Guideline/Guidance Comparison on small molecule bioanalysis

MHLW Guideline (2013)
vs EMA Guideline (2011, updated 2014)
vs FDA Guidance (2018)

Version 3, 28-Aug-2018

Scope

MHLW Guideline (2013)

This guideline is applicable to validation of analytical methods applied to measure concentrations of drugs and their metabolites in biological samples obtained in toxicokinetic studies and clinical trials, as well as to the analyses of study samples using such methods. The information in this guideline generally applies to the quantification of low-molecular-weight drugs (except for endogenous substances), by analytical methods such as liquid chromatography (LC) and gas chromatography (GC) used in combination with mass spectrometry (MS) or with the other detectors.

This guideline is not mandatory for analytical methods used in non-clinical studies that are beyond the scope of "Ministerial Ordinance Concerning the Standards for the Conduct of Non-clinical Studies on the Safety of Drugs (Ministry of Health and Welfare ordinance No. 21, dated March 26, 1997)" but could be used as a reference in conducting a method validation for a non-GxP bioanalysis.

EMA Guideline (2011)

Similar to Japanese Guideline.
A chapter of LBA is given independently.

FDA Guidance (2018)

Similar to Japanese Guideline.
Description about LBA is given.
Biomarker concentration evaluation is mentioned.
Applicability to veterinary drug is mentioned.
Fit-for-purpose concept is given.

Underline: key words in the item

Reference standards

MHLW Guideline (2013)

A reference standard serves as the scale in quantifying an analyte, and is mainly used to prepare calibration standards and quality control (QC) samples, which are relevant blank matrix spiked with a known concentration of the analyte of interest. The quality of the reference material is critical, as the quality affect measurement data. A certificate of analysis or an alternative statement that provides information on lot number, content (purity), and storage conditions should accompany the standard. As a reference standard, it is advisable to obtain a material of known chemical structure from an authenticated source and clarify the expiration date. A certificate of analysis is not necessarily required for an internal standard, but the lack of analytical interference with the analyte should be demonstrated before use as the internal standard.

EMA Guideline (2011)

Similar to Japanese Guideline, but SIL IS mentioned.

FDA Guidance (2018)

Similar to Japanese Guideline.

When the reference standard expires, should not make stock solutions with this lot of standard unless the standard's purity is re-established.

Underline: key words in the item

Full validation

MHLW Guideline (2013)

A full validation should be performed when establishing a new bioanalytical method for quantification of an analyte/analytes. The objective of a full validation is to demonstrate the assay performance of the method, e.g. selectivity, lower limit of quantification (LLOQ), calibration curve, accuracy, precision, matrix effect, carry-over, dilution integrity, and stability. Generally, a full validation should be performed for each species and matrices (mainly plasma, serum, whole blood, or urine) to be analyzed.

A full validation should also be considered when a new analyte, such as a metabolite, is added to an existing, fully validated analytical method. A full validation is also required when implementing an analytical method from a literature. The matrix used in analytical validation should be as close as possible to the target study samples, including anticoagulants and additives. When an analytical method is to be established for a matrix of limited availability (rare matrix, e.g., tissue, cerebrospinal fluid, bile), a sufficient amount of matrix cannot be obtained from sufficient number of sources (subjects or animals). In such a case, a surrogate matrix may be used to prepare calibration standards and QC samples. However, the use of a surrogate matrix should be rigorously justified in the course of establishing the analytical method.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Items of full validation are similar to Japanese Guideline except for matrix effects.

When using LC/MS methods, the sponsor or applicant should determine the effects of the matrix on ion suppression, ion enhancement, or extraction efficiency in method development.

Performance QCs are included in validation runs to determine if analytical runs are acceptable.

Underline: key words in the item

Selectivity

MHLW Guideline (2013)

Selectivity is an ability of an analytical method to measure and differentiate the analyte and the internal standard in the presence of other components in samples.
Selectivity is evaluated using blank samples (matrix samples processed without addition of an analyte or internal standard) obtained from at least 6 individual sources.
The absence of interference with each analyte and its internal standard should be confirmed. In case of the matrix with limited availability, it may be acceptable to use matrix samples obtained from less than 6 sources.
The evaluation should demonstrate that no response attributable to interfering components is observed in the blank samples or that a response attributable to interfering components is not higher than 20% of the response in the LLOQ for the analyte and also not higher than 5% of the internal standard.

EMA Guideline (2011)

Similar to Japanese Guideline, additionally mentioned some interference cases.

FDA Guidance (2018)

Similar to Japanese Guideline.

