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Review Article

Theme: Best Practices for Bioanalytical Methods: Recommendations from the Global Bioanalysis Consortium
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Repeat Analysis and Incurred Sample Reanalysis: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team

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Abstract. The A7 harmonization team (A7 HT), a part of the Global Bioanalysis Consortium (GBC), focused on reviewing best practices for repeat analysis and incurred sample reanalysis (ISR) as applied during regulated bioanalysis. With international representation from Europe, Latin America, North America, and the Asia Pacific region, the team first collated common practices and guidance recommendations and assessed their suitability from both a scientific and logistical perspective. Subsequently, team members developed best practice recommendations and refined them through discussions and presentations with industry experts at scientific meetings. This review summarizes the team findings and best practice recommendations. The few topics where no consensus could be reached are also discussed. The A7 HT recommendations, together with those from the other GBC teams, provide the basis for future international harmonization of regulated bioanalytical practices.

KEY WORDS: harmonization; incurred sample reanalysis; regulated bioanalysis; repeat analysis.

INTRODUCTION

For decades, bioanalytical practitioners across the globe have relied upon the "Crystal City" (CC) white papers (1–3) and the FDA Guidance (4) for direction and standards on bioanalytical method validation (BMV) and sample analysis. In 2009, upon hearing of the likely release of new or revised BMV guidance from several international regulatory bodies (Food and Drug Administration (FDA), European Medicines Agency (EMA), Agência Nacional de Vigilância Sanitária (ANVISA), Ministry of Health, Labour and Welfare (MHLW)), bioanalysts felt an increased need for a global guidance that would provide a harmonized approach to BMV and be acceptable to all regulatory agencies regardless of where the samples were analyzed or in which country the drug application would be submitted. With this goal in mind, a small group of industry experts, with support from CT Viswanathan (working for FDA at the time) and J Welink (from EMA) formed the Global Bioanalysis Consortium (GBC) in 2010 (5). To achieve their lofty goal, the GBC steering committee assembled 20 harmonization teams, each with members from across the globe and assigned them subtopics for which they were to develop harmonized global recommendations. This paper describes the consensus recommendations of the GBC Repeat Analysis and Incurred Sample Reanalysis harmonization team (A7 HT). The team objective was to make practical and scientifically based recommendations on best practices for repeat analysis, including incurred sample reanalysis (ISR), in an effort to harmonize practices globally within the regulated bioanalytical space. This team used discussions among its members and at international meetings to develop its recommendations. While the resulting recommendations should be applicable to many situations, unique circumstances may require different approaches and some flexibility should

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be allowed based on scientific judgment. Furthermore, consensus could not be reached on a few issues, all of which are pointed out within these recommendations.

BACKGROUND

During the application of a validated bioanalytical method for study sample analysis, repeat analysis of study samples may be needed for various reasons. These reanalyses may be categorized into three major categories, which the A7 HT focused on. These are as follows:

- Repeat analysis for an "assignable cause" or "analytical reasons."
- Repeat analysis due to "incongruous results" or for "pharmacokinetic (PK) reasons."
- Repeat analysis to determine the reproducibility of the analysis, also referred to as "incurred samples reanalysis" (ISR).

Repeat analysis for assignable causes or analytical reasons (*e.g.*, instrument failures, runs that do not meet acceptance criteria, values above the limit of quantitation) are generally not very controversial. It is widely accepted that for samples for which there is no valid result following the original analysis, the first valid reanalysis result should be reported as the final value. These repeats are generally performed with the same number of replicates as the original analysis (typically in singlicate for liquid chromatography/mass spectrometry (LC/MS) assays and duplicate for ligand binding assays). Repeat analysis for reasons associated with analytical errors and failures was addressed in the first CC conference report (1). This type of repeat analysis can become more complex in assays where two or more analytes are quantitated simultaneously. The FDA guidance (4), drafted after the second CC meeting in 2000 (2), clearly states that for multi-analyte assays, the data for analytes that meet run acceptance criteria should not be rejected just because another analyte fails in the same run. The third CC conference report (3) provides further clarity on repeat analysis for multi-analyte assays, indicating that only the data for analytes that failed in the original analysis need be quantitated when samples are reanalyzed. A related topic discussed during the third CC meeting, but for which there was no consensus, was the acceptance criteria for internal standard (IS) response. However, it was noted that if study samples were to be reanalyzed based on IS variability, an objective criteria should be established *a priori*. The EMA guidance (6) takes a similar position in its recommendation for the IS response, as well as other aspects of repeats for analytical failures. Interestingly, there was no mention of "reanalysis" in the first Brazilian bioanalytical guidance released by ANVISA in 2003 (7) and the first inclusion of the expression occurred in 2006, in ANVISA Resolution 1170 (8). The theme was explored further in 2012, in ANVISA Resolution RDC no. 27 (9), which defines what is considered reanalysis and which samples may be subject to reanalysis, but in which no criteria were established.

