Guideline/Guidance Comparison on small molecule bioanalysis

MHLW Guideline (2013)
vs EMA Guideline (2011)
vs FDA draft Guidance (2013)

Version 2, 28-Feb-2014

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FDA draft Guidance (2013)

Similar to Japanese Guideline

A chapter of LBA is given independently

Additionally, Endogenous Compounds, Biomarkers, Diagnostic Kits and New Technologies are mentioned.

Applicability to veterinary drug

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 draft Guidance (2013)

Less detailed definition, leaving judgments to scientist

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

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 draft Guidance (2013)

Similar to Japanese Guideline, but expiration date and purity required for IS.
Stock solutions should not be used if the reference or IS expires.

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 draft Guidance (2013)

Similar to Japanese Guideline, but not mentioned acceptance criteria.
Some interference cases are described.

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 draft Guidance (2013)

Described, but leaving judgments to scientists. No criteria are mentioned.

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 draft Guidance (2013)

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 draft Guidance (2013)

Similar to Japanese Guideline, instruction of exclusion of calibrators

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

FDA draft Guidance (2013)

Similar to Japanese Guideline.
Accuracy of an additional QC level should be assessed before study sample analysis.
It is clearly required to include outliers for calculation of accuracy.
QC concentration is designated not based on calibration curve range, but based on the range of the expected study sample concentration.

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 draft Guidance (2013)

Similar to Japanese Guideline

Precision of an additional QC level should be assessed before study sample analysis. It is clearly required to include outliers for calculation of precision.

QC concentration is designated not based on calibration curve range, but based on the range of the expected study sample concentration.

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

FDA draft Guidance (2013)

Described, but leaving judgments to scientists.

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 draft Guidance (2013)

Described, but leaving judgments to scientists.

Underline: key words in the item

Stability

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..

FDA draft Guidance (2013)

Similar to Japanese Guideline, but both of reinjection reproducibility and processed sample stability are required.

In addition, internal standard solution stability is required.

The storage time in a long-term stability evaluation should equal or exceed the time between the date of first sample collection and the date of last sample analysis.

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 draft Guidance (2013)

Similar to Japanese Guideline
However, reference samples for recovery is different in the two guidelines.
Japanese Guideline: "a biological blank sample that is processed and then spiked with the analyte."
draft FDA 2013: "concentration of the analyte in solvent"
but draft FDA refer 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 draft Guidance (2013)

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

"Change in analytical methodology" is described.

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 draft Guidance (2013)

Described, but leaving judgments to scientists

cross-validation with both spiked matrix standards and subject samples should be conducted at each site or laboratory.

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 draft Guidance (2013)
Similar to Japanese Guideline
more detailed requirement
recommendation for summary table

Underline: key words in the item

Acceptance criteria

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 draft Guidance (2013)

Similar to Japanese Guideline, but it was mentioned samples involving multiple analytes should not be rejected based on the data from one analyte failing the acceptance criteria.

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 draft Guidance (2013)

Similar to Japanese Guideline.

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 draft Guidance (2013)

Similar, but clearly mentioned the number of replicates for reassays.

No special description about safety concerns.

Manager authorization for reanalysis should be documented in bioanalytical report.

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 draft Guidance (2013)

FDA guidance requires not only re-integration data but also original data for reporting.

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 draft Guidance (2013)

Similar to Japanese Guideline, recommended ISR size 7%

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 draft Guidance (2013)

System suitability is requested, with a specific SOP.
Study samples, standards, or QCs within the analytical run should not be used as system suitability samples.
Data should be maintained for inspection.

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 draft Guidance (2013)

Similar to Japanese Guideline
more detailed requirement, recommendation for summary table.
In addition, reporting chromatograms is described in more detail..

Underline: key words in the item