**Validation plan / protocol**

***(Re-)* Validation of the method xxx (Title)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Document Approval** | | | |
|  | **Name (Function / Department) \*** | **Signature** | **Date** |
| Compiled | Xxx (Lab xxx) |  |  |
| Reviewed | Xxx (Head of Lab xxx) |  |  |
| Approved | Xxx (Head of QC) |  |  |
| Approved | Xxx (Head of QA) |  |  |

*\* These are just examples, to be adjusted according to your procedures. There should be an author, a reviewer and a QA.*

|  |  |
| --- | --- |
| **Document History \*** | |
| **Version** | **Description of change** |
| 1.0 | New version |

*\* Might be placed here or e.g. at the end of the document. Can also be added by other columns such as author and date, if no digital document management system is used.*

|  |  |  |
| --- | --- | --- |
| **Further applicable documents and references \*** | | |
| **Document Number** | **Document Title** | **Version** |
| SOP-xxx | Validation of analytical methods *(your internal SOP describing how to perform method validations)* | 1.0 |
| SOP-xxx | Performance of method xxx *(if a SOP describing the method which should be validated already exists e.g. in case of revalidation or a draft version of the new method, this SOP should be listed here)* | 1.0 |
| SOP-xxx | Deviation management *(your internal SOP dealing with deviation management)* | 1.0 |
| xxx | Risk assessment for method xxx *(your internal risk assessment document, if performed and applicable, see chapter 7.9)* | 1.0 |
| ICH Q2(R1) „Validation of Analytical Procedures: Text and Methodology”, 2005 | | |
| FDA “Validation of chromatographic methods” reviewer guidance document, 1994  *only useful to be listed here in case of chromatographic methods* | | |
| *Further documents can be added* | | |

*\* This is not a must, but might be useful especially if an already existing method can be referenced. Otherwise the method can also be referenced in another section or fully described.*

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# Aim of this validation

This validation plan / protocol describes the procedure and the aspects to be evaluated during the validation the method xxx.

The method is intended to be used as identity test / purity test / for the determination of the content / potency of xxx in xxx samples (according to SOP-xxx\*).

*\* if already applicable*

According to SOP-xxx *(your internal validation SOP)* /(and) the ICH guideline Q2(R1) for an identity test specificity and robustness will be evaluated. / specificity, detection limit and robustness\* will be investigated for this limit impurity testing. / for this quantitative impurity test accuracy, repeatability, intermediate precision, specificity, limit of quantitation, limit of detection, linearity, range and robustness will be determined. / for an assay accuracy, repeatability, intermediate precision, specificity, linearity, range and robustness have to be investigated.

*\* if not yet already investigated during method development*

An overview about the parameters to be evaluated during this validation and the corresponding acceptance criteria to be met is provided in Table 1.

Table 1: Parameter overview and acceptance criteria

|  |  |  |
| --- | --- | --- |
| Parameter | Acceptance criteria # | Details in section ^ |
| *# these are just examples, to be adjusted to your method*  *^ The corresponding section describing the details should be referenced here.* | | |
| Accuracy | Spike recovery 100 ± 20% / xxx | 7.1 |
| Repeatability | RSD *or* CV ≤ 20% / xxx | 7.2 |
| Intermediate precision | xxx | 7.3 |
| Instrumental precision \* | xxx | 7.4 |
| *\* in case of chromatographic methods* | | |
| Specificity | No other peaks visible during RT x - y min / xxx | 7.5 |
| Detection limit | S/N ≥ 3 / xxx | 7.6 |
| Quantitation limit | S/N ≥ 10 / xxx | 7.6 |
| Linearity | R2 ≥ 0.99 / xxx | 7.7 |
| Robustness ° | RSD *or* CV ≤ 10% / xxx | 7.9 |
| *° Sample stability (if applicable) might be investigated during robustness or listed as separate point.* | | |

The performance of the method is outlined in SOP-xxx and will not be detailed in this plan / protocol, if not indicated differently in this plan / is described in the following chapter 2. [All equipment used is maintained and calibrated (see attachment 1). The staff involved in this validation is trained in the method and will be referenced in the report (see attachment 2)] \*

*\* optional*

[All attachments provided in this validation plan / protocol will be filled out during execution of the validation experiments and will be part of the report.] \* The results of these validation experiments will be summarized in a validation report; this includes the evaluation against the acceptance criteria defined in this plan / protocol. In case any deviations from the plan / protocol described here will occur, they will also be described in the report and/ corresponding actions will be initiated according to SOP-xxx *(your internal SOP dealing with deviation management)*.