While the first CC conference report (1) recommends a cautious use of "pharmacokinetic fit" as justification for repeat analysis, it does not make any recommendation other than that the protocol for repeat analysis be established *a priori*. The FDA guidance (4) provides a little more clarity on repeat analysis and recommends that reassay for inconsistent PK data be performed in triplicate provided there is enough sample volume. This was later clarified to mean duplicate analysis at the third CC meeting in 2006 (3). While repeat analysis due to incongruous PK data has been allowed by the FDA guidance, the EMA and MHLW bioanalytical guidance take a stronger position on this type of repeat analysis, stating that normal reanalysis for PK reasons is not acceptable in bioequivalence (BE) studies (6,10).

Incurred sample reanalysis became an expectation in regulated bioanalysis after FDA shared concerns over discrepancies observed between original and repeat analysis results from numerous submissions. The topic was discussed at the third CC conference (3) and the resulting recommendation was to reanalyze a subset of the study samples for both clinical and non-clinical studies to verify assay reproducibility. Ironically, Health Canada had previously dropped a requirement for ISR as part of regulated bioanalysis. The third CC conference report recognizes that the variability may be higher in clinical studies due to inherent variability between subjects and therefore recommends that consideration be given to factors such as concentration, patient population, and study phase when conducting ISR. Since the third CC report did not provide recommendations on how to perform ISR and lacked specific details on scope and acceptance criteria, a focused ISR workshop was held in 2008 and the workshop report provides specific recommendations on timing and scope of ISR, as well as sample selection and acceptance criteria (11). Both the EMA and MHLW bioanalytical guidance provide details about sample selection, acceptance criteria, and the number of samples required for ISR (6,10). With a few exceptions, these guidances are generally well aligned with the recommendations from the 2008 ISR workshop. However, the recently release draft of FDA guidance on BMV provides some unique recommendations on the amount of ISR to perform (12). As was the case for repeat analysis, the Brazilian ANVISA Guidance does not address ISR (9).

GUIDING PRINCIPLES AND COMMON APPROACHES

The A7 HT began by setting forth several guiding principles on which to base its work. Key among the principles the team identified were the following:

- Recommendations should enable the application of consistent, scientifically sound criteria across the method validation and method application space.
- Where possible, endorse existing and well-established criteria and approaches.
- Emphasize simplicity in approaches and reduce the number of decision points requiring unique calculations, while still ensuring that a high level of quality is maintained.

These principles were aimed at focusing the attention of the team on issues for which multiple approaches or interpretations of guidance exist or for which there had previously been no clear consensus opinion. The team also wanted to identify and make recommendations on common practices which may not be based on scientific precedence.

With the guiding principles above in mind, common approaches for repeat analysis and ISR were grouped into two major categories:

- Approaches that are readily implemented, logical, and for which there was generally a consensus (categorized as approaches with general consensus).
- Approaches needing refinement because they either lack consensus, are difficult to implement, or do not make scientific sense (categorized as approaches needing refinement).

The recommendations of the A7 HT aim to maintain the common practices that are working well and make sense, and resolve issues with practices that are difficult to implement, do not make sense, or for which a consensus approach is lacking in current guidance documents and white papers. The team's categorization of common practices into these groups follows.