Underline: key words in the item

Lower limit of quantification

MHLW Guideline (2013)

The LLOQ is the lowest concentration of an analyte at which the analyte can be quantified with reliable accuracy and precision.

The analyte response at the LLOQ should be at least 5 times the response of that in a blank sample. Mean accuracy and precision at the LLOQ should be within ±20% deviation of the nominal (theoretical) concentration and not more than 20%, respectively.

EMA Guideline (2011)

Similar to Japanese Guideline.

Recommendation for bioequivalence studies: LLOQ should be not higher than 5% of the Cmax.

FDA Guidance (2018)

Similar to Japanese Guideline.

Underline: key words in the item

Calibration curve

MHLW Guideline (2013)

A calibration curve demonstrates the relationship between a theoretical concentration and a response of an analyte.
A calibration curve needs to be prepared for each analyte. The calibration curve should be prepared using the same matrix as the intended study samples, whenever possible, by spiking the blank matrix with known concentrations of the analyte. A calibration curve should be generated with a blank sample, a zero sample (blank sample spiked with internal standard), and at least 6 concentration levels of calibration standards, including an LLOQ sample. In general, the simplest model that adequately describes the concentration-response relationship should be used for regression equation and weighting conditions of the calibration curve. A non-linear regression equation may be used. Blank and zero samples should not be included in the determination of the regression equation for the calibration curve. The validation report should include the validated regression equation.
The accuracy of back calculated concentrations of each calibration standard should be within ±20% deviation of the theoretical concentration at the LLOQ, or ±15% deviation at all the other levels. At least 75% of the calibration standards, with a minimum of 6 levels, including the LLOQ and the highest levels, should meet the above criteria.

EMA Guideline (2011)

Similar to Japanese Guideline.
At least 50% of calibration standards per level needs to meet in case of replicates, detailed recommendation for adaption (analyze in replicate, use freshly spiked samples, stored samples can be used if appropriate stability data support).

FDA Guidance (2018)

Similar to Japanese Guideline.
Description of surrogate matrices is added; When surrogate matrices are necessary, the sponsor should justify and validate the calibration curves.

Underline: key words in the item

Accuracy

MHLW Guideline (2013)

Accuracy of an analytical method describes the degree of closeness between analyte concentration determined by the method and its theoretical concentration.

Accuracy and precision are assessed by performing analysis with QC samples, i.e., samples spiked with known amounts of the analyte. In the validation, QC samples with a minimum of 4 different concentrations (LLOQ and low-, mid-, and high-levels) within the calibration range are prepared. The low-level should be within 3 times the LLOQ, the mid-level is around the midpoint on the calibration curve, and the high-level should be at least 75% of the upper limit of the calibration curve.

Within-run accuracy and precision should be evaluated by at least 5 replicates at each concentration level in a single analytical run. Between-run accuracy and precision should be evaluated by the analysis in at least 3 analytical runs.

The mean accuracy at each concentration level should be within ±15% deviation of the theoretical concentration, except at the LLOQ, where it should be within ±20%.

EMA Guideline (2011)

Similar to Japanese Guideline.

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

FDA Guidance (2018)

Similar to Japanese Guideline.

Fresh calibration curve is required for evaluation and QC sample is preferred to be fresh as well.

No QC acceptance criteria is necessary for accuracy and precision evaluation runs as run acceptance.

Underline: key words in the item

Precision

MHLW Guideline (2013)

Precision of an analytical method describes variation between individual concentrations determined in repeated measurements.

Accuracy and precision are assessed by performing analysis with QC samples, i.e., samples spiked with known amounts of the analyte. In the validation, QC samples with a minimum of 4 different concentrations (LLOQ and low-, mid-, and high-levels) within the calibration range are prepared. The low-level should be within 3 times the LLOQ, the mid-level is around the midpoint on the calibration curve, and the high-level should be at least 75% of the upper limit of the calibration curve. Within-run accuracy and precision should be evaluated by at least 5 replicates at each concentration level in a single analytical run. Between-run accuracy and precision should be evaluated by the analysis in at least 3 analytical runs. Precision of concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.

Fresh calibration curve is required for evaluation and QC sample is preferred to be fresh as well.

No QC acceptance criteria is necessary for accuracy and precision evaluation runs as run acceptance.

Underline: key words in the item

Matrix effect

MHLW Guideline (2013)

Matrix effect is an alteration of the analyte response due to matrix component(s) in the sample. Matrix effect should be assessed when using mass spectrometric methods.

Matrix effect is evaluated by calculating the matrix factor (MF). The MF is determined by comparing the analyte response in the presence of matrix with that in the absence of matrix. MF should be calculated using matrix from at least 6 different sources. The MF may be normalized by its internal standard. The precision of the MF calculated should not exceed 15%.

