BIOANALYTICAL METHOD VALIDATION – M10

Step 2 document – to be released for comments

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

Legal Notice

Background

- This document has been signed off as *Step 2* document (26 Feb. 2019) to be issued by the ICH Regulatory Members for public consultation
- This document was developed based on a Concept Paper (7 Oct. 2016) and a Business Plan (7 Oct. 2016)
- Anticipating finalization as *Step 4* document to be implemented in the local regional regulatory system: Nov. 2020.

Key Principles

- This proposed new multidisciplinary guideline will address the validation of bioanalytical assays.
- This guideline will provide recommendations on how to validate a bioanalytical assay for drug quantification and how to apply validation during study sample analysis.
- This guideline does not provide recommendations which analyte should be analysed. This is covered in other ICH and regional regulatory documents.

Key Principles (continued)

- The validation principles are applicable for drug measurements in pivotal nonclinical toxicokinetic (TK)/pharmacokinetic (PK) studies and clinical trials (including comparative bioavailability/bioequivalence (BA/BE) studies) used to make regulatory decisions.
- This guideline applies to the quantitative analysis by chromatographic methods and by ligand binding assays (LBA).
- Bioanalysis of biomarkers and bioanalytical methods for evaluation of immunogenicity are not within the scope of this guideline.

Guideline Objectives

- The objective of validation of bioanalytical assays is to demonstrate that the method is sensitive, accurate and precise and that the method is suitable for its intended purpose.
- Adherence to the validation principles will improve the quality and consistency of bioanalytical data.
- This guideline will result in the harmonisation of current regional guidelines/guidance and related documents, reduce the need for additional validation experiments and support streamlined global drug development.

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Summary of Guideline Content

Chromatography

- The Reference standard should be obtained from an authentic and traceable source and a Certificate of Analysis (CoA) or equivalent alternative is required.
- A suitable Internal Standard (IS) should be added, however a CoA is not required, but its suitability should be demonstrated.
- In case of Mass Spectrometry (MS) detection, use of a stable isotope-labelled IS is recommended.

Summary of Guideline Content

Chromatography (continued)

- **Validation**
  - Selectivity should normally be demonstrated in blank samples from 6 individual sources. Interference response should be ≤20% of the analyte response at the lower limit of quantification (LLOQ) and ≤ 5% of IS response.
  - For specificity, the response of possible interfering substances should be ≤20% of the analyte response at LLOQ and ≤ 5% of IS response
  - The matrix effect should be evaluated using at least 3 replicates of low and high Quality Control samples (QCs) from at least 6 different sources. The accuracy within ±15% and precision within ±15% in all individual sources.

Summary of Guideline Content

Chromatography (continued)

- **Validation**
  - The calibration curve consists of a blank sample, a zero sample and at least 6 calibration standards, including the LLOQ and the upper limit of quantification (ULOQ).
    - Accuracy of calibration standards should be evaluated over at least 3 runs. Accuracy should be within ±20% at LLOQ and within ±15% at the other standard levels, for at least 75% (with a minimum of 6) of calibration standards.

Summary of Guideline Content

Chromatography (continued)

- **Validation**
  - For accuracy and precision evaluation, QCs should be used at 4 concentration levels, i.e. at the LLOQ, within 3 times the LLOQ (i.e. Low QC (LQC)) around 30 – 50% of the calibration curve range (i.e. Medium QC (MQC)) and at least at 75% of the ULOQ (i.e. High QC (HQC)).
    - Within-run accuracy and precision should be demonstrated for at least 5 replicates per QC level and
    - Between-run accuracy and precision for at least 3 runs.

Accuracy and precision should be within ±20% respectively at the LLOQ and within ±15% respectively at the other QC levels.

Summary of Guideline Content

Chromatography (continued)

- **Validation**
  - Carry-over should be evaluated in a blank sample which is injected after the ULOQ standard. The response should be ≤20% of the response of analyte response at LLOQ and ≤ 5% of IS response.
  - For dilution integrity, a QC level above the ULOQ should be diluted with blank matrix, at least covering the dilution ratio applied to study samples. Using at least 5 replicates per dilution factor, the mean accuracy should be within ±15% and the precision within ±15%.