Repeat Analysis—Approaches with General Consensus

The following principles and approaches to repeat analysis of study samples are considered well established and appropriate as outlined in current guidance and white papers:

- Procedures for the repeat analysis of study samples should be described *a priori* in a standard operating procedure (SOP). The procedures should include acceptable reasons for reanalysis, acceptance criteria for repeats, reporting criteria, and documentation requirements.
- Repeats for analytical cause represent cases where a valid analytical result was not obtained in the initial analysis. Examples of repeats for analytical cause include the following: instrument failures, documented method deviations, run failures (acceptance criteria not met), poor chromatography in LC/MS assays, incongruence of replicate results in ligand binding assays (LBAs), results above the limit of quantitation (ALQ), results below the limit of quantitation (BLQ) after dilution, or a BLQ result when the lowest standard is dropped. Repeats for analytical cause should be performed with the same number of replicates as the original analysis (*e.g.*, singlicate for typical LC/MS assays and duplicate for typical LBAs). Sample reinjection requires reinjection reproducibility and stability be established during validation of LC/MS assays.
- Repeats for PK reasons represent cases where a valid analytical result was obtained in the initial analysis but where the result is pharmacokinetically incongruous. Examples of repeats for PK reasons include quantifiable analyte levels in pre-dose, control animal, or placebo subject samples. Other examples may include a BLQ result bracketed by readily quantifiable values in the middle of a concentration-time profile or results suggesting an obvious sample switch. Repeats for PK reasons are discouraged in BE studies unless part of a formal bioanalytical investigation. This ensures appropriate documentation around the decisions taken. When repeats for PK reasons are conducted as part of non-BE studies, they should be guided by an SOP or investigation plan. SOP-guided selection of PK repeats in non-BE studies may be performed by either the pharmacokineticist or, in certain cases, bioanalytical staff (*e.g.*, non-clinical analysis of control animal samples).
- Samples subject to repeat analysis due to PK reasons should be documented in the report along with the initial value, reason for reanalysis, reanalysis results, the reported value, and the reason for selection of the reported value.

Repeat Analysis—Approaches Needing Refinement

The following principles and approaches to repeat analysis are considered as either difficult to implement, not making sense, or lacking consensus on approach:

- When to report results for all analytes *versus* only an individual analyte during the conduct of repeat analysis for multi-analyte assays.
- Acceptance criteria for replicate repeats when repeat analysis is conducted for PK reasons.
- Criteria for selecting the reported value after replicate repeat analysis for PK reasons. For example, whether to report the original value, median, or mean under various scenarios.
- Requirements for reinjection of partial analytical runs in LC/MS assays.

Incurred Sample Reanalysis—Approaches with General Consensus

The A7 HT share a consensus view that ISR experiments contain both scientific and quality control components and that there are situations where the performance of standards and quality controls (QCs) may not mimic that of study samples. It is also recognized that ISR adds to the cost of drug development and as such, its application as a quality control measure should be limited to studies where traditional process controls (performance of standards and QCs) may not identify issues and to the most critical studies where PK information is obtained (*e.g.*, BE studies).

The following principles and approaches to ISR of study samples are considered well established and appropriate as outlined in current guidance and white papers:

- ISR should be conducted using procedures defined in an SOP or study analytical plan.
- In general, ISR should be conducted on individual study samples and in a separate run from the original analysis.
- ISR should be performed early and throughout the conduct of a study where practical (*e.g.*, large clinical studies).
- For non-clinical good laboratory practice (GLP) studies, ISR only needs to be conducted once per species for each method, matrix, and laboratory.
- ISR should be conducted on samples that represent both the high and low concentration range of results obtained, thereby capturing samples near the C_{max} and elimination phase.
- ISR should be done as part of the conduct of the following clinical studies: first-in-human trials (single and multiple dose studies), first-in-patient trials, special population trials where changes in metabolism may be expected (*e.g.*, trials in hepatic or renal impaired subjects), BE, and biocomparability trials.

- ISR should be performed following transfer of an analytical method to a new laboratory and after significant changes to the method.
- For ISR results to be acceptable, at least two thirds (66.7%) of ISR results should meet acceptance criteria.