*\* optional*

# Principle / Description of the method

*(e.g. for an identity test):* To proof the identity of xxx, peptide mapping is applied. Therefore, the sample is tryptically digested into specific peptides which are then separated by RP-HPLC, resulting in a sample-specific peak profile. This profile is compared with the known profile of the reference standard.

*A short overview of the method’s principle is enough in case a method describing SOP is already in place. In case no method describing SOP exists yet (and cited in chapter 1), this chapter can be used to provide a detailed description on how to perform the method.*

# Schedule, roles and responsibilities \*

*\* optional, mostly applied in case a contract manufacturing or research organization (CMO / CRO) is performing the method validation or during method transfers in case of co-validation is used*

The activities to be performed for this validation, the timeline and the corresponding responsibilities are outlined in Table 2:

Table 2: Tasks, timeline and responsibilities

|  |  |  |
| --- | --- | --- |
| **Task** | **Due date** | **Responsibility** |
| Performance of all validation experiments \* | xx.xx.2020 | Lab x |
| Compilation of the validation report including presentation and evaluation of all results, as well as justification of potential deviations | xx.xx.2020 | Lab x |
| Review of the validation report and corresponding raw data | xx.xx.2020 | Client x |
| Approval of the validation report | xx.xx.2020 | Client x and Lab x |
| *\* in case of a co-validation during a method transfer it can be detailed here which lab is performing the evaluation of which validation parameter* | | |

In case changes to the specified procedure of the validation plan / protocol are necessary to be made during the experimental phase a written consent of client x is required and an amendment to the validation plan / protocol must be provided by lab x before further validation experiments will be executed.

# Samples to be applied in this validation

Table 3 shows which test substances are planned to be used as samples in this validation:

Table 3: Overview about the test substances to be used for validation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name and description** | **Lot** | **(Protein) / xxx content** | **Buffer** | **Storage conditions** |
| Y25a  (Reference standard) | L3412 | 12 mg / mL | x M Phosphate buffer | 2-8°C |
| X23  (Intermediate product of step xxx) | Xxx  (manufactured the xx.xx.2020) | 20 mg / mL | xxx | 2-8°C |
| H48  (Drug product) | Xxx  (manufactured the xx.xx.2020) | 12 mg / mL | x M Phosphate buffer | 2-8°C |
| Placebo solution (Formulation buffer) | - | - | x M Phosphate buffer | 2-8°C |

# Calculations to be applied

The calculations which will be used within this validation are depicted in Table 4.

Table 4: Overview about the calculations applied *(some examples)*

|  |  |
| --- | --- |
| **Parameter \*** | **Formula** |
| Arithmetic mean |  |
| Standard deviation |  |
| Relative standard deviation *or* Coefficient of variation [%] |  |
| Coefficient of determination |  |
| Percent Recovery [%] |  |
| Difference [%] |  |
| *\* more formulae e.g. for correlation coefficient, RSS, confidence interval can be added if required* | |

# Abbreviations

All abbreviations used in this validation plan / protocol are explained in Table 5.

Table 5: Abbreviations *(some examples, to be adjusted individually, in alphabetical order)*

|  |  |
| --- | --- |
| **Abbreviation** | **Definition** |
| AC | Acceptance criterion |
| CV \* | Coefficient of variation |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| RT | Retention time |
| RSD \* | Relative standard deviation |
| RSS | Residual sum of squares |
| SD | Standard deviation |
| S/N | Signal to noise ratio |
| … |  |

*\* CV and RSD express the same. Choose the wording you prefer.*

# Execution of the validation and parameters to be evaluated

As outlined in chapter 1 accuracy, repeatability, intermediate precision, specificity, limit of quantitation, limit of detection, linearity, range and robustness will be assessed *(to be adjusted according to the type of your method)*.

## Accuracy (to be evaluated for quantitative impurity tests and assays)

Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

*Regarding the procedure, the ICH Q2(R1) guideline distinguishes between impurities and assays as well as between drug substance and drug product and offers several possibilities. One possibility for an impurity test is described here:*

To assess accuracy, spiking experiments will be performed. Therefore, placebo solution will be spiked with different concentrations of impurity xxx, corresponding to 80%, 100% and 120% of the target concentration *(as in this example the range should cover 80 to 120%)*. For each concentration, 3 individual replicates are prepared and analyzed.