Summary of Guideline Content

Chromatography (continued)

- **Validation**
  - Stability should cover the processing and storage conditions of the study samples. At least 3 low and 3 high concentration QCs should be analysed at time zero and after the applied storage condition. The mean accuracy should be within ±15% of the nominal concentration at each QC level.
    - If multiple analytes are present in study samples (e.g., due to a fixed combination product or a specific drug regimen) then stability should be tested including all analytes in the matrix.
    - Stability tests should be evaluated for stock and working solutions, freeze-thawing the matrix, bench top stability, processed sample stability and long term stability.

Summary of Guideline Content

Chromatography (continued)

- **Study sample analysis**
  - An analytical run consists of a blank sample, a zero sample, at least 6 calibration standard concentration levels, at least 3 QCs in duplicate (or 5% of the number of samples) and the study samples.
    - The QCs should be divided over the run and study samples should be bracketed by the QCs.
  - Acceptance criteria for an analytical run:
    - Accuracy of the calibration standards should be within ±20% at LLOQ and within ±15% at the other standard levels, for at least 75% (with a minimum of 6) of calibration standards.

Summary of Guideline Content

Chromatography (continued)

- **Study sample analysis**
  - Acceptance criteria for an analytical run (continued):
    - Per run, accuracy of at least 2/3 of total QCs and at least 50% at each concentration level should be within ±15%. The overall accuracy and precision should be within ±15%.
  - Calibration range
    - The calibration curve range should cover the expected concentrations of the study samples. The range and/or QCs should be adapted, if needed.
    - At least 2 QC levels should fall within the range of concentrations measured in study samples.

Summary of Guideline Content

Chromatography (continued)

- **Study sample analysis**
  - Reanalysis of study samples:
    - Reasons, number of replicates and criteria should be predefined in a protocol.
    - For comparative BA/BE studies, reanalysis of study samples for a PK reason is not acceptable.
  - Reinjection of study samples
    - Reinjection of calibration standards or QCs simply because they failed is not acceptable.
  - Integration of chromatograms
    - Reintegrated chromatograms should be reported.

Summary of Guideline Content

Ligand Binding assays

- The Reference standard should be well characterised and documented. It is recommended to use the same batch during study and analysis.
- Critical reagents should be identified and defined in the assay method. Reliable procurement should be considered early in method development. Changes may need additional validation experiments.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Validation**
  - For specificity, the response of possible interfering substances should be below the LLOQ. The accuracy should be within ±25% of the analyte response.
  - Selectivity should normally be demonstrated in blank samples from 10 individual sources. Interference response should be below the LLOQ in 80% of the blank individual sources. Accuracy should be within ±25% at LLOQ and within ±20% at high QC level.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Validation**
  - The calibration curve consists of a blank sample and at least 6 calibration standards, including the LLOQ and ULOQ. Anchor point samples may be used to improve curve fitting.
    - Accuracy of calibration standards should be evaluated over at least 6 runs. Accuracy should be within ±25% at LLOQ and ULOQ and within ±20% at the other standard levels, for at least 75% (with a minimum of 6) of calibration standards.

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Summary of Guideline Content

Ligand Binding assays (continued)

- **Validation**
  - For accuracy and precision evaluation, QCs should be used at 5 concentration levels, i.e. at the LLOQ, within 3 times the LLOQ (LQC), around the geometric mean of the calibration curve range (MQC), at least at 75% of the ULOQ (HQC) and at the ULOQ.
    - Within-run accuracy and precision should be demonstrated for at least 3 replicates per QC level and between-run accuracy and precision for at least 6 runs. Accuracy and precision should be within±25% at the LLOQ and the ULOQ and within±20% at the other QC levels. Total error should be ≤40% at LLOQ and ULOQ and 30% at the other QC levels.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Validation**
  - For dilution linearity, a QC level above the ULOQ should be diluted with blank matrix, at least covering the dilution factor applied to study samples. Undiluted and diluted sample should be analysed for presence of hook effect.

