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Summary

Charles River has established incurred sample reanalysis (ISR) as part of its bioanalytical process to support both nonclinical and clinical studies. ISR ensures that we continue to provide the highest quality study data, while meeting or exceeding industry and regulatory guidelines.

SAFETY ASSESSMENT

Incurred Sample Reanalysis

Background

Bioanalytical methods are validated to support both nonclinical and clinical studies. The validation process includes assessment of parameters such as accuracy and precision, and short- and long-term matrix stability. These method validations are performed using drug-free control matrix from animals or healthy human volunteers that is spiked with the test compound. However, samples from dosed subjects (incurred samples) could differ from the control matrix due to factors such as incurred instability, sample non-homogeneity, the presence of metabolites, protein binding, test compound recovery issues, and mass spectrometer ionization effects, among others. Therefore, quality control (QC) standards prepared by spiking the test compound into control matrix may not always mimic the activity of the test compound in incurred samples.

Discussions regarding the necessity of performing reanalysis of incurred samples began at the May 2006 AAPS/FDA Bioanalytical Workshop (Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays). The US Food and Drug Administration (FDA) had become increasingly concerned with the reproducibility of bioanalytical methods because, in some studies, results for reanalyzed samples did not match the original analysis results. Additional data reviewed from multiple bioequivalence (BE) and pharmacokinetic (PK) studies showed that, in some instances, the results from reanalyzed samples differed by as much as 350% from the original results. While the reasons for the performance of the ISR assessment were enumerated, few details were provided on the execution and acceptance criteria to be employed.

At the February 2008 AAPS/FDA Bioanalytical Workshop (Workshop Report and Follow-Up – AAPS Workshop on Current Topics in GLP Bioanalysis: Assay Reproducibility for Incurred Samples - Implications of the Crystal City Recommendations. Fast et. al., The AAPS Journal, Vol 11, No. 2, June 2009), industry leaders met to initiate discussions on the general procedures for conducting ISR. In May 2009, the workshop report and follow-up document on ISR was published, outlining the expectations for the conduct, documentation, and reporting of the ISR assessments.

The European Medicines Agency's Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr.) was the first regulatory guideline to detail how and when ISR should be conducted, along with the acceptance criteria for the assessment, with a 2013 FDA draft document providing similar (but not identical) guidance.

Conduct

For nonclinical studies, ISR is required once per species per method per laboratory, and the assessment is typically performed during the first definitive toxicology study with responsive dose. For clinical studies, ISR is required for all bioequivalence studies and for any studies in which PK evaluation of the drug is a primary endpoint of the study. To ensure that any method reproducibility issues are detected as soon as possible, it is expected that ISR is performed early in the study. Up to 10% of study samples should be selected for reanalysis. Sample selection should be performed based on the PK profile of the drug, with samples being selected from timepoints near the C_{max} and from near the end of the elimination phase.

These individual samples should be analyzed within proven stability using the same bioanalytical method (including number of replicates and any applied dilution factor) that was used for the original analysis. For the sample to be considered acceptable, at least two-thirds (67%) of the ISR results must be within 20% of the original sample result for small molecule bioanalysis, and within 30% of the original sample results for large molecule bioanalysis.

If any of the individual sample results do not meet the acceptance criteria, there is currently no regulatory requirement to perform additional analyses on those samples; however, scientific judgment is applied in any case where the difference between the two results is substantially different. If the overall ISR assessment does not meet acceptance criteria, then the sample analysis should be halted and an investigation performed and documented.

Investigation

Charles River has the expertise to perform investigative work that may be required as the result of an ISR failure. Even though the purpose of ISR is to evaluate the performance of the bioanalytical method, the first step of an investigation is to verify there were no errors in the execution of the study. Some of the items that would be explored during such an investigation are:

- Physical examination of the sample(s)
- Review of raw data (including second-person sample verification, dilution factors, chromatography, and internal standard response)
- Recent method modifications or updates
- Recent instrument maintenance
- Changes in the analytical reference material
- Changes in the calibration or QC standards
- Changes in the matrix supplier

If operational errors are ruled out, scientific parameters of the method are evaluated. These can be broad-ranging in scope, depending on the details of the bioanalytical method. Some of the scientific parameters that might be explored during such an investigation are:

- Incurred sample stability
- Chromatographic separation
- Ion suppression
- Extraction recovery

The conclusion to an investigation may result in modifications to the bioanalytical method, which may require additional validation. Sample analysis would be repeated with the updated method, along with ISR, confirming that the modified method is reproducible.

Conclusion

Charles River remains focused on client and regulatory expectations. Procedures for performing, reporting and investigating ISR are documented in our organization's standard operating procedures (SOPs). These SOPs are regularly reviewed and, where appropriate, updated to ensure that we meet industry and regulatory expectations and provide the most reliable and highest quality study data.

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