*[In case no impurities for spiking are available, nor any alternative method, accuracy can also be inferred from linearity data. This is described in these* [*two*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/normalization) *blog* [*articles*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/recovery)*.]*

*One example for an assay is shown below:*

To assess accuracy, the data generated in the linearity experiments (see chapter 7.7) will be evaluated. Therefore, the xxx *e.g. protein* content of the 80%-, 100%- and 120%-solution *(as in this example the range should cover 80 to 120%)* will be calculated applying the regression equation obtained in the linearity study.

For each replicate its theoretical value, the measured / back-calculated value and the percent recovery has to be reported. Additionally, the percent recovery for each concentration level will be calculated. [The data will be recorded using the datasheet provided in attachment 3. \*]

*\* optional, as often lab data are recorded in laboratory notebooks or LIMS and calculations are performed using Excel, Minitab, etc. …*

Acceptance criterion:

A percent recovery of x to y% \* has to be met.

*\* no values can be provided here as this belongs to your method conditions, the experiences you have made during method development and your drug substance / drug product specification*

## Repeatability / Intra-assay precision (to be evaluated for quantitative impurity tests and assays)

Repeatability is one part of precision and by precision, the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions is expressed. Repeatability covers precision under the same operating conditions over a short interval of time.

To evaluate repeatability, six individually prepared sample solutions at 100% target concentration\* will be analyzed according to the method by operator 1 *(the preparation and analysis of all 6 solutions should be done within 1 day)*. *\* Another option are 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/3 replicates each). This can be a good option when the accuracy experiment is set up like this. Then accuracy data can also be used for repeatability evaluation.* [For evaluation, the relative peak areas will be considered. #]

*# optional, can be specified in case of HPLC methods*

[Record the results *(e.g. RT, peak height, absolute and relative peak area)* on the datasheet provided in attachment 4. \*] *\* optional, see comment above* Calculate the arithmetic mean, SD and RSD *or* CV.

Acceptance criterion:

RSD *or* CV must be ≤ x%.

## Intermediate precision / Inter-assay precision (to be evaluated for quantitative impurity tests and assays)

Like repeatability, intermediate precision in another part of precision. Intermediate precision expresses within-laboratories variations: different days, different operators, different instruments, etc.

Within-laboratory variations will be evaluated by the following matrix approach (Table 6) considering all aspects mentioned above:

Table 6: Matrix approach for intermediate precision

|  |  |  |  |
| --- | --- | --- | --- |
| **Experiment** | **Operator** | **Day** | **Instrument \*)** |
| 1 | 1 | 1 | 1 |
| 2 | 2 | 1 | 2 |
| 3 | 1 | 2 | 1 |
| 4 | 2 | 2 | 2 |
| 5 | 1 | 3 | 2 |
| 6 | 2 | 3 | 1 |
| \* two different photometers / HPLC systems / etc. | | | |

For each experiment, the sample is analyzed at 100% target concentration. [For evaluation, the relative peak areas will be considered. \*] *\* optional, can be specified in case of HPLC methods*

[Record the results (e.g. RT, peak height, absolute and relative peak area) on the datasheet provided in attachment 5. \*] *\* optional, see comment above* Calculate the arithmetic mean, SD and RSD *or* CV.

Acceptance criterion:

RSD *or* CV must be ≤ x%.

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*another option:*

To evaluate intermediate precision, 6 analyses as described in section 7.2 will be performed by a second operator on a second instrument *(and e.g. different column lot)* and compared to the ones of operator 1 (covering operator-to-operator precision as well as instrument-to-instrument precision). Additionally, 6 new analyses will be performed by operator 1 on a different day and also compared to the first results (day-to-day precision).

[Record the results *(e.g. RT, peak height, absolute and relative peak area)* on the datasheet provided in attachment 5. \*] *\* optional, see comment above* Calculate for each type of intermediate precision the arithmetic mean, SD and RSD *or* CV. Additionally, the overall precision is determined. Therefore, arithmetic mean, SD and RSD *or* CV of all 18 analyses will be calculated. Furthermore, a confidence interval considering all 18 analyses is determined. \*

*\* Although required by the ICH Q2(R1) for every single kind of precision, this only makes sense for overall precision. Details can be found* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/confidence-interval-method-validation)*.*

Acceptance criteria:

RSD *or* CV of both types of intermediate precision as well as of overall precision must be ≤ x%.