    Using at least 3 runs, for each dilution, the mean accuracy and precision should be within ±20%.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Validation**
  - Stability should cover the processing and storage conditions of the study samples. At least 3 low and 3 high concentration QCs should be analysed at time zero and after the applied storage condition. The mean accuracy should be within± 20% of the nominal concentration at each QC level.
    - Stability tests should be evaluated for freeze-thawing the matrix, bench top stability and long term stability.
    - It is acceptable to apply a bracketing approach, e.g., demonstrated stability at -70˚C and at -20˚C, also applies to in between temperatures.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Study sample analysis**
  - An analytical run consists of a blank sample, at least 6 calibration standard concentration levels, at least 3 QCs in duplicate (or 5% of the number of samples) and the study samples.
    - The QCs should be divided over the microtiter plates and run and study samples should be bracketed by the QCs.
  - Acceptance criteria for an analytical run:
    - Accuracy of the calibration standards should be within ±25% at LLOQ and ULOQ and within ±20% at the other standard levels, for at least 75% (with a minimum of 6) of calibration standards.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Study sample analysis**
  - Acceptance criteria for an analytical run (continued):
    - Accuracy of at least 2/3 of total QCs and at least 50% at each concentration level should be within ±20%.
      The overall accuracy and precision should be within ±20%.
  - Calibration range
    - The calibration curve range should cover the expected concentrations of the study samples. The range and/or QCs should be adapted, if needed.
    - At least 2 QC levels should fall within the range of concentrations measured in study samples.

Summary of Guideline Content

Ligand Binding assays (continued)

- **Study sample analysis**
  - Reanalysis of study samples:
    - Reasons, number of replicates and criteria should be predefined in a protocol.
    - For comparative BA/BE studies, reanalysis of study samples for a PK reason is not acceptable.

Summary of Guideline Content

- **Incurred sample reanalysis (ISR)**
  - Should be performed in case of:
    - main nonclinical TK studies once per species.
    - all pivotal comparative BA/BE studies
    - first clinical trial in subjects
    - pivotal early patient trial(s), once per patient population
    - first or pivotal trial in patients with impaired hepatic and/or renal function
  - Number of samples for ISR: 10% of the first 1000 study samples, and additional 5% of the number of study samples above 1000.

Summary of Guideline Content

- **Incurred sample reanalysis (continued)**
  - Calculation of ISR:

$$\% \text{ difference} = \frac{\text{repeat value} - \text{initial value}}{\text{mean value}} \times 100$$

  - Criteria:
    - chromatographic methods: the percent difference should be ≤ 20% for at least 2/3 of the repeats.
    - for LBAs: the percent difference should be ≤ 30% for at least 2/3 of the repeats.

Summary of Guideline Content

- **Partial and Cross Validation:**
  - Partial validation can range from as little as one within-run accuracy and precision determination, to a nearly full validation.
  - Cross validation is required in case of:
    - Data are obtained from different fully validated methods within a study.
    - Data are obtained from different fully validated methods across studies that are going to be combined or compared.
    - Data are obtained within a study from different laboratories with the same bioanalytical method.

Summary of Guideline Content

- **Additional considerations:**
  - Endogenous compounds: In case of matrices without interference are not available, 4 approaches are suggested:
    - Standard addition approach
    - Background subtraction approach
    - Surrogate matrix approach
    - Surrogate analyte approach
  - Parallelism of LBA should be evaluated on a case-by-case basis, e.g., where interference caused by a matrix component is suspected during study sample analysis.

Summary of Guideline Content

- **Additional considerations (continued):**
  - Recovery experiments are recommended to be performed.
  - If applicable, the minimum required dilution should be determined during method development
  - Commercial and diagnostic kits should be validated for its intended use confirming to the drug development standards described in this guideline.
  - The use of new technology in regulated bioanalysis should be supported by acceptance criteria established a priori based on method development and verified in validation.

Considerations

- General and specific SOPs and good record keeping are essential to a properly validated analytical method. The data generated for bioanalytical method validation should be documented and available for data audit and inspection.
- All relevant documentation necessary for reconstructing the study as it was conducted and reported should be maintained in a secure environment.
- Summary information should be included in Section 2.6.4/2.7.1 of the CTD.

Conclusions

- **This harmonised guidance on the validation of bioanalytical methods and analysis of study samples, reduces the need for carrying out additional bioanalytical experiments. This may accelerate development and drug approval and may lower the costs.**

Contact

- **For any questions please contact the ICH Secretariat:**

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