_2 = (C_1 + C_3)/2$	$C_3 = (C_1 + C_5)/2$	$C_4 = (C_3 + C_5)/2$	Max. Konc*1.25
Replicates	6	2	6	2	6

Appendix 2. Parameters, that should be calculated for a calibration curved calculated by means of linear regression.

- Slope & Intercept according to eqs 11 and 12.
- Residuals according to eq 19, with residual plots, both type a and b.
- A plot of the regression line with **all** points included.
- R^2 according to eq 25.
- The correlation coefficient as the square root of R^2, sign included, eq 25b.
- Optionally an analysis of variance table like table 1.

Appendix 2. Parameters, that should be calculated for a calibration curved calculated by means of linear regression.

- An estimated standard deviation for the slope, eq 26, and the confidence interval, eq 27.
- An estimated standard deviation for the intercept, eq 28, and the confidence interval, eq 29.
- Standard deviation for the estimated Y-values, eq 30, and the confidence interval, eq 31, 32 or 33, preferably also plotted as confidence bands on the plot of the regression line.
- F-test for significance of the regression, eq 34.
- Test for significance of regression/lack of fit, eq 41.

Reporting the Calibration

Appendix 2. Parameters, that should be calculated for a calibration curved calculated by means of linear regression.

- Evaluation of the quality of the regression line, eq 43.
- Check if the calibration curve passes through 0,0
- LOD and LOQ calculated from the calibration curve, the parameter s (table 1), as a check (LOD and LOQ are determined independently).

Reporting the Calibration

If the calibration curve is used to calculate X-values (concentrations) from Y-values (measured response), the confidence interval for the result is calculated using eqs 42a and 42b.

The Calibration Curve

Summing up

The Calibration Curve

Standard Solution	1	2	3	4	5
Concentration	2·LOQ	(C1+C3)/2	(C1+C5)/2	(C3+C5)/2	$C_{max} \cdot 1.25$
Replicates	6	2	6	2	6

The Calibration Curve

Standard Solution	1	2	3	4	5
Concentration	2·LOQ	(C1+C3)/2	(C1+C5)/2	(C3+C5)/2	C_{max} ·1.25
Replicates	6	2	6	2	6

What is most important:

The Calibration Curve

Standard Solution	1	2	3	4	5
Concentration	2·LOQ	(C1+C3)/2	(C1+C5)/2	(C3+C5)/2	C_{max} · 1.25
Replicates	6	2	6	2	6

What is most important:

- Getting the exact values calculated for the concentrations

The Calibration Curve

Standard Solution	1	2	3	4	5
Concentration	2·LOQ	(C1+C3)/2	(C1+C5)/2	(C3+C5)/2	C_{max} ·1.25
Replicates	6	2	6	2	6

What is most important:

-Getting the exact values calculated for the concentrations

-Knowing the exact values of the concentrations

The Calibration Curve

Standard Solution	1	2	3	4	5
Concentration	2·LOQ	(C1+C3)/2	(C1+C5)/2	(C3+C5)/2	C_{max} ·1.25
Replicates	6	2	6	2	6

What is most important:

-Getting the exact values calculated for the concentrations

-Knowing the exact values of the concentrations