ICH M10:

JBF Workshop Report

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14th JBF Symposium

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JBF ICH M10 Workshop

- **Date:** 20-21 October 2022
- **Place:** Miyako Messe convention center, Kyoto
- **Participants:** 71 people, total
  - 49 peoples from 32 Industries and CROs
  - 22 JBF steering committee members

Day 1	
12:00~	Entry
12:30~13:30	**Opening & Chrom/LBA**
13:30~13:40	Break
13:40~14:30	**Chrom/LBA**
14:30~14:40	Break
14:40~15:30	**Chrom/LBA**
15:30~15:40	Break
15:40~16:30	**Chrom/LBA**
16:30~16:40	Break
16:40~18:00	**Chrom/LBA**

Day 2	
8:40~	Entry
9:00~10:00	**Other chapters**
10:00~10:10	Break
10:10~11:00	**Other chapters**
11:00~11:10	Break
11:10~12:00	**Other chapters & Closing remarks**

Discussion points

- **Survey results:**

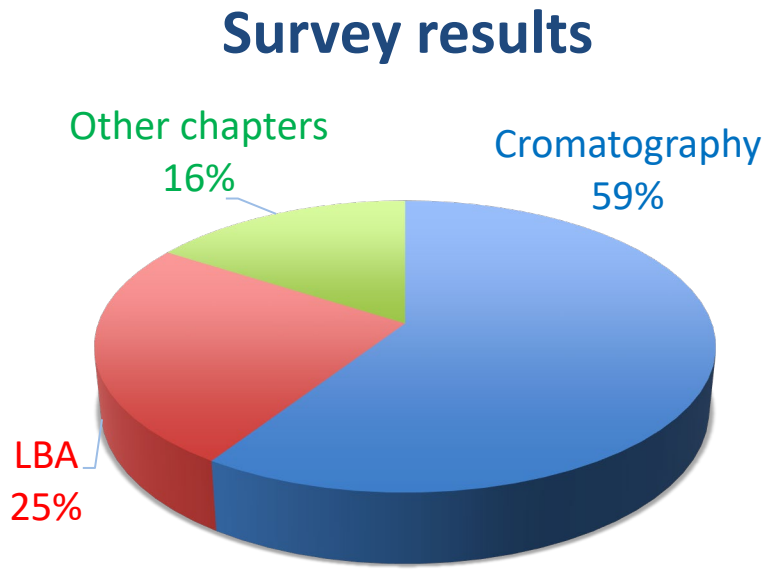
  478 discussion points/questions were collected. (283 for Chromatography, 119 for LBA, 76 for other chapters)

  JBF SC categorized and prioritized issues (High, Middle, Low), and the issues of high importance and attention were discussed at the Workshop.

  - **Chromatography** **High: 10**, Middle: 11, Low: >50
  - **LBA** **High: 18**, Middle: 16, Low: 21
  - **Other chapters** **High: 6**, Middle: 13, Low: 13

ICH M10 Guideline

1. INTRODUCTION
2. GENERAL PRINCIPLES
3. CHROMATOGRAPHY
4. LIGAND BINDING ASSAYS
5. INCURRED SAMPLE REANALYSIS (ISR)
6. PARTIAL AND CROSS VALIDATION
7. ADDITIONAL CONSIDERATIONS
8. DOCUMENTATION



1. INTRODUCTION

1.3 Scope

The guideline is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of GLP, **nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies**, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions.

*Which **Nonclinical PK Studies** are within the scope of this guideline?*

- *Referring to FAQ, most of conventional Nonclinical PK Studies noted in CTD (including in vitro studies) are out of the scope of M10 guideline. However, we could not conclude whether the PK/PD study using animal model is the scope of M10 guideline or not.*

2.2.1 Full Validation

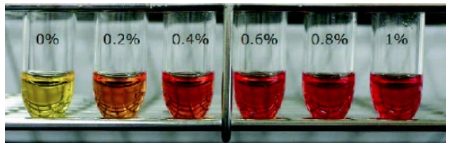
The choice of surrogate matrix should be scientifically justified. Matrix differences within species (e.g., age, ethnicity, gender) are generally not considered different when validating a method.