Matrix effect can also be evaluated by analyzing QC samples, each prepared using matrix from at least 6 different sources. The precision of determined concentrations should not be greater than 15%.

In case the matrix is of limited availability, it may be acceptable to use matrix obtained from less than 6 sources.

EMA Guideline (2011)

Similar to Japanese Guideline.

Both MF and IS normalized MF need to be calculated, more detailed recommendation with dosing solution and specific matrix.

Concentrations (low and high) are described.

FDA Guidance (2018)

Described, but evaluation procedures or acceptance criteria are not mentioned as matrix effect.

Underline: key words in the item

Carry-Over

MHLW Guideline (2013)

Carry-over is an alteration of a measured concentration due to a leftover analyte in the analytical instrument.
The carry-over should be evaluated by analyzing a blank sample following the highest concentration calibration standard.
The response in the blank sample obtained after the highest concentration standard should not be greater than 20% of the analyte response at the LLOQ and also not greater than 5% of the response of internal standard.
If the criteria cannot be met, the impact of carry-over needs to be examined, and appropriate procedures should be taken to avoid any biases during the analysis of actual study samples.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
No criteria for IS are indicated.

Underline: key words in the item

Dilution integrity

MHLW Guideline (2013)

If samples require dilution before analysis, the dilution procedure should be tested to confirm no impact on the measured concentration of the analyte.
Dilution integrity should be evaluated by at least 5 replicates per dilution factor after diluting a sample with blank matrix to bring the analyte concentration within the calibration range. The dilution factors should be selected by considering the dilution method used for study samples. Mean accuracy and precision in the measurements of diluted samples should be within ±15% deviation of the theoretical concentration and not more than 15%, respectively.
If a surrogate matrix is used for sample dilution, the impact on the accuracy and precision should be demonstrated in the same manner.

EMA Guideline (2011)

Similar to Japanese Guideline.
The concentration of QC sample is required to exceed the ULOQ.

FDA Guidance (2018)

Similar to Japanese Guideline.
The concentration of QC sample is required to exceed the ULOQ.

Underline: key words in the item

Stability (1 of 2)

MHLW Guideline (2013)

Analyte stability should be evaluated to ensure that the concentration is not affected by the samples through each step of the process from the sample collection to the analysis. The stability of the samples should be assessed under conditions that are as close to the actual circumstances, e.g. sample storage and sample analysis as much as possible. Careful consideration should be given to the solvent or matrix type, container materials, and storage conditions used in the stability-determination process.

Validation studies should determine analyte stability after freeze and thaw cycles, after short-term (at room temperature, on ice, or under refrigeration) and long-term storage; stability in the processed samples should also be considered. All stability experiments should be performed on samples that have been stored for a time that is longer than the actual storage period.

Stability of the analyte in the stock and working solutions is usually evaluated using solutions near the highest and lowest concentration levels. The evaluation is performed by at least 3 replicates at each concentration level.

Stability of the analyte in the studied matrix is evaluated using low- and high-level QC samples. The QC samples should be prepared using a matrix that is as close as possible to the actual study samples, including anticoagulant and additives.

Stability is evaluated by at least 3 replicates per concentration level with QC samples before and after storage. The mean accuracy in the measurements at each level should be within ±15% deviation of the theoretical concentration, in principle. If the other criteria are more appropriate for the evaluation of specific analyte, they could be used.

EMA Guideline (2011)

Similar to Japanese Guideline, but no definition on number of repeat per conc.

ISS may be used but not considered sufficient.

SIL stability is not required.

In F/T stability, at each cycle, samples should be frozen for at least 12 hours before they are thawed.

Whole blood stability is not required on a routine basis.

Underline: key words in the item

Stability (2 of 2)

FDA Guidance (2018)

Similar to Japanese Guideline, but the following descriptions are included.

- When the matrix is rare, the use of suitable surrogate matrices is accepted.
- For drugs administered as fixed combinations, or part of a specific drug regimen, the stability of the analyte should be assessed in the presence of the other drug.
- The stability of the analyte in the presence of other co-medications that are known to be regularly administered to patients for the indication of the drug under development should be considered.
- Stability in whole blood is mentioned.
- All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix.
- For autosampler stability, the sponsor should demonstrate the stability of extracts in the autosampler only if the autosampler storage conditions are different or not covered by extract (processed sample) stability.
- For freeze/thaw stability, a minimum of 3 cycles should be assessed. QC samples should be frozen for at least 12 hours between cycles.
- The long-term stability of the sample should cover a period of time equal to or exceeding the time between the date of first sample collection and the date of last sample analysis.
- Determination of stability at minus 20ºC would cover stability at colder temperatures.
- For stock solution stability, stock solutions should not be made from reference materials that are about to expire unless the purity of the analyte in the stock solutions is re-established.

