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ICH M10 Guidance: Harmonization and Modification to Bioanalytical Method Variation

Global regulatory standards govern bioanalytical method development, validation and subsequent assays. When those standards are updated, industry stakeholders should actively partake in their evolution and prepare for the ensuing changes. On May 24, 2022, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) adopted the harmonized guideline "Bioanalytical Method Validation and Study Sample Analysis M10". The guideline has been implemented by many national regulatory authorities such as NMPA, US FDA, EMA, Health Canada, Swissmedic et al. Drug developers need to prepare for the changes of M10 in bioanalysis and adjust resources, methods and testing to comply with this latest regulatory standard.

ICH synchronizes regulatory expectations in an increasingly global drug development industry. The new multidisciplinary guidance, ICH M10, aims to help the industry further harmonize bioanalytical method validation standards internationally.

Harmonization can help drug developers maximize the potential of their team's development efforts, streamline regulatory approvals and ease the strain of keeping up with regulatory standards' discrepancies. However, teams must stay updated on approaching changes or new regulatory guidance.

Bioanalytical experts at WuXi AppTec analyzed M10 and pinpointed the most salient aspects that differ from current guidance and will impact bioanalytical programs. WuXi AppTec's goal is to ensure drug developers are aware of the variations and prepared to adopt and implement the new guidance seamlessly.

Significant Changes for Drug Developers

ICH M10, effective May 24, 2022, has drug developers and laboratories searching to understand how it will affect their programs and bioanalytical processes. Though the document is extensive, not all the information is new. The following sections identify the most compelling insights within the standard. By proactively applying them to drug development pathways, teams can get up to speed and account for modifications when planning and testing.

Bioanalytical Method Development

Proper method development work should design suitable bioanalytical assays and test assay conditions to support the studies' intended needs. Prior to method development, the bioanalyst should thoroughly understand the analyte of interest (physicochemical properties of the drug, mechanism of action and protein binding characteristics) and the limitations of reagents, biomatrix and study materials.

Bioanalytical Method Validation

Guidance on bioanalytical method validation has been in flux for years as industry experts debate its most beneficial and superfluous aspects. ICH expects M10 to integrate the advantageous components of similar guidance and harmonize with global standards.

Proper validation is central to any drug development program because it supports data reliability, assay performance acceptability, preparation for pharmacokinetic and toxicokinetic studies, and more. Full, partial and cross validations are necessary in the following situations:

- ***Full validation*** is performed when establishing and implementing a bioanalytical method in pivotal nonclinical studies and clinical studies.
- ***Partial validation*** is performed when there are modifications to a fully validated method.
- ***Cross validation*** is performed when a comparison of validation parameters is needed because two or more bioanalytical methods are used within a study or across studies. This can occur when multiple laboratories, using an identical method, supply data using the same procedures.

When issues arise in estimated dose levels, drug concentrations, assay sensitivities and ranges during data review (e.g., varying results that challenge original expectations), bioanalytical method validations can demonstrate the applicability of a developed method. Bioanalytical validation guidance outlined in ICH M10 aims to mitigate concerns about addressing variation in results and allows for a relevant interpretation of the data.

Cross validation procedures have gained the most attention in M10 guidance. Each laboratory or drug developer often has their own approach to cross validation. Guidance helps to detail when to perform cross validation by providing scenarios and requirements.

Page 36 of the ICH M10 guidance states that cross validation is required to compare data under certain circumstances. They include:

- Data are obtained from different fully validated methods within a study.
- Data are obtained within a study from different laboratories using the same bioanalytical method.
- Data are obtained from different fully validated methods across studies that are going to be combined or compared to support special dosing regimens or regulatory decisions regarding safety, efficacy, and labeling.

- Data are obtained within a study from different laboratories with the same bioanalytical method.

If any of these scenarios apply to your testing program, Section 6.2 in M10 provides guidance on approaching cross validation and acceptance criteria. For example, data obtained from different laboratories within a study triggers the need for cross validation, especially when supporting dosing regimen evaluation or regulatory decisions around safety, efficacy and labeling.