Incurred Sample Reanalysis—Approaches Needing Refinement

The following principles and approaches to ISR are considered as either difficult to implement, not making sense, or lacking consensus on approach:

- Selection of ISR samples based on visual inspection, random selection, or a defined algorithm. Also, whether to select samples that were diluted or near the lower limit of quantitation (LLOQ).
- Selection of ISR samples for multi-analyte assays and whether to prioritize selections based on a particular analyte.
- The number of samples to reanalyze for ISR assessments. Application of a tiered approach (*e.g.*, 10% of samples are reanalyzed for the first 1,000 study samples and an additional 5% of samples are reanalyzed for study samples in excess of 1,000, as recommended in the EMA guidance (6)) *versus* a simpler single-tier approach (*e.g.*, 7% of all unknown samples, as recommended in the draft of FDA guidance (12)). Application of a minimum number of ISR samples.
- Whether to conduct ISR on all drug-drug interaction studies.
- The appropriate limits for acceptable variability between results (*e.g.*, 20% for typical LC/MS methods and 30% for typical LBAs).
- When to place a method on hold after an ISR failure. Individual run *versus* complete study failure.
- Whether and how to address individual ISR failures.
- How to deal with multi-analyte assay results when ISR fails.
- Requirements for ISR investigations.

REPEAT ANALYSIS AND ISR BEST PRACTICE RECOMMENDATIONS

This section provides the A7 HT best practice recommendations for repeat analysis and ISR. The practices described represent the consensus views of the team and are viewed as appropriate for typical bioanalytical methods (both LC/MS and LBA assays). As always, unique circumstances may require different approaches (*e.g.*, when working with complex modalities). In these cases, good scientific judgment and appropriate documentation of the methods and decisions made are important.

It is very important that SOPs, including within-study analysis SOPs or study-specific analysis plans (in particular the activities at CROs), be in place prior to the start of study sample analysis activities. These SOPs and/or plans should guide all aspects of repeat analysis, ISR, and associated investigations.

Reanalysis of Study Samples

In general, sample reanalysis for known and documented analytical reasons are typically acceptable. Sample reanalysis for incongruous results or for pharmacokinetic reasons is discouraged, especially in BE studies, and should be supported by a SOP and/or investigation with documented justification.

Repeats for Analytical Reasons (Assignable Cause)

The following are the common analytical reasons that trigger reanalysis of study samples using the same number of replicates as the original method describes (*e.g.*, typically singlicate for LC/MS and duplicate for LBAs). In these cases, only the results from the reanalysis should be reported.

- Rejected bioanalytical assay runs where the batch acceptance criteria were not met (*e.g.*, unacceptable calibration standards, QC samples, or IS results).
- The calculated analyte concentration is above the upper limit of quantification (ULOQ).
- The calculated analyte concentration is below the adjusted LLOQ in an analytical assay run where the LLOQ was raised because the results of the original LLOQ calibration standard failed to meet the batch acceptance criteria.
- The obtained analyte concentration from a pre-diluted sample is BLQ.
- Loss of sample during processing.
- Incongruence of replicate results in LBAs.
- Documented (observed) sample processing errors or deviations from the validated method including but not limited to the following: (1) improper sample mixing, (2) use of wrong reagents, and (3) failure to add a critical reagent (*e.g.*, detection reagent in LBAs or IS in LC/MS).
- Poor chromatography in LC/MS assays.
- Instrument malfunction.
- For multi-analyte assays, reanalysis of study samples is performed for a specific analyte or all analytes depending on the assigned cause.
- During LC/MS analysis, reanalysis of samples based on unexpected IS response (*e.g.*, no IS peak), trends in IS response, or differences between incurred and spiked sample IS response should be driven by SOP or an investigation. Due to the variety of scenarios which could warrant reassay consideration, consensus on a single set of criteria for selecting, reanalyzing, and reporting these samples was not reached. Nonetheless, the same scientific principles for sample reanalysis should be applied for IS issues. For example, a sample with no IS response may be treated as a repeat for analytical reasons (no IS spiked) and could be repeated in singlicate, as no valid bioanalytical result was obtained in the initial analysis.