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## Instrumental precision (for chromatographic methods)

Instrument(al) precision, sometimes also termed injection repeatability / injection reproducibility is not required by the ICH Q2(R1) guideline but is part of the “Validation of chromatographic methods” reviewer guidance document, already issued by the FDA in 1994 but still in force. It is an important parameter for system suitability testing.

To assess instrumental precision, the reference standard solution at 100% target concentration is injected x times. *(x may be e.g. 5, 6 or 10-times depending on the regulatory document to be respected. In most cases, 6 injections are performed according to USP <621>, while 10 are recommended by the FDA’s reviewer guidance mentioned before)*.

[Record the results *(e.g. RT, peak height, absolute and relative peak area)* on the datasheet provided in attachment 6. \*] *\* optional, see comment above* Calculate the arithmetic mean, SD and RSD *or* CV.

Acceptance criterion:

RSD *or* CV must be ≤ x%. *(1 – 2% depending on the corresponding regulatory requirement, some details can be found* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/sst)*)*

## Specificity (to be evaluated for all kinds of methods)

Specificity is defined as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, this might include the drug itself, impurities, degradants, buffer components etc.

*One exemplary procedure for an identity test:*

For the demonstration of specificity, the sample, a solution of substance xxx (closely related to the sample), a placebo solution and the reference standard are analyzed individually according to the method and the electropherograms / chromatograms / western blots will be compared.

Acceptance criteria:

* The electropherograms / chromatograms / western blots of the placebo solution and the substance xxx solution don’t show any interfering peaks / bands in the corresponding pI / RT / molecular weight range.
* The electropherogram / chromatogram of the sample is identical to the reference standard with a pI of x ± y / a RT of the main peak of x ± y min. / The molecular weight of the sample has to be between x and y kDa and the band must be visible at the same height as the one of the reference standard.

*One exemplary procedure for an assay (with impurities available):*

Specificity will be shown by spiking experiments. Therefore, one analysis will be performed with the drug product sample at 100% target concentration without any additional impurities (= normal condition). Further analyses will be done by spiking the drug product sample at 100% target concentration with x, y and z mg/mL of impurity xxx (corresponding to x, y, z% *- increasing concentrations*). 3 replicates will be analyzed for each condition.

[Record the results on the datasheet provided in attachment 7. \*] *\* optional, see comment above* Calculate the arithmetic mean, SD and RSD *or* CV for each condition. Finally, the “spiked” results are compared to the normal unspiked condition by calculation of the percent difference.

Acceptance criterion:

The spiked assay results must be unaffected by the presence of the spike. A percent difference of ± x% is acceptable.

*One exemplary procedure for an impurity test (with no impurities available):*

To evaluate specificity, a sample will be analyzed at 100% target concentration according to the method in triplicates. Additionally, a second sample from the same batch will be analyzed according to the independent, validated method xxx (reference method; *must be a well-characterized procedure, could also be a compendial method*). Furthermore, samples stored under appropriate stress conditions (light, heat, humidity, acid/base hydrolysis, oxidation, freeze/thaw cycles) will also be analyzed by both kinds of methods. The selected stress conditions known to generate degradation products will be detailed in the validation report.

[Record the results on the datasheet provided in attachment 7. \*] *\* optional, see comment above* Calculate the arithmetic mean, SD and RSD *or* CV for both methods. Finally, the results of this method are compared to the ones obtained by the reference method by calculation of the percent difference.

Acceptance criteria:

* The results obtained by the method under validation must be comparable to the ones of the reference method. A percent difference of ± x% is acceptable.
* For the selected stress conditions, differences such as e.g. more peaks or a changed composition in the chromatogram must be visible / an increase of impurity xxx should be visible when compared to the unstressed condition.

## Limit of detection and limit of quantitation (LOD & LOQ for quantitative impurity tests; for impurity limit tests only LOQ is required)

The detection limit is defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value whereas the lowest amount of analyte to be quantitatively determined with suitable precision and accuracy is defined as quantitation limit.

*The ICH Q2(R1) guidelines offers several approaches depending on the kind of method. Details can be found* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/limit-of-detection-quantifcation)*.*

*Procedure for LOQ; HPLC method as example:*

For the determination of the quantitation limit, the signal to noise ratio will be used. Based on the results during method development, six injections of x µg/mL, corresponding to 0.x% of the nominal concentration *(a very low concentration suspected / known to be the LOQ based on method development or pre-validation experiments with serial dilution)* of individually prepared sample solutions will be analyzed.