Underline: key words in the item

Recovery

MHLW Guideline (2013)

Recovery is a measure of the efficiency at which an analytical method recovers the analyte through the sample-processing step. In order to elucidate the nature of analytical method, it is advisable to evaluate the recovery.
The recovery is determined by comparing the analyte response in a biological sample that is spiked with the analyte and processed, with the response in a biological blank sample that is processed and then spiked with the analyte. It is important to demonstrate the reproducibility, rather than to show a higher recovery rate.

EMA Guideline (2011)

Not addressed.

FDA Guidance (2018)

Similar to Japanese Guideline.
FDA Guidance refers to perform recovery experiment at three concentrations (low, medium and high).

Underline: key words in the item

Partial validation

MHLW Guideline (2013)

Partial validation may be performed when minor changes are made to an analytical method that has already been fully validated. The items in a partial validation are determined according to the extent and nature of the changes made to the method.

Typical bioanalytical method changes subjected to a partial validation are as follows: analytical method transfers between laboratories, changes in analytical instruments, changes in calibration range, changes in sample volume used for analysis, changes in anticoagulant, changes in sample-processing procedures or analytical conditions, changes in sample storage conditions, confirmation of impact by concomitant drugs, and use of rare matrices.

Acceptance criteria used in partial validation should be the same as those employed in the full validation in principle.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline, but OK with partial for matrix change within species & species change within matrix.

”Change in analytical methodology" is described and "Changes in sample storage conditions" is not described as one of typical example.

Underline: key words in the item

Cross validation

MHLW Guideline (2013)

Cross validation is primarily conducted when data are generated in multiple laboratories within a study or when comparing analytical methods used in different studies, after a full or partial validation. The same set of QC samples spiked with the analyte or the same set of study samples is analyzed at both laboratories or by both analytical methods, and the mean accuracy at each concentration level or the assay variability is evaluated.

In the cross validation among two or more laboratories within a study, the mean accuracy of QC samples (low-, mid-, and high-levels) evaluated by at least 3 replicates at each level, should be within ±20% deviation of the theoretical concentration, considering intra- and inter-laboratories precision. When using a set of study samples, the assay variability should be within ±20% for at least two-thirds of the samples.

In the cross validation between different analytical methods based on different assay principles, both validation procedure and acceptance criteria (i.e., mean accuracy or assay variability) should be separately defined based on scientific judgment according to the type of the analytical methods.

EMA Guideline (2011)

Similar to Japanese Guideline, but 15% for QCs is acceptable.

FDA Guidance (2018)

Described, but leaving judgment to scientists.

Cross-validation with spiked matrix QCs and non-pooled subject samples should be conducted at each site or laboratory

Pooled incurred samples can be used when insufficient volume exists.

Underline: key words in the item

Reporting

MHLW Guideline (2013)
Validation report
- Summary of the validation
- Information on the reference standards
- Information on the blank matrices
- Analytical method
- Validated parameters and the acceptance criteria
- Validation results and discussion
- Rejected runs together with the reason for rejection
- Information on reanalysis
- Deviations from the protocol and/or SOP, along with the impact on study results
- Information on reference study, protocol, and literature
- Representative chromatograms

EMA Guideline (2011)
Similar to Japanese Guideline.
More detailed requirement.
No chromatogram needed for validation report.

FDA Guidance (2018)
Similar to Japanese Guideline.
More detailed requirement.
Recommendation for summary table.

Underline: key words in the item

Analysis of study samples

MHLW Guideline (2013)

Study samples are biological specimens that are obtained from toxicokinetic studies and clinical trials. Analysis of study samples should be carried out using a fully validated analytical method. In the analysis, study samples should be handled under conditions that are validated for adequate stability, and analyzed within a confirmed stability period, along with a blank sample, a zero sample, calibration standards at a minimum of 6 concentration levels, and QC samples.
Validity of the analytical method during study sample analysis should be evaluated in each analytical run by using the calibration curve and QC samples. In studies that serve pharmacokinetic data as a primary endpoint, reproducibility of the analytical method should be confirmed for each representative study per matrix by performing incurred sample reanalysis (ISR: reanalysis of incurred samples in separate analytical runs on different day to determine whether the original analytical results are reproducible).
If carry-over is a concern for the study samples analyzed, the evaluation of validity should also include the item.