Reinjection of Extracted Samples and Rescanning LBA Plates

Under certain circumstances the reinjection of previously extracted study samples either as a full or partial batch may be justified provided reinjection reproducibility and/or on-instrument stability have been demonstrated during method validation of LC/MS methods. Partial batch reinjection must

include a system suitability test and minimum of two passing QCs (ideally bracketing the placement of unknown samples) for which acceptance criteria must be defined in the sample analysis SOP or bioanalytical study plan. Overall run acceptance criteria must also be met. The SOP should also address the treatment of any data obtained during the original batch injection. Common reasons for reinjection of extracted samples may include but are not limited to the following:

- Documented (observed) sample injection failures (consecutive samples).
- Instrument malfunction.
- Poor chromatography as a result of use of a wrong/bad column and/or wrong mobile phase, in which case the entire batch should be reinjected.

Reinjection of a full analytical run or of individual calibration standard samples or QC samples simply because the calibration standards or QCs failed, without any identified analytical cause, is not acceptable. Furthermore, selection of individual study samples for reinjection without a valid analytical reason and supporting documentation should be avoided. For multi-analyte assays, reinjected samples should be analyzed for all analytes.

Similarly, on rare occasions, it may be acceptable to reread a LBA plate in the course of sample analysis. This may be the result of an instrument failure or an inappropriate instrument setting (*e.g.*, incorrect wavelength). In this case, consideration should be given to the length and impact of the delay in reading the plate. The original (if obtained) and final data should be documented along with a description of the event.

Repeats for Incongruous Results or for Pharmacokinetic Reasons

As mentioned previously, sample reanalysis for incongruous results or for pharmacokinetic reasons should be minimized. Acceptable reasons for reanalysis related to incongruous results may include but are not limited to the following:

- Measurable concentrations in control and placebo samples.
- BLQ or no concentration in the middle of a concentration-time profile.
- Possible sample switch (documented switches should be treated as analytical errors).

Such cause should be supported by a SOP and/or laboratory investigation with justification. All repeat analyses due to incongruity should be conducted with duplication (*e.g.*, analyzed in duplicate in the case of LC/MS and with two sets of duplicates in the case of LBAs) and where feasible prior to analytical data lock. As mentioned previously, repeats for PK reasons are discouraged in BE studies unless part of a formal bioanalytical investigation. This ensures appropriate documentation around the decisions taken.

Repeat Analysis Acceptance Criteria

Criteria for the acceptance and reporting of repeat analyses should be pre-defined in an SOP or protocol prior to the start of sample analysis. The document governing repeat analysis should provide clear guidance for the acceptance of the repeat analysis and the selection of the reported result.

A recommended decision paradigm for repeat analysis is provided below:

- If the sample is repeated for an analytical reason and without replication, the reassay value should be the reported value.
- If the sample is repeated as incongruous and with replication, the following calculations and comparisons should be used to accept the repeated results and select the reported value:
  - For the repeat analysis to be acceptable, the difference between the duplicate repeat values should be <30% (<40% for LBA assays) as calculated below:

$$[(\text{repeat } 1 - \text{repeat } 2)/\text{mean}] \times 100 < 30\%(40\% \text{ for LBA})$$

    If this condition is not met, reject the reanalysis and perform repeat analysis again or report "no result" (NR, e.g., in case of insufficient sample volume).
  - If the repeat values are acceptable, the selected value to report should be the median of the original value and the two repeat values (if the original value was BLQ, report the smaller of the two repeat values). The median value is recommended as it provides a statistically meaningful result without a complex decision tree and additional calculations, thereby reducing the potential for reporting errors.
- In cases where the formula above cannot be applied due to lack of numeric values (*e.g.*, BLQ or ALQ):
  - Report BLQ if both reassay values are BLQ.
  - If both values are ALQ, reject the reanalysis and perform repeat analysis again with dilution or report NR.
  - If one repeat value is numeric and the other is BLQ or ALQ, select the reported value based on good scientific judgment and a comparison of all three values (original and repeats) or report NR. In this case, a clear rationale for the selected value should be provided in the data file and report (*e.g.*, selected value represents median of resulting data).