*How should we choose **Surrogate Matrix** scientifically?*

- *Water and buffer are often chosen because they do not contain endogenous molecules to be measured. However, it is not recommended that these matrices are selected from the beginning.*
- *Step by step confirmation is advisable from similar matrices, such as a buffer containing albumin.*
- *Recovery and parallelism are required for validating the LBA method using surrogate matrices.*
- *In addition to the routine validation items including selectivity, we should take care the effect of absorption.*

3.2.1 Selectivity

For the investigation of **selectivity in haemolysed matrices** at least one source of matrix should be used. Haemolysed matrices should be obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.



http://www.chiringi.or.jp/k_library/kaishi/kaishi2012_3/index.html

*How do we prepare **Haemolysed Matrices**?*

- *The most common method is to prepare haemolysed blood by sonication or freeze-thawing.*

Is it necessary to consider the degree of hemolysis in the study sample analysis?

- *Data handling of study samples should be judged comprehensively based on the physical properties and characteristics of the compound being measured.*

*Is the selectivity using **Haemolysed Matrices** needed for both of clinical and non-clinical?*

- *For non-clinical studies, evaluation of the effects of hemolysis is less important, but it should be considered based on the physical properties and characteristics of the compound.*

3.2.1 Selectivity

For the investigation of **selectivity in lipaemic matrices** at least one source of matrix should be used. To be scientifically meaningful, the matrix used for these tests should be representative as much as possible of the expected study samples. A naturally lipaemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipaemic matrix from donors, if this is difficult to obtain, matrix can be **spiked with triglycerides** even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidaemic, the use of spiked samples is discouraged. This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidaemic.

*How much **triglyceride** concentration is considered "high levels"?*

- *Many participants select >150 or >300 mg/dL, which is generally considered hyperlipidemic.*

If lipaemic matrices are created by spiking, is spiking lipid a single or multiple components?

- *The opinions from the participants diverged.*

3.2.2 Specificity

Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities, degradation products formed during sample preparation, or concomitant medications that are expected to be used in the treatment of patients with the intended indication).

*When is an evaluation for **Specificity** needed?*

- *Specificity should be evaluated when there is concern about impact on the analysis and the reference material is available.*
- *Evaluation for Specificity is needed for combination drugs or drugs administered concurrently in DDI studies. The concentration should be determined considering the expected concentration in the clinical study.*

3.2.2 Specificity

The possibility of back-conversion of a metabolite into the parent analyte during the successive steps of the analysis (including extraction procedures or in the MS source) should also be evaluated when relevant (e.g., potentially unstable metabolites such as ester analytes to ester/acidic metabolites, unstable N-oxides or glucuronide metabolites, lactone-ring structures). It is acknowledged that this evaluation will not be possible in the early stages of drug development of a new chemical entity when the metabolism is not yet evaluated. However, it is expected that this issue should be investigated, and partial validation performed if needed. The extent of back-conversion, if any, should be established and the impact on the study results should be discussed in the Bioanalytical Report.

*When is an evaluation for **metabolite back-conversion** performed?*

- *Consider evaluating if there are concerns based on experimental data on in-vitro metabolism or information from the original drug product.*
- *Particular attention should be paid when the metabolite is significantly higher in concentration than the parent drug.*

3.2.3 Matrix Effect

The matrix effect **should** also be evaluated in **relevant patient populations or special populations** (e.g., hepatically impaired or renally impaired) when available. An additional evaluation of the matrix effect is **recommended** using **haemolysed or lipaemic matrix samples** during method validation on a case-by-case basis, especially when these conditions are expected to occur within the study.

*Evaluation of **Matrix Effect** for hepatic/renal impairment patient plasma and hemolyzed/hyperlipidemic plasma was discussed.*

- *When study samples are obtained from the target patient population or special patient populations (e.g., hepatic or renal impairment) in clinical trials, Matrix Effect should be evaluated in these populations.*
- *It is recommended that Matrix Effect with hemolytic or hyperlipidemic matrix samples be additionally evaluated in analytical method validation, depending on the individual circumstances, especially when Matrix Effect are expected to occur in the study.*
- *Most participants did not consider the ethnic differences on Matrix Effect.*

3.2.5.2 Evaluation of Accuracy and Precision

To enable **the evaluation of any trends over time within one run**, it is recommended to demonstrate accuracy and precision of the QCs over at least one of the runs **in a size equivalent to a prospective analytical run** of study samples.