For assay validation, ICH M10 provides detailed instructions for matrix usage, calibration standards, and QC preparation. It is recommended that the matrix used for bioanalytical method validation should be the same as the matrix of the study samples, including anticoagulants and additives. In cases where it is difficult to obtain an identical matrix to that of the study samples (e.g., rare matrices such as tissue, cerebrospinal fluid, bile or in cases where free drug is measured), surrogate matrices may be acceptable for analytical method validation. Beyond that, matrix differences within species (e.g., age, ethnicity, gender) are generally not considered different when validating a method.

It is also suggested that calibration standards and QCs should be prepared from separate stock solutions for the chromatographic analysis method. However, calibration standards and QCs may be prepared from the same stock solution provided the accurate preparation and stability of the stock solution have been verified. Besides, internal standards (IS) should be added to all calibration standards, QCs and study samples during sample processing. The absence of an IS should be justified.

Selectivity

In the selectivity section, ICH M10 emphasizes that selectivity should be evaluated using blank samples obtained from at least six individual sources/lots (non-hemolyzed and non-lipemic) for the chromatographic analysis method. The use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated.

For the LBA method, selectivity is evaluated using blank samples obtained from at least 10 individual sources, spiking the individual blank matrices at the LLOQ and the high QC level. The use of fewer sources may be acceptable in the case of rare matrices. The response of the blank samples should be below the LLOQ in at least 80% of the individual sources. Selectivity should be assessed in samples from relevant patient populations (e.g., renally or hepatically impaired patients, inflammatory or immuno-oncology patients, if applicable). In the case of relevant patient populations, there should be at least five individual patients.

It is also advised that selectivity should be evaluated in lipemic samples. A naturally lipemic matrix with abnormally high levels of triglycerides should be obtained from donors. When it is difficult to obtain a lipemic matrix from donors, it can be spiked with triglycerides even though it may not represent study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidemic, the use of spiked samples is discouraged. This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism or is administered in a hyperlipidemic animal strain. Selectivity should be evaluated in hemolyzed matrices with at least one matrix source.

Stability

In the stability section of ICH M10, stability evaluations should be carried out to ensure that every step taken during sample preparation, processing and analysis, as well as the storage conditions used, do not affect the concentration of the analyte. Since sample dilution may be required for many LBA methods due to a narrow calibration range, the concentrations of the study samples may be consistently higher than the ULOQ of the calibration curve. If this is the case, the concentration of the QCs should be adjusted, considering the applied sample dilution, to represent the actual sample concentration range. The same principle applies to chromatographic analysis as well. On the other side, it is recognized that this may not be possible in nonclinical studies due to solubility limitations.

For the stability validation of the chromatographic analysis method, the stability of the analyte in processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. For example:

- Stability of the processed sample under the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase).
- On-instrument/autosampler stability of the processed sample at injector or autosampler temperature.

The total time that a processed sample is stored must be concurrent (i.e., autosampler and other storage times cannot be added together).

Incurred Sample Reanalysis (ISR)

In the ISR section, it is suggested that ISR should be performed at least in the following situations:

- For nonclinical studies within the scope of this guideline, ISR should generally be performed at least once per species.
- All pivotal comparative BA/BE studies.

- First clinical trial in subjects.
- Pivotal early patient trial(s), once per patient population.
- First or pivotal trial in patients with impaired hepatic and/or renal function.

An investigation of ISR failure has been discussed in this section. It is advised that if the overall ISR results fail the acceptance criteria, an investigation should be conducted, and the causes remediated. If ISR meets the acceptance criteria yet shows large or systemic differences between results for multiple samples, this may indicate analytical issues, and it is advisable to investigate this further. Examples of trends that are of concern may include:

- All ISR samples from one subject fail.
- All ISR samples from one run fail.

Individual samples that are quite different from the original value (e.g., > 50%, "flyers") should not trigger reanalysis of the original sample and do not need to be investigated.

One area of testing unaddressed by ICH M10 is the use of automation during method validation.

Automation can remove the potential for human error and improve efficiency in sample analysis. Yet, most guidance lacks acknowledgment of this technology. As the industry puts these standards into practice, it is important to keep in mind the rate of evolving testing tools compared to the relatively slower pace it takes to update guidance.

