Validating Drug Assays in Tissues

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If you are looking to validate tissue assays for your **drug project**, you should read about our approach below.

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Designing a tissue assay in accordance to the FDA bioanalytical method validation (BMV) guidance involves three key questions. First, can control tissue be easily obtained in bulk quantities or should a surrogate tissue approach be considered? Second, what sample processing procedures should be considered relative to the known stability of the analyte? Finally, how should the matrix be fortified with the analyte(s) of interest and internal standard to best approximate tissue samples from dosed animals or human subjects?

The ability to design and successfully validate complex tissue assays for drugs and/or their metabolites can often differentiate the technical strength of bioanalytical laboratories. Here we discuss the three important method development aspects that we consider based on your unique program.

RARE/LIMITED MATRICES

Tissues with small sample weights or limited availability pose the greatest challenge in establishing a suitable control matrix for system calibration during validation. Examples of tissues KCAS has worked with include: human prostate tumor biopsy samples (50-100 mg), rabbit retinal tissue (40 mg) and canine synovial fluid (<1 mL). The FDA BMV guidance clearly speaks to the use of surrogate matrices in these cases, but finding a suitable matrix where Standards and Quality Controls (QC) in surrogate matrix match the recovery and response of the analyte in authentic tissue matrix can be challenging.

In particular, it can be extremely difficult for laboratories to obtain even the smallest of human tumor tissues for use in preparing authentic QC samples. Tumor banks are very reluctant to provide tissues for what is viewed as non-research purposes, preferring to retain these tissues for higher research or academic purposes (e.g. DNA sequencing and biomarker research). In these cases, the assay may have to rely on a single authentic tumor QC and use non-tumor tissue to prepare Standards and remaining QCs for validation. In the example of the human prostate tumor assay, non-cancerous dog prostate tissue was used as an appropriate surrogate matrix.

Non-clinical tissues of very limited sample weights, such as ocular tissue, would require obtaining tissues from hundreds of animals to prepare matched Standards and QCs for validation. We have found that rabbit plasma closely approximates retinal QC samples in system calibrations, and serves as a more humane and cost-effective approach.

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PROCESSING PLATFORMS

The stability of the drug and its metabolites can, under various conditions, drive the approach used to process tissue samples prior to extraction.

For the analysis of alkaline stable analytes (e.g., Risedronate – an osteoporosis therapy; Terbinafine and Fluconazole – antifungals), in bone and nail tissue, the matrices can be "softened" or completely dissolved when incubated in 1 M NaOH prior to processing.

Thermally unstable analytes may be optimally processed using dry ice / cryogenic milling of tissues. Once the tissue sample is pulverized, the dry ice evaporates, leaving tissue powder that can be directly weighed for further processing.

Large intact tissues, such as bovine liver or human feces, are best processed using commercial homogenizers. The tissue is subdivided into smaller pieces and combined with an appropriate solvent into a large container for homogenization. If the resulting homogenate is not sufficiently fine to avoid the clogging of pipet tips, we often find that further processing using a micro bead homogenizer is effective. The disadvantage of using a commercial homogenizer (including dry ice mills), is the potential for analyte carry-over from sample to sample even when employing a robust cleaning protocol. Therefore, it is important that carry-over evaluations be included in all methods that use highly fortified sample homogenates.

Routine tissue processing is best accomplished using a bead homogenizer platform. In the KCAS tissue lab we prefer the Precellys system, which also provides benefits for processing small tissue samples. The tissue is placed in a microfuge tube, along with solvent and beads. Depending on the tissue density, either ceramic, glass, or stainless steel beads can be used. Samples can be processed in as little as 15-30 seconds, and the unit has sub ambient temperature control which can be beneficial for temperature-sensitive analytes. The benefits of this platform are the rapid processing of up to 24 samples simultaneously and the elimination of sample-to-sample carryover, since the beads and tubes are disposable.

TISSUE/HOMOGENATE VALIDATION

Fortification of tissue samples for Standard and QC preparation to best approximate the amount present in the authentic, intact tissues can be problematic. Very few tissues can be fortified directly with the target analytes, since analyte in direct spiked solutions remains at the tissue surface rather than being dispersed within the tissues as in study samples. This can result in different analyte recoveries in the fortified and study tissues. Some exceptions are isolated cells (e.g. RBCs) feces and complex biological fluids (e.g. synovial fluid), where the drug can be added directly to the tissue. More often, tissue homogenates are fortified for method validation. However, fortification of a homogenate may not approximate how intact drug might bind to tissue components in the absence of the chosen solvent employed during homogenization.

KCAS prefers to use aqueous-based spiking solutions for homogenates to mitigate this potential concern. If non-aqueous solvents (e.g., Methanol, DMSO) are required for homogenate fortification, the amount of solvent in spiking procedures should always be minimized for the same reasons.

Tissue assays can be difficult to validate and often a fit-for-purpose approach needs to be considered when multiple tissues (e.g., aqueous and vitreous humors, lens, retina, choroid, cornea, iris-ciliary body) are requested from each animal. Our experience indicates there is not a "one-size fits all" approach. Each tissue is unique, and KCAS can help you design and troubleshoot your tissue assays to support your small molecule development program.

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