EMA Guideline (2011)

Following descriptions are added:

- The calibration standards and QC samples should have been spiked independently using separately prepared stock solutions, unless the nominal concentration(s) of the stock solutions have been established.
- For bioequivalence studies it is advised to analyse all samples of one subject together in one analytical run to reduce the variability in outcome.

FDA Guidance (2018)

Following evaluation items or recommendations are added:

- Selectivity and sensitivity in the blank and zero calibrators.
- QC results should be included in the estimation of accuracy and precision.
- Acceptance of separation batch of the analytical run.
- The IS response variability.
- Drift should be monitored and its impact on the accuracy should be addressed, if any.
- All study samples from a subject should be analyzed in a single run, especially for studies designed with repeated measures from individual subjects (e.g., crossover or sequential design required for BE studies).
- If a unique or disproportionately high concentration of a metabolite is discovered in human studies, assay method validation may be needed for the metabolite.

Underline: key words in the item

Acceptance criteria (1 of 2)

MHLW Guideline (2013)

A calibration curve used in study sample analysis should be generated for each analytical run by using the validated analytical method. The same model as in the bioanalytical method validation should be used for the regression equation and weighting conditions of the calibration curve. The accuracy of back calculated concentrations of calibration standards at each level should be within ±20% deviation of the theoretical concentration at the LLOQ, or ±15% deviation at all other levels. At least 75% of the calibration standards, with a minimum of 6 levels, should meet the above criteria.
In case the calibration standard at the LLOQ or the highest level did not meet the criteria in study sample analysis, the next lowest/highest-level calibration standard may be used as the LLOQ or the upper limit of the calibration curve. Even though narrowed, the modified calibration range should still cover at least 3 different QC sample levels (low-, mid-, and high-levels).
QC samples with a minimum of 3 different concentration levels (low-, mid-, and high-levels) within the calibration range are analyzed in each analytical run. Usually, the low-level is within 3 times the LLOQ, the mid-level is in the midrange of the calibration curve, and the high-level needs to be at least 75% of the upper limit of the calibration curve. The analysis requires 2 QC samples at each QC level or at least 5% of the total number of study samples in the analytical run, whichever is the greater. QC samples should be placed before and after study sample analysis.
The accuracy in the measurements of QC samples should be within ±15% deviation of the theoretical concentrations. At least two-thirds of the QC samples and at least 50% at each concentration level should meet the criteria.

EMA Guideline (2011)

Similar to Japanese Guideline, except EMA Guideline stated the case an analytical run is acceptable for one analyte in simultaneous determination of multiple analytes.

Underline: key words in the item

Acceptance criteria (2 of 2)

FDA Guidance (2018)

Basic concept is same as Japanese Guideline, but more detailed descriptions are added;

QC:

- If distinct processing batches are consisted in the analytical runs, QC acceptance criteria should be applied for the whole run and for each distinct batch within the runs.
- For study samples involving multiple analytes, a valid result for one analyte should not be rejected because of another analyte failing the acceptance criteria.

Selectivity:

- In each analytical run, the blank and zero calibrators should be free of interference at the retention times of the analyte and the internal standard
- In each analytical run, the internal standard response in the blank should not exceed 5% of average internal standard response of the calibrators and QCs.

Specificity:

- Check as needed.

Sensitivity:

- The analyte response at the LLOQ should be ≥ five times the analyte response of the zero calibrator.

Underline: key words in the item

Calibration range

MHLW Guideline (2013)

If concentration data obtained during the analysis of study samples are found within a narrow range of the calibration range, it is advisable to redefine the concentration levels of QC samples accordingly.
In case the calibration range is changed, partial validation should be performed.
However, it is not necessary to reanalyze the study samples that have been quantified prior to the change (the calibration range, levels or number of QC samples).

EMA Guideline (2011)

Similar to Japanese Guideline, along with broad recommendation on inadequate calibration range.

FDA Guidance (2018)

Similar to Japanese Guideline.
Changes in the response-function relationship between the validation and study sample analyses indicate potential problems. A SOP should be developed a priori to address such issues.