[Record the results *(e.g. peak height, base line, S/N, absolute peak area)* on the datasheet provided in attachment 8. \*] *\* optional, see comment above* Calculate for the absolute peak area the arithmetic mean, SD and RSD *or* CV and determine the recovery relating the mean of the x µg/mL concentration (0.x % level) to the 100% nominal value (data for the 100% nominal value will be taken from linearity experiments).

Acceptance criteria:

* The signal to noise ratio must be ≥ 10.
* The RSD *or* CV for the absolute peak area must be ≤ x% (evaluation of precision at LOQ).
* The percent recovery of x to y% has to be met (evaluation of accuracy at LOQ). \*

*\* Details regarding the calculation can be found* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/loq-accuracy-precision)*.*

*Procedure for LOD; ELISA as example:*

The assessment of the limit of detection is based on the standard deviation of the response and the slope. For the standard deviation of the response, blank is used. Serial dilutions of the standard are prepared according to the method and applied in triplicates. Six blanks are applied.

[Record all absorbance values on the datasheet provided in attachment 9. \*] *\* optional, see comment above* Calculate for each concentration of the standard the arithmetic mean, perform regression analysis and calculate the slope of the regression line. For the blank, calculate the arithmetic mean of the six values.

Acceptance criterion:

The LOD is calculated using the following formula:



(Where σ = the standard deviation of the blank; S = the slope of the calibration curve).

## Linearity (to be evaluated for quantitative impurity tests and assays)

Linearity of an analytical procedure is defined as its ability to obtain test results which are directly proportional to the concentration / amount of analyte in the sample (within a given range).

To assess linearity, 5 concentrations will be prepared for evaluation. Therefore, the reference standard containing a known amount of impurity xxx *(for quantitative impurity methods)* / with a known xxx *e.g. protein* content *(for assays)* will be diluted to x1%, x2%, x3%, x4% and x5% of the target concentration, corresponding to x – y µg/mL *(this can be e.g. 80 – 120% depending on the range to be analysed)*. For each concentration, 3 individually prepared solutions will be analysed.

*This following section only applies to quantitative impurity tests:* In addition, linearity will also be evaluated in the range of LOQ. Therefore, 5 concentrations starting from LOQ to y% (LOQ, y1%, y2%, y3%, y4%) will analysed using individually prepared solutions in triplicates.

For evaluation the response is recorded [on the datasheet provided in attachment 10 - *optional, see comment above*] and set into relation to its concentration. Calculate for each concentration the arithmetic mean of the response. A plot of absolute peak area / absorbance / activity / xxx (y-axis) against concentration (x-axis) will be analysed by linear regression applying the method of the least squares. *(In some cases, mathematical transformation is required prior to regression analysis, Details can be found* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/linear-versus-non-linear-regression)*.)* In addition to a plot, regression equation, correlation coefficient, y-intercept, slope of the regression line and residual sum of squares will be provided in the report. (*The ICH Q2(R1) furthermore suggests that an evaluation of the data of the regression line itself or more specific an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating the degree of linearity. The influence of the single data points can be analysed using Hat values and Cook’s distance as described* [*here*](http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/sum-of-quares-part2)*.*)

Acceptance criteria:

* The regression line spanning the range from x1% to x5% must show a correlation coefficient R (*in other cases also the coefficient of determination R2 is used*) of *e.g.* ≥ 0.98 *(can also be ≥ 0.95, ≥ 0.97 or ≥ 0.99, depending on your type of method and analyte(s))*.
* The regression line covering the range from LOQ to y4% must show a correlation coefficient R *(in other cases also the coefficient of determination R2 is used)* of *e.g.* ≥ 0.95 *(same comment as above but it is usually lower compared to the working range)*.

## Range (to be evaluated for quantitative impurity tests and assays)

The range of an analytical procedure is defined as the interval between the upper and lower concentration / amount of analyte in the sample for which a suitable level of precision, accuracy and linearity has been demonstrated. It will be inferred from the corresponding experiments carried out for linearity, precision and accuracy, considering the acceptance criteria for these parameters.