Dilutional Linearity

Dilutional linearity studies aim to determine whether study samples with spiked analyte concentrations above the upper limit of quantitation (ULOQ) can be diluted into the assay range for an accurate measurement. Guidance, including ICH M10, requires specificity to be evaluated as an element of a full validation. So, the demonstration of the analyte's dilutional linearity in a particular biological matrix is critical in developing and validating a bioanalytical method.

The same matrix as the study sample should be used to prepare the QCs for dilution.

In performing dilutional linearity tests, bioanalysts spike the analyte into the biological matrix as a known quantity above the ULOQ.

The high-concentration analyte sample will be diluted into dilution linearity samples at different concentrations. Then, the dilution linearity samples will be measured and compared with the spiked quantity to evaluate bioanalytical recovery.

Historically, regulatory agencies enforced differing requirements regarding dilutional linearity, and the introduction of ICH M10 will help harmonize global standards. ICH M10 has shared the recommended approach and acceptance criteria of dilutional linearity in developing bioanalytical assays. The analyte level of study samples from the high-dose group often exceeds the assay range (ULOQ) and requires dilution before the bioanalysis. Scientists test a series of dilution factors to verify the consistent correlation between analyte dilution and bioanalytical measurement. When needed, scientists evaluate the hook effect (i.e., a signal suppression caused by high analyte concentrations) using dilution linearity results, including the high drug level samples from the estimated maximum concentration (Cmax) level.

M10 guidance states: "For each dilution factor tested, at least 3 independently prepared dilution series should be performed using the number of replicates that will be used in sample analysis."

The calculated mean concentration for each dilution should be within ±20% of the nominal concentration after correction for dilution, and the precision should not exceed 20%.

Endogenous Analytes

Similar to dilutional linearity, ICH M10 features endogenous analytes, a popular discussion topic among laboratories and developers. Because calculating an analyte's endogenous concentrations in matrices is a complex process that requires immense scientific expertise, ICH M10 outlines various testing considerations.

Because biological systems naturally contain endogenous analytes, it is challenging to differentiate the therapeutic(s) from the body's chemicals.

For example, the human body already produces testosterone, but testosterone is also a marketed drug, and when it is in the biological system, scientists cannot distinguish the two sources.

ICH M10 – more thoroughly than comparable guidance – outlines four possible approaches to quantify the endogenous analyte:

- **Surrogate Matrix Approach**. As the most common method, this approach requires using an authentic analyte in an analyte-free matrix, or in other words, replacing the genuine matrix with an artificial one. These replacements can range in complexity and similarity to the biological matrix, from water or salt solution to a stripped matrix with the endogenous analyte removed. Scientists must then implement parallelism to demonstrate that there is no difference when using a surrogate matrix to the genuine matrix of the sample.

This technique applies primarily to LC-MS. Ligand binding assays (LBA) should use the same matrix as the one in the study, though in cases where that matrix unavailable, the surrogate matrix is acceptable.

- ***Surrogate Analyte Approach.*** This quantification method is similar to the surrogate matrix approach but instead uses the genuine matrix and the analyte replacement. The replacement adds a stable-isotope labeled (SIL) analog to the drug in place of the authentic analyte. This approach requires at least two SILs, one for preparing the calibration curve and quality control samples and the other as the internal standard. The SILs should theoretically have the same physical-chemical properties as the analyte, so the method response gives scientists the same result. Remember, the surrogate analyte is unique to LC-MS assays because only MS can differentiate between the analyte and the SIL analogs.
- ***Standard Addition Approach.*** In this approach, scientists analyze the sample by adding the analyte to it at different concentration levels. Laboratories must split the samples into aliquots, which allow scientists to extrapolate a trend line to find the analyte's actual value in the specimen. Keep in mind the standard addition approach requires a large sample volume and tedious work for each sample so this approach may be impractical for samples with large analytical batches.
- ***Background Subtraction Approach.*** To offset the endogenous background concentrations of analytes, scientists can deduct the initial concentration from the blank samples (not spiked) and then use the subtracted concentrations to create the calibration curve.

Quantifying endogenous analytes is complex. For teams that don't have the specific bioanalytical expertise to understand the impact of study results fully, it is critical to rely on a trusted testing partner to interpret the data and varying analogs.

