Critical Recommendations from the Third AAPS/FDA Bioanalytical Workshop

Background

In 1990 leaders in the field of bioanalytical testing met at Crystal City Conference Center in Arlington, VA. to discuss important issues in the field of bioanalysis. A consensus paper was published[1] from this meeting which set the industry standard for bioanalysis and provided the foundation for the FDA guidelines which were promulgated in 2001[2]. A third meeting occurred in May 2006 and the working group from this 3rd AAPS/FDA Bioanalytical Workshop has published a consensus report[3] which is likely to be as standard-setting as the original report was 15 years ago.

Sponsors need to ensure that the laboratories where they have bioanalytical testing performed are aware of these recommendations and are taking steps to come into compliance with them. The FDA was represented at the conference and it is reasonable to expect that the FDA will start requiring compliance with some or all of these recommendations, even if it is slow to publish its own follow-up guidelines.

There are many individual recommendations published in the paper for both ligand-binding assays and chromatographic assays. Here are a few of the pivotal recommendations for chromatographic assays with advice on their implementation.

Calibration Range and Quality Control (QC) Samples

Text: For studies involving pharmacokinetic profiles spanning all or most of the calibration curve, three QC samples run in duplicate (or at least 5% of the unknown samples), spaced across the standard curve as per the FDA Guidance

Advice on Implementation: There are 6 QC samples analyzed in a typical bioanalytical batch. This number is adequate for batches of up to 120 samples (6 = 5% of 120). Many methods are employing 96-well plate formats and this becomes problematic when more than 1 plate in employed for a batch. It has always been a good idea to document the batch size during method validation, but given this specific recommendation not only should the batch size be documented, but if multiple 96-well plates will constitute one batch, then a *set number of QC samples per plate should be assigned* – typically 6 QC samples per 96-well plate.

Text: If a narrow range of analysis values is unanticipated, but observed after start of the sample analysis, it is recommended that the analysis be stopped and either the standard curve narrowed, existing QC concentrations revised, or QC samples at additional concentrations added to the original curve prior to continuing with sample analysis. It is not necessary to reanalyze samples analyzed prior to optimizing the standard curve or QC concentrations.

Advice on Implementation: This reinforces the 483's that the FDA has issued over the past several years for bioanalytical methods used to support bioequivalence studies, cited for too great an analytical range. While laboratories like to make calibration ranges as wide as linearity permits, it is clear that the FDA expects calibration ranges to be equal to the *actual* range of concentrations found in the study samples and that the low, medium and high QC samples represent the actual concentration range of samples analyzed within the batch. This means that labs will have to take one of two approaches: validate a series of ranges, each with three levels of QC concentrations; or validate a very wide range but with multiple QC concentrations spread over the entire range.

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[1] Shah, V.P., et al, *Pharm. Res*., 9 (1992) 588-592
[2] Guidance for Industry – Bioanalytical Method Validation, 2001
[3] Viswanathan, C.T., et al, *AAPSJ*, 9 (2007) Article 4

Incurred Sample Reproducibility (Duplicate Sample Analysis)
Text: A proper evaluation of incurred sample reproducibility and accuracy needs to be performed on each species used for GLP toxicology experiments. It is not necessary for additional incurred sample investigations to be performed in toxicology species once the initial assessment has been performed. Incurred sample evaluations performed using samples from one study would be sufficient for all other studies using that same species.

Advice on Implementation: Laboratories should plan on randomly selecting samples from each treatment arm of the first scheduled study for each species to perform this evaluation. Incurred sample-to-sample precision should be evaluated using the same criteria as the method validation. The results of all duplicate samples should be presented in the study report and evaluated in an addendum to the method validation report.

Text: The final decision as to the extent and nature of the incurred sample testing is left to the analytical investigator, and should be based on an in-depth understanding of the method, the behavior of the drug, metabolites, and any concomitant medications in the matrices of interest. There should be some assessment of both reproducibility and accuracy of the reported concentration.

Advice on Implementation: As a candidate compound progress through the clinical study program and more becomes known about metabolites and potential concomitant medications, the sponsor and the laboratory should proactively determine if and when additional duplicate sample information should be collected.

Text: In selecting samples to be reassayed, it is encouraged that issues such as concentration, patient population and special populations (e.g., renally impaired) be considered, depending on what is known about the drug, its metabolism and its clearance. First in human, proof of concept in patients, special population and bioequivalence studies are examples of studies that should be considered for incurred-sample concentration verification. The study sample results obtained for establishing incurred sample reproducibility may be used for comparison purposes, and do not necessarily have to be used in calculating reported sample concentrations.

Advice on Implementation: It will not be sufficient to just randomly select samples to be evaluated as duplicates. Samples may be randomly selected within sub groups of a study or studies to ensure that all possible treatment arms, concentrations, and sub-populations are adequately evaluated. Laboratories should use their previously established sample reanalysis SOP to determine how specific duplicate samples should be reported for a specific study, but the results of all duplicate samples should be presented in the study report and evaluated in an addendum to the method validation report.

Text: The results of incurred sample reanalysis studies may be documented in the final bioanalytical or clinical report for the study, and/or as an addendum to the method validation report.

Advice on Implementation: Driven by the decision to collect additional duplicate sample information, the results of this testing should be published in the individual study report (as duplicate sample reporting) and as an addendum to the method validation report as support of the method robustness over a range of conditions and matrices for the method.