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Questions and Answers (Q&A) for the Guideline on Bioanalytical Method Validation in Pharmaceutical Development

<< Reference standard >>

Q1. How should I use a reference standard when the expiration date has not been established?

A1. When the expiration date is not established, an appropriate quality control for the reference standard by setting a retest date or by using other measures should be employed.

<< Selectivity >>

Q2. Selectivity is one of the parameters to be assessed in an analytical method validation. Is it different from "specificity"?

A2. "Selectivity" is listed as a parameter to be assessed according to the guidelines for analytical method validation. It is defined as the ability of an analytical method to detect the target analyte and its internal standard without having any interference from other components in the samples. "Selectivity" is equivalent to "specificity" which is mentioned in "Text on Validation of Analytical Procedures" (Notification No. 755 of Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, dated July 20, 1995). The term "selectivity" is widely used in bioanalytical method validations using chromatography; in addition, "selectivity" has been used in overseas guidances/guidelines. Thus, the term "specificity" in an old dataset can be regarded to an equivalent parameter to "selectivity" mentioned in this guideline.

<<Stability>>

Q3. Can I use an index other than mean accuracy for stability assessment?

A3. In principle, stability of an analyte should be assessed by the mean concentration against its nominal value considering an assay error in the measurement of pre-storage samples. If the other indices are more appropriate for evaluating the stability of a specific analyte in view of

assay precision, indices such as residual ratio could be used for evaluation. When indices such as residual ratio are used, the procedures and acceptance criteria should be predefined in the protocol or the standard operating procedure (SOP) for the evaluation.

Q4. How should I assess stability of an analyte after freeze and thaw cycles?

A4. Quality control (QC) samples stored under the target frozen state are thawed under the same condition as that used for study sample analysis. After the samples are completely thawed, the samples are frozen again under the same condition. The samples should be frozen for at least 12 hours. A series of process from freezing to thawing is defined as 1 cycle, and the QC samples are measured after the same number of freeze-thaw cycles applied to the study samples or more. The accuracy of QC samples should be within ±15% deviation of the theoretical concentration,.

<< Cross validation >>

Q5. What is a specific example of cross validation comparing analytical methods used in different studies?

A5. Cross validation is performed to compare different analytical methods based on different analytical principles (for example, LC-MS/MS and ELISA). In this case, both the validation procedure and the acceptance criteria (i.e., mean accuracy or assay variability) should be separately defined on the basis of scientific justification by considering the nature of the analytical methods.

If analytical methods with the same analytical principle with a minor modification are used in different studies, cross validation may be not performed in most cases, because the validity of the modified analytical method is usually verified by a partial validation.

Q6. Why does this guideline state, "the mean accuracy…at each level should be within ±20% deviation of the theoretical concentration"?

A6. The guideline requires that the mean accuracy of an analytical method at each concentration level should be within ±15% deviation of the theoretical concentration. For cross validation, acceptance criteria is set at 20% considering intra- and inter-laboratory precision.

If study samples are analyzed by different laboratories in the single study, an effort to minimize

inter-laboratory variations is necessary in addition to the analytical method validation. A handling of study samples and reference standards should be defined in the protocol or SOP for the analysis.

<< Incurred samples reanalysis (ISR) >>

Q7. Is ISR required for urine samples?

A7. ISR is mandatory for urine samples as well as for blood samples, if drug concentrations in urine are used as a primary endpoint in bioequivalence studies since no drug is detected in the blood. The need for ISR depends on the significance of urine concentrations.

Q8. How should I perform ISR in toxicokinetic studies?

A8. In a GLP toxicokinetic study, ISR should be performed once per matrix for each animal species. If an analytical method is modified or analysis is performed in a different laboratory, ISR should be performed again.

In addition, ISR can be performed during a bioanalytical method validation using study samples obtained from a non-GLP study such as a dose-finding study performed before a GLP toxicokinetic study. In this case, the study design, including dose and regimen, should be comparable to that of the GLP study.

Q9. How should I perform ISR in clinical trials?

A9. ISR should be performed in representative clinical trials whose pharmacokinetic data as a primary endpoint. To evaluate the validity of an analytical method in an early stage, ISR should be performed as early as possible in the process of pharmaceutical development.

In a clinical trial with a different population of subjects with altered matrix composition, ISR should be performed again. In a bioequivalence study which serves pharmacokinetic data as the primary endpoint, ISR should be performed in the study.

Q10. If study samples obtained from clinical trials are already available at the time of analytical method validation, can I use the samples for ISR?

A10. If you have already obtained study samples from a clinical trial at the time of analytical method validation, you can use the samples for ISR. For example, a metabolite is added to the analyte(s), or reanalysis is performed with an improved analytical method after a failure to meet ISR acceptance criteria. However, an informed consent must be obtained from each subject who provides the study samples. The procedures of ISR and related items should be predefined.

Q11. If overall results meet the ISR acceptance criteria, but the assay variability of a specific sample exceeds the threshold of ±20%, is it required to reanalyze the samples to correct first value?

A11. ISR is intended to confirm the validity of an analytical method using study samples. Therefore, reanalysis of individual study samples is not required to correct the first value, even though the assay variability exceeds the threshold of ±20% when overall result meets the ISR acceptance criteria.

Q12. Where in a report is appropriate to provide ISR results?

A12. When the ISR is performed in the study sample analysis, ISR results should be reported in a study sample analytical report to prove the validity of an analytical method. When the ISR is performed in the analytical method validation, ISR results should be reported in a validation report.

<< Carry-over during study sample analysis >>

Q13. Is it required to repeat assessment of carry-over during study sample analysis even if it is examined in the analytical method validation?

A13. The extent of carry-over may alter depending on the state of the analytical instrument used and the total number of samples analyzed. Thus, carry-over after the analytical method validation should be paid attention. In particular, carry-over should be assessed during study sample analysis, if carry-over cannot be avoided completely in the analytical method validation.

It is not required to report the carry-over in each assay run in a report of study sample analysis.

<< Reanalysis >>

Q14. What issues should be addressed in reanalysis for a pharmacokinetic reason?

A14. Reanalysis of study samples for a pharmacokinetic reason should be avoided, whenever possible, in order to maintain objectivity. If reanalysis due to pharmacokinetic reason is performed, the selection of reanalysis samples should be carefully considered, for example, are included the one before and one after blood sampling points of the questionable sample in the analytical run. In addition, procedures for reanalysis should be predefined, including the number of repeat and the selection of report values, in the protocol or SOP.

In principle, reanalysis of the study samples based on the analytical results obtained is not acceptable in a study using bioanalytical concentrations as a primary endpoint, such as bioequivalence studies. However, this does not restrict reanalysis for investigation and verification which does not replace the concentration data from first results.

<< Others >>

Q15. How should I perform analytical method validation for endogenous substances?

A15. This guideline does not cover the validation of an analytical method for an endogenous substance (e.g., vitamins, amino acids) in biological samples, even though it is administered as drugs; because such validation may involve some issues that are not appropriate for the application of specifications in this guideline. However, it is recommended to perform appropriate validation according to the specifications in this guideline.

It is acceptable to use an appropriate surrogate matrix to measure concentrations of endogenous substances in biological samples. In this case, the validity of the surrogate matrix should be shown in analytical method validation.