Critical Reagents

Critical reagents play a crucial role in immunoassays, which developers primarily use when developing large molecule drugs. Critical reagents bind to the analyte of interest, generating the instrument signal for the quantitation of large molecule drugs. This means the assay's accuracy, precision, and robustness directly depend on critical reagents' quality. To ensure consistent physical, chemical and biological characterization of critical reagents across the bioanalytical testing, characterizing critical reagents is expected to deliver high-quality bioanalytical data.

ICH M10 highlights the importance of critical reagents in assay development. It also recommends defining the critical reagents in ligand binding assays. The identity, source, batch number, purity, concentration, storage conditions and stability should be recorded and monitored in the bioanalytical assays, whether in-house or commercially purchased. When critical reagents are chemically modified, scientists need to investigate the physical, chemical and biological characterization.

Critical reagent quality and makeup fluctuate from lot to lot, making managing lot changes challenging. Bioanalysts must ensure critical reagent quality during major and minor changes, as lot variation can impact the assay and subsequent results. Lot changes may require a partial validation to guarantee the new lot's sufficiency and account for any deviations.

The M10 guidance applies to major and minor lot changes, depending on the impact on the assay. A minor change, such as using a previously qualified stock, only requires an assessment for comparative accuracy and precision. Conversely, a major change, like deriving critical reagents from a new production method, requires additional validation experiments.

Lot changes may be necessary, but drug developers can reduce their occurrence by generating and managing critical reagents for preclinical assays in the early stages. Partnering with a trusted laboratory that can manage critical reagents throughout their life cycle and also has a thorough understanding of necessary validation for regulatory requirements can condense timelines for the overall program.

Reporting

Proper reporting practices are a core element of implementing ICH M10 guidance. Regulators cannot appropriately review the requirements above without reporting templates that satisfy the latest expectations. ICH M10 aims to harmonize reporting across member states and increase efficiency during the review process.

Drug developers may find themselves challenged by ICH M10's standardized reporting, and this shift may be difficult since companies often have preferred report formats. Standardization and easy-to-read templates can help condense the time it takes to review submissions and bring drugs to market quickly.

Under M10, bioanalytical study reports supporting bioavailability (BA) and bioequivalence (BE) studies are required to produce an internal standard (IS) response plot (specific to the LC-MS technique) for each analytical run. This enables laboratories and regulators to assess the quality and reliability of sample batches at a glance. Reporting IS response plots provides benchmarks and added clarity. Outliers or defective analytical batches also become more

evident to laboratories reporting IS responses, thus creating opportunities to investigate and remediate tests before incurring excessive costs or delays.

The amount of data shared under M10 compared to other standards, like the U.S Food and Drug Administration (FDA) BMV guidance, is a sizable jump and speaks to a trend of increasing information sharing between companies and regulators. Laboratories that have recently conducted comparative BA/BE studies to submit to the U.S. FDA may have reported around 20% of chromatograms in serially selected subjects. ICH M10, however, supports reporting 100% of accepted and failed runs.

Additional reporting changes under M10 generally differ in the methods used to evaluate large and small molecules, making identifying these needs more logical. Also, LBAs and chromatography assays

contain varying approaches because each compound category is subject to specific analytical theories and techniques. While reporting changes is seemingly simple compared to other parts of M10 guidance, it also acts as the final connection of harmonization to global regulators.

Obtaining Harmonization

When regulators alter or introduce new guidance, companies must first decipher what the changes entail. Secondly, and perhaps most importantly, they must understand how to leverage the changes to meet business goals.

Harmonizing bioanalytical method validation standards is a continuous global effort, and drug developers who invest in maximizing these changes will reap the rewards. The regulatory harmonization that ICH M10 is after aims to streamline regulatory pathways by eliminating variations between agencies. By reducing these variations, drug developers and laboratories can more easily account for differences in testing and reporting requirements around the world, reducing timelines and expenses.

Companies and teams seeking the benefits of M10 may find it easier to expand product reach since harmonization should make it easier to submit applications to a broader set of regulators. Harmonization can also help those looking to enter other markets and deliver health solutions to more people.

All in all, there will be a lot of new processes and procedures to learn, implement and test within ICH M10. Only through constant education and transparency can the industry enable drug development programs to meet regulatory compliance, advance life sciences and deliver life-changing medicines.

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