*How do we conduct **batch size** evaluation*

- *The intervening samples among the QCs should contain matrix-derived components.*
- *A method that can evaluate the accuracy and precision of the QC at the time the prospective batch size has elapsed is desirable.*

3.2.8 4) Stability of the Analyte in Whole Blood

Sufficient attention should be paid to the **stability of the analyte in the sampled matrix (blood)** directly after collection from subjects and prior to preparation for storage to ensure that the concentrations obtained by the analytical method reflect the concentrations of the analyte in the subject's blood at the time of sample collection. If the matrix used is plasma, the stability of the analyte in blood should be evaluated during method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

*Is **Whole Blood Stability** for non-clinical samples needed to evaluate?*

- *For non-clinical samples, it is less important because the plasma separation is performed promptly after blood collection, however, desirable to evaluate it in a preliminary study.*

*Is **Whole Blood Stability** in Serum NOT needed to evaluate?*

- *If the matrix to be measured is serum, it is not possible to evaluate stability in whole blood in the same environment as the study sample because blood in the absence of anticoagulant will quickly coagulate. In addition, since the study sample is collected in a vacuum blood collection tube containing a coagulant and quickly transferred to serum, evaluation of stability in whole blood is considered unnecessary.*

3.3.2 Acceptance Criteria for an Analytical Run

Analytical runs containing samples that are diluted and reanalysed **should include dilution QCs** to verify the accuracy and precision of the dilution method during study sample analysis. The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor. If multiple dilution factors are used in one analytical run, then dilution QCs need only be diluted by the highest and lowest dilution factors. The within-run acceptance criteria of the dilution QC(s) will only affect the acceptance of the diluted study samples and not the outcome of the analytical run.

*How do we conduct **Dilution QC** evaluation in the Analytical Run? (number and criteria)*

- *When measuring the study samples, at least N=3 of Dilution QCs diluted by the same dilution procedure as the dilution of the study samples should be measured to evaluate the accuracy and precision. The acceptance criteria are the same as dilution integrity in the validation study.*

*Is it necessary to evaluate the stability of the **Dilution QCs**?*

- *Since it is possible that the product may recrystallize during storage, it is recommended to evaluate the stability of the Dilution QCs if there are concerns about solubility.*

4.1.1 Reference Standard

It is recommended that **the manufacturing batch of the reference standard used for the preparation of calibration standards and QCs is derived from the same batch of drug substance as that used for dosing** in the nonclinical and clinical studies whenever possible.
If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out with QCs from the original material and the new material prior to use to ensure that the performance characteristics of the method are within the acceptance criteria.

*For most participants, it is often difficult to unify **Investigational Drug batches** and **Analytical Reference batches** in clinical trials compared to non-clinical studies.*

- *Considering that there is no problem if the API is the same manufacturing method, Reference Standard used for bioanalysis is prepared and guaranteed by CoA.*
- *When the lot is changed, it is confirmed that the old and new lots are equivalent by COA, and one batch with old and new lots is measured and confirmed by bioanalysis.*

4.1.2 Critical Reagents

The data sheet for the critical reagent **should include at a minimum identity, source, batch/lot number, purity (if applicable), concentration (if applicable) and stability/retest date/storage conditions** (Refer to Table 1). Additional characteristics may be warranted.
Retest dates and validation parameters should be documented in order to support the extension or replacement of the critical reagent. Stability testing of the reagents should be based upon the performance in the bioanalytical method and upon general guidance for reagent storage conditions. It can be extended beyond the expiry date from the supplier.

*How is **Stability Data** collected and noted for the quality assurance of Critical Reagent?*

- *The stability of the Critical Reagents should be evaluated for the entire bioanalytical method, not for individuals.*

*How is **Stability/Retest date** set and recorded?*

- *If the calibration curve/QC results of the study sample analysis meet the criteria, it can be judged that the Critical Reagent is working (stable). It is necessary to create a data sheet. The Retest date is set for just temporary stability period.*

4.2 Validation

Most often microtitre plates are used for LBAs and **study samples can be analysed using an assay format of 1 or more well(s) per sample**.
If method development and method validation are performed using 1 or more well(s) per sample, then study sample analysis should also be performed using 1 or more well(s) per sample, respectively.

*In what cases is a **Single sample analysis** performed? Are there any problem with **singlet assays** for calibration curves and QCs?*

- *Most participants commented that singlet assays was difficult due to large CV in LBA especially.*
- *Duplicate is appropriate when measuring manually, but if the automation is performed, singlet may be discussed more.*
- *Screening tests and study sample analysis with a large number of samples have the advantage of being performed with singlet.*

4.2.2 Selectivity

Use of fewer sources may be acceptable in the case of rare matrices.

