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ICH guideline M10 on bioanalytical method validation and study sample analysis – Frequently Asked Questions (FAQ)

Step5

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To support the implementation of ICH M10, the Expert Working Group has developed a series of FAQs.

Guideline section	Questions	Answers
1	The guideline states that it is applicable to "nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies…" Please provide an example for such studies.	One example which includes nonclinical PK data to support human dosing is rescue agents for acute radiation syndromes or anthrax etc., under the Animal Rule (FDA, United States).
2	In situations where a matrix is unavailable (e.g., shortage, 3Rs - Reduce, Refine, Replace) can a similar surrogate matrix (e.g., human plasma) be used to dilute samples?	Yes, as long as the use of the surrogate matrix meets the requirements of the guideline, including accuracy and precision, lack of interferences, etc. and the dilution quality control samples (QCs) are processed in the same way. The rationale needs to be well justified because the approach might be questioned.
2, 3, 4	When adding a new QC concentration level during study sample analysis without changing the calibration curve range in either chromatographic assays or ligand binding assays, is it necessary to validate the new QC concentration level with a partial validation?	The precision and accuracy of the new QC concentration level should be demonstrated before use in study sample analysis. This can be documented either as a partial validation or as a note to the bioanalytical report.
3	Is it acceptable to demonstrate the absence of analytical interference of the IS itself, any impurities or its isotopic stability based on the analytical results of the zero sample?	Yes, this is applicable for both method validation and study sample analysis.
3	For long-term stability, does a failed time-point mean you should not continue with longer time-points?	Additional time-points can be evaluated. Any failure should be investigated to identify the root cause and the impact on the stability assessment.
3	Can the physicochemical properties of the related substances be used to justify that the related substances do not co-elute or interfere with the analyte measurement during mass spectrometry (MS) analysis?	Yes, but if co-elution of the related substance and the analyte is not excluded, additional investigations are needed to demonstrate chromatographic separation (e.g., for isomers). If the analyte and the related substance co-elute, matrix effect (ion suppression/enhancement) and back-conversion should be evaluated.
3	What does the guideline mean with respect to "concentrations" in the following sentence: "The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution	The diluted concentrations should fall within the validated calibration curve range.

Guideline section	Questions	Answers
	factors and concentrations evaluated during validation"?	
3	What is the purpose of measuring the concentration of the QC at time zero?	To confirm the QCs were correctly prepared. Stability in the matrix (e.g., bench-top, long-term, freeze-thaw) should be evaluated by comparing with the nominal value.
3	How is the accurate preparation of the stock solution verified?	By comparing two independently prepared stock solutions and demonstrating that the difference of their measured responses is within 5%. % difference=(|Stock solution 1 - Stock solution 2|)/(mean value) × 100
4	Is there a requirement to test specificity in validation with an irrelevant immunoglobulin molecule when the analyte is an immunoglobulin and the assay contains analyte specific reagents (e.g., use of anti-idiotypic antibody(ies) as capture and/or detection reagents)?	There is no requirement to assess specificity in validation with an irrelevant immunoglobulin as long as the specificity of the reagent(s) has been evaluated during reagent characterisation.
5	How should trends of concern or incurred sample reanalysis (ISR) failure be investigated?	The investigation should be driven by an SOP and should take into account the entire process, including sample handling, processing and analysis. This should also include a scientific assessment of whether there are issues impacting the bioanalytical method, such as interferences and instability.
6	Given that M10 allows partial validation for matrices within species or same matrix across species, is an N-in-1 approach (multiple species or matrices in 1 validation) allowed for chromatographic methods for nonclinical studies?	Possibly this approach may be used. However, caution should be taken in using this approach; the rationale needs to be well justified, because the approach might be questioned.