Underline: key words in the item

QC samples

MHLW Guideline (2013)

A calibration curve used in study sample analysis should be generated for each analytical run by using the validated analytical method. The same model as in the bioanalytical method validation should be used for the regression equation and weighting conditions of the calibration curve. The accuracy of back calculated concentrations of calibration standards at each level should be within ±20% deviation of the theoretical concentration at the LLOQ, or ±15% deviation at all other levels. At least 75% of the calibration standards, with a minimum of 6 levels, should meet the above criteria.
In case the calibration standard at the LLOQ or the highest level did not meet the criteria in study sample analysis, the next lowest/highest-level calibration standard may be used as the LLOQ or the upper limit of the calibration curve. Even though narrowed, the modified calibration range should still cover at least 3 different QC sample levels (low-, mid-, and high-levels).
QC samples with a minimum of 3 different concentration levels (low-, mid-, and high-levels) within the calibration range are analyzed in each analytical run. Usually, the low-level is within 3 times the LLOQ, the mid-level is in the midrange of the calibration curve, and the high-level needs to be at least 75% of the upper limit of the calibration curve. The analysis requires 2 QC samples at each QC level or at least 5% of the total number of study samples in the analytical run, whichever is the greater. QC samples should be placed before and after study sample analysis.
The accuracy in the measurements of QC samples should be within ±15% deviation of the theoretical concentrations. At least two-thirds of the QC samples and at least 50% at each concentration level should meet the criteria.

EMA Guideline (2011)

Similar to Japanese Guideline, except EMA Guideline stated the case an analytical run is acceptable for one analyte in simultaneous determination of multiple analytes.

FDA Guidance (2018)

Similar to Japanese Guideline, but FDA Guidance added description as follows:
A distinct batch or batches in an analytical run may be rejected when it fails to meet QC acceptance criteria, but the remaining batches may pass provided that the analytical run meets the overall QC acceptance criteria.

Underline: key words in the item

Reanalysis of study samples

MHLW Guideline (2013)

Possible reasons and procedures for reanalysis, as well as criteria for handling of concentration data should be predefined in the protocol or standard operating procedure (SOP).

Examples of reasons for reanalysis are as follows: calibration curve or QC samples failed to meet the criteria for the validity of analytical run; the obtained concentration was higher than the upper limit of the calibration range; the analyte of interest was detected in pre-dose or placebo samples; improper sample processing or malfunction of equipment; defective chromatogram; and causal investigation on the abnormal value.

Reanalysis of study samples for a pharmacokinetic reason should be avoided, whenever possible. In bioequivalence studies, it is not acceptable to reanalyze study samples only because the initial data were pharmacokinetically questionable in order to replace the concentration data. However, reanalysis of specific study samples are acceptable when, for instance, the initial analysis yielded an unexpected or anomalous result that may affect the patient safety in a clinical trial.

In any case, when reanalysis is performed, the analytical report should provide information of the reanalyzed samples; the reason for reanalysis; the data obtained in the initial analysis, if any; the data obtained in the reanalysis; and the final accepted values and the reason and method of selection.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.

No special description about reanalysis due to safety concerns.

No confirmatory reanalysis for BE studies is accepted.

Underline: key words in the item

Integration

MHLW Guideline (2013)

Procedures for chromatogram integration and re-integration should be predefined in the protocol or SOP.
In case chromatogram re-integration is performed, the reason for re-integration should be recorded and the chromatograms obtained both before and after the re-integration should be kept for future reference.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
Original and re-integrated data should be reported.

Underline: key words in the item

Incurred samples reanalysis

MHLW Guideline (2013)

In bioanalysis, it can happen that the results of analyses of study samples are not reproducible, even when the method validation is successfully conducted and the validity of at each analytical run is confirmed by calibration standards and QC samples.
Such failures can be attributed to various factors, including inhomogeneity of study samples, contamination and other operational errors, and interference of biological components unique to the study samples or of unknown metabolites. ISR refers to reanalysis of incurred samples in separate analytical runs on different days to check whether the original analytical results are reproducible.
Confirmation of the reproducibility by ISR improves the reliability of the analytical data. In addition, a failure to demonstrate the reproducibility of the original data in the ISR can trigger a cause investigation and remedial measures for the analytical method.
Usually, ISR is performed for representative studies selected for each matrix in studies that use pharmacokinetic data as the primary endpoint. For instance, ISR should be conducted in the following situations: toxicokinetic studies for each different species; clinical studies representative pharmacokinetic studies for healthy volunteers and patients with renal/hepatic impairment, as well as in bioequivalence studies. For non-clinical studies, ISR may be performed with samples obtained in a independent non-GLP study, if the study design is similar to the relevant toxicokintics study, e.g. sampling conditions.
ISR should be performed with samples from as many subjects or animals as possible and should usually include those of near the maximum blood concentration (Cmax) and the elimination phase. ISR should be performed within a time window that ensures the stability of the analyte. As a guide, approximately 10% of the samples should be reanalyzed in cases where the total number of study samples is less than 1000 and approximately 5% of the number of samples exceeding 1000 samples.
The results of ISR are evaluated using assay variability. Assay variability can be calculated as the difference between the concentration obtained by ISR and that in the original analysis divided by their mean and multiplied by 100. The assay variability should be within ±20% for at least two-thirds of the samples analyzed in ISR. In case the ISR data failed to meet the above criteria, cause investigation should be conducted for the analytical method and necessary measures should be taken by considering the potential impact on study sample analysis.
It should be noted that ISR is performed to monitor assay variability. The original data should never be discarded or replaced with the reanalysis data even if the assay variability exceeds ±20% in a specific sample.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
Description to usable bulk frozen calibration curve is added. Incurred samples should not be pooled. In addition to Japanese Guideline, ISR is expected for all pivotal pharmacodynamic and biomarker studies.