## Robustness (to be evaluated for all kinds of methods in case not yet performed during method development)

The robustness is defined as a measure of a method’s capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

[According to a previously performed risk assessment (document no. xxx – *please cite your internal risk assessment document or the number thereof here*) / according to the following risk evaluation (*please add e.g. a small table (with e.g. risk factor, potential consequences, evaluation, activity for method validation if applicable) showing the risk evaluation of your method*), critical performance parameters of the method will be investigated during the robustness studies detailed hereafter.] \*

*\* optional*

*HPLC method as an example:*

For the assessment of robustness, the following method parameters will be modified:

* pH of mobile phase x: *e.g.* 5.8 and 6.2 *(e.g. original: 6.0)*
* Composition of mobile phase x: *e.g.* 0.25 M and 0.75 M *(e.g. original: 0.5 M)*
* Column temperature: *e.g.* 25 and 35°C *(e.g. original: 30°C)*
* Flow rate: *e.g.* 0.8 and 1.2 mL/min *(e.g. original: 1.0 mL/min)*
* Detection wavelength: ± 2 nm
* Sample stability: Analysis of the sample after x *(e.g. 48)* h of storage in the autosampler / 3 days at room temperature / 3 days at -20°C /… \*

*\* Sample stability might be part of the robustness study as shown here, but might also be listed as a separate point*

* …

Different column lots / suppliers will not be evaluated for robustness as already included in intermediate precision.

All evaluations will be performed three times using reference standard and/or sample xxx at 100% target concentration. The results obtained when applying the modified conditions will be compared to three runs performed under original method conditions (at time 0).

[Record the results *(e.g. RT, peak height, absolute and relative peak area)* on the datasheet provided in attachment 11 *or* attachment 12. \*] *\* optional, see comment above* Determine the mean content of xxx and calculate the percent difference between each variation and the original *or* calculate SD and RSD / CV.

Acceptance criteria:

* The percent difference of the result obtained of each variation and the original condition is not more than ± x%.

*or:*

* The acceptance criteria for intermediate precision must be met, i.e. RSD *or* CV must be ≤ x%.

*or – in case of an identity test:*

* The RT / pI / molecular weight range of the reference standard and/or sample under modified condition must meet the actual batch release specification i.e. show a RT / pI / molecular weight of not more than x ± y\* min / kDa *\*This value (y) is typically 2 or 3SD of the reference standard.*

In case an acceptance criterion will not be met by one (or more) modified conditions, a remark to pay special attention to that point will be incorporated into the method’s description.

## System suitability (for all kinds of methods)

The tentative system suitability parameters defined in the method (see chapter 2 *in case no SOP exists yet and the whole method is described in that chapter*) will verified after method validation. Therefore, all analyses performed with reference standard during this validation will be evaluated accordingly and final acceptance criteria will be set.

(Remark: For chromatographic methods, this includes of course chapter 7.4 and the corresponding regulatory requirements must be considered.)

# Attachments

**Attachment 1: Overview of equipment used in this validation**

|  |  |  |
| --- | --- | --- |
| **Equipment Name** | **Serial # or internal equipment #** | **Date of last calibration** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

**Attachment 2: Overview of personnel involved in this validation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of the operator** | **Involved in validation experiment(s)** | **Date of last training** | **Training reference document #** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Attachment 3: Accuracy data**

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Level [%]** | **Theoretical conc. [xxx]** | **Response [xxx] *e.g. Absorbance [-]*** | **Measured / back-calculated conc. [xxx]** | **Recovery [%]** | **Mean recovery [%]** | **AC** | **Evalua-tion** |
| 80 |  |  |  |  |  | x% ≤ Recovery ≥ y% | passed |
|  |  |  |  |  |
|  |  |  |  |  |
| 100 |  |  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| 120 |  |  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**Attachment 4: Repeatability data**

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Repli-cate** | **Response [xxx] *e.g. Absorbance [-] or relative peak area [Area%]*** | **Calculated conc. [xxx] \*** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** | **AC** | **Evalua-tion** |
| 1 |  |  |  |  |  | RSD ≤ x% | passed |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  |

*\* optional, might not be necessary in every case*

**Attachment 5: Intermediate precision data**

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Experi-ment** | **Response [xxx] *e.g. Absorbance [-] or relative peak area [Area%]*** | **Calculated conc. [xxx] \*** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** | **AC** | **Evalua-tion** |
| 1 |  |  |  |  |  | RSD ≤ x% | passed |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  |

*\* optional, might not be necessary in every case*

*another option:*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Repli-cate** | **Response [xxx] *e.g. Absorbance [-] or relative peak area [Area%]*** | **Calculated conc. [xxx] #** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |  | |
| **Operator 1, \***  **instrument 1** | 1 |  |  |  |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  |
| **Operator 2,**  **instrument 2** | 1 |  |  |  |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  | **AC** | **Evaluation** |
|  | Intermediate precision (operator-to-operator + instrument-to-instrument); n = 12 | | |  |  |  | RSD ≤ x% | passed |