- Selectivity should be evaluated in **lipaemic samples** and haemolysed samples.
- Selectivity should be assessed in **samples from relevant patient populations (e.g., renally or hepatically impaired patients, inflammatory or immuno-oncology patients if applicable)**. In the case of relevant patient populations, there should be **at least five individual patients.**

*Selection criteria and population of **hyperlipidemia and other patient-derived samples** were discussed.*

- *Patient samples have become available recently, and it is common for the participants to prepare patient samples to assess Selectivity.*
- *Although it may be difficult to align the number of individuals for each disease, Selectivity can be evaluated by performing partial validation using Predose samples obtained from actual clinical trials.*

4.2.4.2 Evaluation of Accuracy and Precision

Within-run accuracy and precision data should be reported for each run. If the within-run accuracy or precision criteria are not met in all runs, **an overall estimate of within-run accuracy and precision for each QC level should be calculated.** Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.

*It was discussed why the **Overall estimate** was added in the guideline.*

- *How is Overall Estimate handled?*
- *What is the difference between "overall estimate of within-run accuracy and precision" and "between-run accuracy and precision“?*

- *No definitive conclusions could be reached. It is desirable that specific views on the overall estimate be presented through the training materials, etc.*

4.2.7 Stability

For **fixed dose combination products and specifically labelled drug regimens,** the freeze-thaw, benchtop and long-term **stability tests** of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds, on a case-by-case basis.

*Stability evaluation for **combination drugs** was discussed.*

- *It was suggested that concomitant drugs with molecules not structurally related (e.g. biologics and small molecules) are not needed to evaluated for Stability.*
- *It might be easier to evaluate the Stability if it can be evaluated using the Incurred samples, however, the validity of the ISS is not recognized.*
- *When assessing stability with follow-up, there is a concern about the risk of not meeting the criteria.*

4.3.3 Calibration Range

At least 2 QC sample levels should fall within the range of concentrations measured in study samples. At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and **either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range** before continuing with study sample analysis.

*How should **at least 2 QC sample levels fall within the range of concentrations** measured in study samples?*

- *Neither participant was able to present a clear policy on how to consider and respond.*
- *It was considered important to add QC samples of an appropriate concentration including Dilution QCs in consideration of the study sample concentration.*
- *It is desirable that specific views be presented through training materials etc.*

6. PARTIAL AND CROSS VALIDATION

6.1 Partial Validation

Change from one matrix within a species to another (e.g., switching from human plasma to serum or cerebrospinal fluid) or **changes to the species within the matrix** (e.g., switching from rat plasma to mouse plasma)

*What situations should we conduct **Partial Validations**?*

1) *From human plasma to serum or cerebrospinal fluid.*
2) *From rat plasma to mouse plasma.*
3) *From ICR mouse plasma to rasH2 mouse plasma (switch of strain).*

- *In cases of 1) and 2), Full Validation is needed, shown as Caution for N-in-1 approach (multiple species or matrices in 1 validation) in FAQ for the M10 guideline.*
- *In case of 3), various approaches were proposed; Full Validation, Partial Validation, and bridging by QC samples in study sample analysis without conducting Partial Validation.*

6. PARTIAL AND CROSS VALIDATION

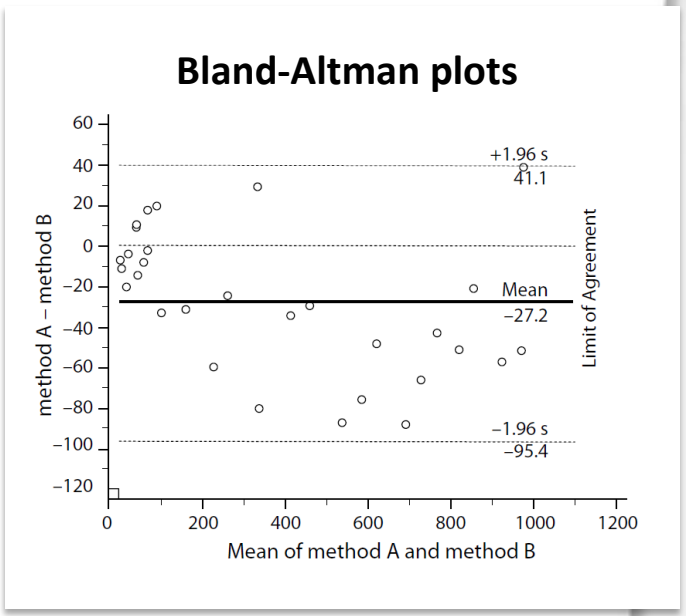
6.2 Cross Validation

Bias can be assessed by Bland-Altman plots or Deming regression. Other methods appropriate for assessing agreement between two methods (e.g., concordance correlation coefficient) may be used too. Alternatively, the concentration vs. time curves for study samples could be plotted for samples analysed by each method to assess bias.