In cases where insufficient volume exists for duplicate analysis, repeat analysis in singlicate may be performed. If original result and repeat value are within 30% of their mean (40% for LBA), the median (which in this case would be the mean value) should be reported. In cases where the two values are not within 30% (40% for LBA), the reported value should be selected based on good scientific judgment, or report NR. Again, a clear rationale for the selected value should be provided in the data file and report.

Documentation and Reporting of Repeat Analysis Results

Study documentation should include the reason for the repeat analysis, the initial (where available) and repeat analysis results, the reported result, assay run identification, and the rationale for selecting the reported result. Samples reanalyzed due to run failures do not need to be included in

the sample reanalysis table in the report; however, the fact that a run failed and the reason should be included in the report. Any deviations, justifications, and investigations in relation to repeat analysis should also be discussed in the analytical report.

Incurred Sample Reanalysis

Incurred sample reanalysis (ISR) has both scientific and quality control components when properly applied to regulated studies. For small molecules measured by LC/MS, ISR provides an indirect assessment of analyte and metabolite stability under the conditions of the assay. In the case of large molecules measured by LBAs, ISR may also identify unique issues related to factors such as aggregation or interference from anti-drug antibodies. In any case, ISR ensures the bioanalytical method has been applied in a manner that provides consistently reproducible results. Generally, ISR should be conducted at the same dilution as the original analysis. Unless ISR results suggest the need for further investigation, ISR results should not replace the original reportable result and should not be used in pharmacokinetic analysis. Recommendations on the timing, amount, selection, and acceptance criteria for ISR are provided below.

Timing of ISR

It is recommended that ISR be performed early and on an ongoing basis throughout the study to allow timely detection of assay problems. Generally, ISR should be carried out in an independent analytical run from the original analysis. Exceptions should be detailed in the bioanalytical method or bioanalysis plan prior to the start of analysis (*e.g.*, if limited freeze/thaw stability has been demonstrated). ISR can be conducted in a single run for smaller studies or in multiple runs on a rolling basis for large studies. ISR samples may be analyzed in the same run as previously unanalyzed samples. Use of additional sample aliquots (sometimes referred to as "splits") for ISR is acceptable.

Sample Selection for ISR

The following principles are recommended when performing sample selection for ISR:

- Samples for ISR analysis may be selected based on visual inspection of concentration-time profiles.
- Samples should be selected across the concentration-time profile in such a manner that they are representative of the range of results obtained during initial analysis. As such, samples providing results across the observed concentration range (including the lower and upper range) should be selected.
- When possible, selection of samples <3× LLOQ and >80% of the ULOQ should be avoided as these sample may result in BLQ or ALQ results, rendering the interpretation of reanalysis impossible. However, if such samples are reassayed during ISR, samples resulting in BLQ or ALQ should not be included in the statistical evaluation and do not require additional analysis.
- For multi-analyte assays performed using LC/MS, selection of samples for ISR should be based on the primary active entity. It might be unavoidable to have one or more secondary analytes (*e.g.*, metabolite or prodrug) with a majority of samples <3× LLOQ. For these analytes, the results of ISR will be reported but not subject to statistical analysis and acceptance should be based on the primary active entity.
- For multi-analyte assays containing two or more major active entities (*e.g.*, co-medication), consideration should be given to ensuring that samples are selected across the measurable concentration-time profiles for all active entities (analytes). As mentioned earlier, if concentrations for some analytes fall below 3× LLOQ (by necessity), the results of ISR for those samples will be reported but not used in the statistical analysis.

Number of Samples Used in ISR

ISR should be conducted on at least 5% of the study samples analyzed in applicable studies. This approach is consistent with the number of QC samples run during a typical bioanalysis run and should provide enough information to achieve both the scientific and quality goals of ISR. A minimum of 6 samples should be selected for ISR in all studies, although it is noted that many on the team use a greater number in order to avoid investigation based on a small number of results (*e.g.*, minimum of 21 or 24).