*\* here the data of repeatability should be presented once more # optional, might not be necessary in every case*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Repli-cate** | **Response [xxx] *e.g. Absorbance [-] or relative peak area [Area%]*** | **Calculated conc. [xxx] #** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |  | |
| **Operator 1, \***  **day 1** | 1 |  |  |  |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  |
| **Operator 1,**  **day 2** | 1 |  |  |  |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  | **AC** | **Evaluation** |
|  | Intermediate precision (day-to-day); n = 12 | | |  |  |  | RSD ≤ x% | passed |

*\* here the data of repeatability should be presented once more # optional, might not be necessary in every case*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Repli-cate** | **Response [xxx] *e.g. Absorbance [-] or relative peak area [Area%]*** | | **Calculated conc. [xxx] #** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |  | |
| **Operator 1, \***  **day 1** | 1 |  | |  |  |  |  |
| 2 |  | |  |
| 3 |  | |  |
| 4 |  | |  |
| 5 |  | |  |
| 6 |  | |  |
| **Operator 2,**  **instrument 2** |  |  | |  |  |  |  |
|  |  | |  |
|  |  | |  |
|  |  | |  |
|  |  | |  |
|  |  | |  |
| **Operator 1,**  **day 2** | 1 |  | |  |  |  |  |
| 2 |  | |  |
| 3 |  | |  |
| 4 |  | |  |
| 5 |  | |  |
| 6 |  | |  | **AC** | **Evaluation** |
|  | Overall precision; n = 18 | | | |  |  |  | RSD ≤ x% | passed |
|  | Confidence interval | |  | |  | | | | |

*\* here the data of repeatability should be presented once more # optional, might not be necessary in every case*

**Attachment 6: Instrumental precision**

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Injection** | **Response [xxx]**  ***e.g. relative peak area [Area%]*** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** | **AC** | **Evaluation** |
| 1 |  |  |  |  | RSD ≤ x% | passed |
| 2 |  |
| 3 |  |
| 4 |  |
| 5 |  |
| *6 \** |  |
| *7 \** |  |
| *8 \** |  |
| *9 \** |  |
| *10 \** |  |

*\* to be deleted depending on the corresponding regulatory requirements*

**Attachment 7: Specificity**

*One exemplary procedure for an assay (with impurities available):*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experi-ment** | **Response [xxx] *e.g. relative peak area [Area%]*** | **Calculated conc. [xxx] \*** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |  | | |
| Normal condition |  |  |  |  |  |
|  |  |
|  |  | **Diff [%]** | **AC** | **Evaluation** |
| Spike x% |  |  |  |  |  |  | Diff ± x% | passed |
|  |  |
|  |  |
| Spike y% |  |  |  |  |  |  |  |
|  |  |
|  |  |
| Spike z% |  |  |  |  |  |  |  |
|  |  |
|  |  |

*\* optional, might not be necessary in every case*

*One exemplary procedure for an impurity test (with no impurities available):*

*[Remark: The data record for the analysis of stressed samples is not shown here.]*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experi-ment** | **Response [xxx] *e.g. relative peak area [Area%]*** | **Calculated conc. [xxx] \*** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |  | | |
| Normal method |  |  |  |  |  |
|  |  |
|  |  | **Diff [%]** | **AC** | **Evaluation** |
| Reference method |  |  |  |  |  |  | Diff ± x% | passed |
|  |  |
|  |  |

*\* optional, might not be necessary in every case*

**Attachment 8: Limit of quantitation**

*One exemplary procedure for an HPLC method:*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Theor. conc. [xxx]** | **Injec-tion** | **Peak height**  **[xxx]** | **Baseline**  **[xxx]** | **S/N** | **AC** | **Evaluation** |
| x µg/mL | 1 |  |  |  | S/N ≥ 10 | passed |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Level [%]** | **Injec-tion** | **Absolute peak area** | | | | **AC** | **Evaluation** |
| **Singe result [xxx]** | **Mean [xxx]** | **SD [xxx]** | **RSD [%]** |
| 0.x | 1 |  |  |  |  | RSD ≤ x% | passed |
| 2 |  |
| 3 |  |
| 4 |  |
| 5 |  |
| 6 |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Level [%]** | **Injec-tion** | **Absolute peak area [xxx]** | **Mean [xxx]** |  | | | |
| 100 \* | 1 |  |  |
| 2 |  |
| 3 |  | **Calculated level [%]** | **Reco-very [%]** | **AC** | **Evaluation** |
| 0.x | 1 |  |  |  |  | x% ≤ Recovery ≥ y% | passed |
| 2 |  |
| 3 |  |
| 4 |  |
| 5 |  |
| 6 |  |