Recommended evaluation methods for cross validation were discussed.

- *What is **Bland-Altman plots**?*

- *Participants have not experienced bias assessment, but ISR-based assessment.*
- *The evaluation method by Bland-Altman plot has been shown.*
- *It is difficult to judge Pass/Fail with setting the criteria in advance like ISR.*
- *It is necessary to consider comprehensively if the data is acceptable with other factors in addition to the Bias.*



7. ADDITIONAL CONSIDERATIONS

7.2 Parallelism

Although lack of parallelism is a rare occurrence for bioanalytical methods for PK evaluation, **parallelism of LBA should be evaluated on a case-by-case basis**, e.g., where interference caused by a matrix component (e.g., presence of endogenous binding protein) is suspected during study sample analysis.
Parallelism investigations, or the justification for its absence, should be included in the Bioanalytical Report.
Some methods may demonstrate parallelism for one patient population, but lack it for another population.

*Points to consider to evaluate **Parallelism for bioanalytical methods for PK** was discussed.*

- *How to incorporate Parallelism evaluation in bioanalytical method validation and how to deal with problems with Parallelism should be needed continuously in JBF with the industries.*
- *It is desirable that specific views be presented through training materials etc.*

8. DOCUMENTATION

Table 1: Documentation and Reporting

Analysis — Bioanalytical Report

For All Studies

- **QCs graphs trend analysis** encouraged

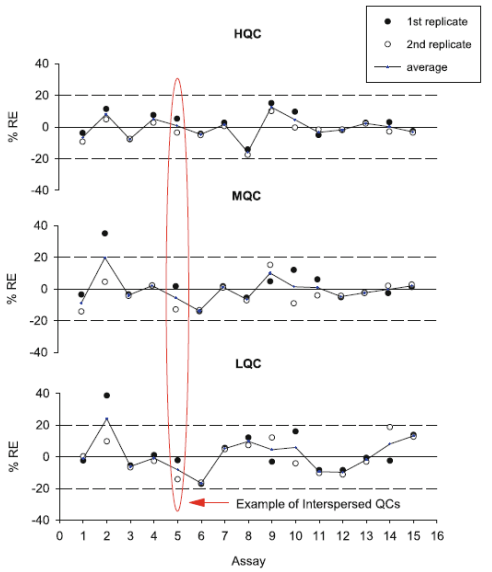
Additionally, for Comparative BA/BE Studies also include:

- **IS response plots for each analytical run, including failed runs**

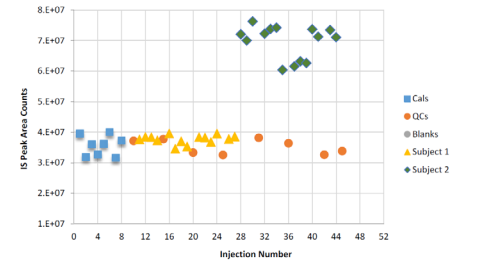
*Recommended drawing methods for "**QCs graphs trend analysis**" and "**IS response plot**" were discussed.*

- *Examples of QCs graph and IS response plot are shown.*
- *Most participants do not feel negative for creating plots and visualized them since accuracy of QC samples and IS response have already been evaluated, however, there seems to be various opinions whether all plots are shown in Bioanalytical Report because of "encouraged".*

QC trend



IS plot



Summary

- *The 478 discussion points/questions were provided by 32 Industries and CROs.*
- *The issues of high importance and attention which JBF SC categorized and prioritized were discussed at the JBF ICH M10 Workshop.*
- *Many opinions were exchanged at the workshop, and we were able to deepen our understanding of the points of discussion.*
- *As for some points of discussion that could not be concluded, it is desirable that specific views be presented through training materials etc.*

Detail contents are exhibited as a poster at the Poster & Booth Venue (2F)

Acknowledgement

All participants from Industries and CROs
JBF steering committee members