Application of ISR (Study Selection)

At a minimum, ISR should be performed for the studies described below:

- For non-clinical GLP studies (*e.g.*, regulated non-clinical toxicokinetic evaluations), ISR should be conducted at least once per species for each method, matrix, and laboratory.
- ISR is recommended for the following clinical evaluations:
  - First-in-human trials (single and multiple dose)
  - First-in-patient trials
  - Drug-drug interaction trials
  - Trials in hepatic or renal impaired subjects
  - Bioequivalence and biocomparability trials
  - Pivotal phase III trials
  - First time use of a method in a new laboratory

ISR Acceptance Criteria

The difference between the concentration obtained for the initial analysis and the concentration measured during ISR should be within ±20% (±30% for LBA) of the mean of the two values for at least two thirds (67%) of ISR results. The difference is calculated as shown below.

$$[(\text{original} - \text{ISR})/\text{mean}] \times 100 \text{ within} \pm 20\% (30\% \text{ for LBA})$$

ISR Investigations

The decision on when to initiate an ISR investigation depends on numerous factors including the ISR strategy employed (*e.g.*, rolling or at end of study), the size of the

study, and the details of the ISR results. For example, when conducting ISR on a rolling basis, it may not be necessary to halt analysis and initiate an investigation based on the "failure" of a single run if a small number of ISR samples were analyzed and the results were not highly aberrant. Furthermore, for multi-analyte assays performed by LC/MS, ISR failure on one analyte should trigger investigation on that analyte only and not necessarily analytes for which ISR passed. Scientific judgment should be employed and certainly an investigation should be considered if ISR results from two successive runs fail to meet acceptance criteria. In any case, all ISR investigations should be fully documented and guided by an SOP.

When conducting an investigation to elucidate the root cause of ISR failure, initial steps to consider may include the following:

- Review of assay documentation to identify any errors in the conduct of the method, sample sequence, use and expiration of critical reagents, sample dilutions, sample extraction (*e.g.*, LC/MS), or instrument set-up.
- For LC/MS methods, review of chromatograms with a focus on data processing, peak responses, and possible contamination.
- Special attention should be paid to trends apparent within or between individual subjects, runs, or even studies, taking into consideration potential impact of specific matrix effects.

A variety of approaches may be taken to resolve ISR failures depending on the initial investigation findings. Key decision points and acceptance criteria should be detailed in the investigation plan, which should be updated as additional information becomes available. The investigation plan and summary should be subject to appropriate review and approval. It is recognized, however, that each case will be unique and the investigation approach and path forward may vary.

Corrective actions taken to address investigation findings will also vary from case to case but could include one or more of the following:

- Repeat of one or more ISR runs.
- Reassay of all samples initially assayed in a production run that is confirmed problematic by ISR and investigation.
- Change of sample handling procedure to minimize the impact of instability of analyte(s) or their metabolites (*e.g.*, phase II conjugates).
- Redevelopment of the bioanalytical method (sample preparation, critical reagent selection, chromatography, *etc.*) followed by assay revalidation and sample reanalysis.
- Conduct of additional personnel training.

In general, any event not pre-defined in a SOP should be addressed in the investigation based on scientific rationale, and related corrective action should be discussed in the analytical report.

The A7 HT discussed the treatment of ISR failures for individual samples at length but could not reach consensus on a recommendation for dealing with these results when the overall ISR meets acceptance criteria. Two of the common approaches for dealing with individual ISR failures that were discussed are provided below:

- Conduct investigations or reanalysis of samples where the difference between the original and ISR result is >x% of their mean (preference for the value of x varied between team members, but values between 30 and 50% were most common).
- No investigation or reanalysis of individual ISR failures is performed unless a pharmacokineticist requests reanalysis due to incongruity.

One key area of debate was the selection of a non-arbitrary threshold for triggering the investigation of an individual ISR failure. Also discussed was the concept that ISR is conducted to identify systematic errors and investigation of individual ISR failures expands the quality control aspects of ISR beyond that scope and, therefore, may not be warranted.

CONCLUSION

The A7 HT used white papers, regulatory guidance, and discussions at scientific meetings to review and assess common practices for repeat analysis and ISR during regulated bioanalysis. Through these efforts, the team has developed best practice recommendations for repeat analysis and ISR with the aim of progressing toward international harmonization. While a few topics remain where a single consensus approach was not reached, alignment was achieved on the majority of issues, and the team feels the current recommendations provide the foundation for alignment of international practices during regulated bioanalysis.

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