*\* 100% level values were taken from the corresponding linearity experiments (see chapter / table xxx)*

**Attachment 9: Limit of detection**

*One exemplary procedure for an ELISA:*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |
| --- | --- | --- | --- |
| **Conc. [xxx]** | **Response [xxx] *here: Absorbance [-]*** | **Mean Response [xxx] *here: Absorbance [-]*** | **SD σ [xxx]**  ***here: Absorbance [-]*** |
| Blank |  |  |  |
|  |
|  |
|  |
|  |
|  | **Slope S [xxx]** | **LOD [xxx]** |
| v |  |  |  |  |
|  |
|  |
| w |  |  |
|  |
|  |
| x |  |  |
|  |
|  |
| y |  |  |
|  |
|  |
| z |  |  |
|  |
|  |

**Attachment 10: Linearity data**

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Level [%]** | **Conc. [xxx, *e.g. µg/mL*]** | **Response [xxx] *e.g. Absorbance [-]*** | **Mean Response [xxx] *here: Absorbance [-]*** | **Regression equation,**  **y-intercept, slope, RSS** | **Correlation coefficient R** | **AC** | **Evalua-tion** |
| LOQ\* | a |  |  |  |  | R of ≥ 0.9x *(e.g. 0.95)* | passed |
|  |
|  |
| y1\* | b |  |  |
|  |
|  |
| y2\* | c |  |  |
|  |
|  |
| y3\* | d |  |  |
|  |
|  |
| y4\* | e |  |  |
|  |
|  |
| 80 (x1) | v |  |  |  |  | R of ≥ 0.9x *(e.g. 0.98)* | passed |
|  |
|  |
| 90 (x2) | w |  |  |
|  |
|  |
| 100 (x3) | x |  |  |
|  |
|  |
| 110 (x4) | y |  |  |
|  |
|  |
| 120 (x5) | z |  |  |
|  |
|  |

*\* the first part of this table (LOQ to y4)* *only applies to quantitative impurity tests and can thus be deleted for others*

**Attachment 11: Robustness data *(example 1)***

*One exemplary procedure for an HPLC method:*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Response [xxx] *e.g. relative peak area [Area%]*** | **Calculated conc. [xxx]** | **Mean [xxx]** |  | | |
| Normal method condition |  |  |  |
|  |  |
|  |  | **Diff [%]** | **AC** | **Evaluation** |
| Mobile phase pH 5.8 |  |  |  |  | Diff ≤ ± x% | passed |
|  |  |
|  |  |
| Mobile phase pH 6.2 |  |  |  |  | passed |
|  |  |
|  |  |
| Mobile phase 0.25 M |  |  |  |  | passed |
|  |  |
|  |  |
| Mobile phase 0.75 M |  |  |  |  | failed |
|  |  |
|  |  |
| Column temp. 25°C |  |  |  |  | passed |
|  |  |
|  |  |
| Column temp. 35°C |  |  |  |  | passed |
|  |  |
|  |  |
| Flow rate 0.8 mL/min |  |  |  |  | passed |
|  |  |
|  |  |
| … |  |  |  |  | passed |
|  |  |
|  |  |

**Attachment 12: Robustness data *(example 2)***

*One exemplary procedure for an HPLC method:*

Lab book #: \_\_\_\_\_, electronic file(s) name / #: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment** | **Response [xxx] *e.g. relative peak area [Area%]*** | **Calculated conc. [xxx]** | **Mean [xxx]** |  | | | |
| Normal method condition |  |  |  |
|  |  |
|  |  | **SD [xxx]** | **RSD [%]** | **AC** | **Evaluation** |
| Mobile phase pH 5.8 |  |  |  |  |  | RSD ≤ ± x% | passed |
|  |  |  |
|  |  |  |
| Mobile phase pH 6.2 |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |
| Mobile phase 0.25 M |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |
| Mobile phase 0.75 M |  |  |  |  |  | failed |
|  |  |  |
|  |  |  |
| Column temp. 25°C |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |
| Column temp. 35°C |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |
| Flow rate 0.8 mL/min |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |
| … |  |  |  |  |  | passed |
|  |  |  |
|  |  |  |