Underline: key words in the item

System suitability

MHLW Guideline (2013)

Analytical instruments used in bioanalysis should be well maintained and properly serviced. In order to ensure optimum performance of the instrument used for bioanalysis, it is advisable to confirm the system suitability prior to each run, in addition to periodical check. However, confirmation of the system suitability is not mandatory in bioanalysis, because the validity of analysis is routinely checked by evaluation of calibration curves and QC samples in each analytical run.

EMA Guideline (2011)

Not addressed.

FDA Guidance (2018)

If system suitability is assessed, a specific SOP should be used.
System suitability should be determined using samples that are independent of the current study calibrators, QCs, and study samples.
Records should be maintained and available for audits.

Underline: key words in the item

Reporting

MHLW Guideline (2013)

Study sample analysis report

- Summary of the study sample analysis
- Information on the reference standards
- Information on the blank matrices
- Information on receipt and storage of study samples
- Analytical method
- Parameters, acceptance criteria, and results of the validity evaluation
- Results and discussion of study sample analysis
- Rejected runs together with the reason for rejection
- Information on reanalysis
- Deviations from the protocol and/or SOP, along with impact on study results
- Information on reference study, protocol, and literature
- Representative chromatograms, as needed

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
More detailed requirement.
Recommendation for summary table.
In addition, number of QCs, IS response, re-integration, and reported chromatograms are described in more detail.
For chromatograms, 20% of serially selected subjects for BE studies or randomly selected chromatograms from 5% of studies should be included.

Underline: key words in the item

Documentation and Archives

MHLW Guideline (2013)

In order to ensure adequate reproducibility and reliability of bioanalysis, results obtained in analytical method validations and study sample analyses should be documented in a validation report and a study sample analysis report as described below. The reports should be stored along with relevant records and raw data in an appropriate manner.

All relevant records and raw data should be kept, including those obtained in rejected analytical runs, specifically record of reference materials and blank matrices (receipt/release, use, storage), record of samples (receipt/release, preparation, and storage), record of analyses, record of instrument (calibration and settings), record of deviations, record of communications, and raw data such as analytical data and chromatograms.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline, more detailed requirement, recommendation for summary table.

The FDA expects the sponsor to maintain data at the analytical site to support summary data submitted in Validation and Analytical Study Reports.

Underline: key words in the item

Issues not stated in MHLW Guideline (1 of 4)

FDA Guidance (2018)

Endogenous Compounds

For analytes that are also endogenous compounds, the accuracy of the measurement of the analytes poses a challenge when the assay cannot distinguish between the therapeutic agent and the endogenous counterpart. In such situations, the following approaches are recommended to validate and monitor assay performance. Other approaches, if justified by scientific principles, can also be considered.

- The biological matrix used to prepare calibration standards should be the same as the study samples and free of the endogenous analyte. To address the suitability of using an analyte-free biological matrix, the matrix should be demonstrated to have: (1) no measurable endogenous analyte; and (2) no matrix effect or interference when compared to the biological matrix. The use of alternate matrices (e.g., buffers, dialyzed serum) for the preparation of calibration standards should be justified. The QCs should be prepared by spiking known quantities of the analyte in the same biological matrix as the study samples. The endogenous concentrations of the analyte in the biological matrix should be evaluated before QC preparation (e.g., by replicate analysis). The concentrations for the QCs should account for the endogenous concentrations in the biological matrix (i.e., additive) and be representative of the expected study concentrations.

- Parallelism should be evaluated for assays for endogenous compounds.

Underline: key words in the item

Issues not stated in MHLW Guideline (2 of 4)

FDA Guidance (2018)

Biomarkers

The recommendations in this guidance only pertain to the validation of assays to measure in vivo biomarker concentrations in biological matrices such as blood or urine. Considerable effort also goes into defining the biological function of biomarkers, and confusion may arise regarding terminology (e.g. biomarker method validation vs biomarker qualification).

Biomarkers are increasingly used to assess the effects of new drugs and therapeutic biological products in patient populations. Because of the important roles biomarkers can play in evaluating the safety, activity, or effectiveness of a new medical product, it is critical to ensure the integrity of the data generated by assays used to measure them. Biomarkers can be used for a wide variety of purposes during drug development; therefore, a FFP approach should be used when determining the appropriate extent of method validation. When biomarker data will be used to support a regulatory decision making, such as the pivotal determination of safety and/or effectiveness or to support dosing instructions in product labeling, the assay should be fully validated.

For assays intended to support early drug development (e.g., candidate selection, go-no-go decisions, proof-of-concept), the sponsor should incorporate the extent of method validation they deem appropriate.

Method validation for biomarker assays should address the same questions as method validation for drug assays. The accuracy, precision, sensitivity, selectivity, parallelism, range, reproducibility, and stability of a biomarker assay are important characteristics that define the method. The approach used for drug assays should be the starting point for validation of biomarker assays, although the FDA realizes that some characteristics may not apply or that different considerations may need to be addressed.

Underline: key words in the item

Issues not stated in MHLW Guideline (3 of 4)

FDA Guidance (2018)

Diagnostic Kits

Diagnostic kits are sometimes co-developed with new drug or therapeutic biological products as analytical methods that are used during the development of new drugs and therapeutic biologics. The recommendations in this section of the guidance do not apply to commercial diagnostic kits intended for point-of-care patient diagnosis (e.g., companion diagnostic kits), which are addressed in the following CDRH guidance documents:

- Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product
- In Vitro Companion Diagnostic Devices

However, when commercial diagnostic kits are repurposed as analytical methods to measure the concentrations of drugs, therapeutic biologics, or biomarkers in development, the FDA has the following recommendations:

- LBA kits with various detection platforms are sometimes used to determine analyte concentrations in pharmacokinetic or pharmacodynamic studies when the reported results must exhibit sufficient precision and accuracy. Because such kits are generally developed for use as clinical diagnostic tools, their suitability for use in such studies should be demonstrated.
- Diagnostic kit validation data provided by the manufacturer may not ensure that the kit method is reliable for drug development purposes. In such situations, the performance of diagnostic kits should be assessed in the facility conducting the sample analysis. Validation considerations for kit assays include, but are not limited to, the following examples:
  - Site-specific validation should be performed. The specificity, accuracy, precision, and stability of the assay should be demonstrated under actual conditions of use. Modifications from kit processing instructions should be completely validated.
  - Kits that use sparse calibration standards (e.g., one- or two-point calibration curves) should include in-house validation experiments to establish the calibration curve with a sufficient number of standards across the calibration range.
  - Actual QC concentrations should be known. Concentrations of QCs expressed as ranges are not sufficient for quantitative applications. In such cases, QCs with known concentrations should be prepared and used, independent of the kit-supplied QCs.
  - Standards and QCs should be prepared in the same matrix as the subject samples. Kits with standards and QCs prepared in a matrix different from the subject samples should be justified, and appropriate cross-validation experiments should be performed.
  - If the analyte source (i.e., reference standard) in the kit differs from that of the subject samples (e.g., the sample is a protein isoform of the reference standard), testing should evaluate differences in assay performance of the kit reagents.
  - If multiple kit lots are used within a study, lot-to-lot variability and comparability should be addressed for any critical reagents.
  - Individual batches using multiple assay plates (e.g., 96-well ELISA plates) should include sufficient replicate QCs on each plate to monitor the accuracy of the assay. Acceptance criteria should be established for the individual plates and the overall analytical run.

Underline: key words in the item

Issues not stated in MHLW Guideline (4 of 4)

FDA Guidance (2018)

Bridging Data From Multiple Bioanalytical Technologies

Assessing the output of both methods with a set of incurred samples (a minimum of 20 samples) is recommended. In cases where one method produces data with a constant bias relative to the other, concentrations can be mathematically transformed by that factor to allow for appropriate study interpretation.

DBS

Dried blood spot (DBS) technology has been under development for several years. The benefits of DBS include reduced blood sample volumes collected for drug analysis as well as ease of collection, storage, and transportation. Additional validation of this sampling approach is essential before using DBS in regulatory studies. This validation should address, at a minimum, the effects of the following issues: storage and handling temperatures, homogeneity of sample spotting, hematocrit, stability, carryover, and reproducibility, including ISR. Correlative studies with traditional sampling should be conducted during drug development. Sponsors are encouraged to seek feedback from the appropriate FDA review division early in drug development.

Underline: key words in the item