Stability

The following elements of stability must be demonstrated by the completion of the method validation.

Freeze/Thaw Stability

The analyte(s) must be demonstrated to be stable in matrix at during successive cycles of freezing and thawing as is likely to occur during sample collection, shipment, preparation, and even re-preparation. A minimum of three freeze/thaw cycles is required to be evaluated with samples frozen between 12 and 24 hours and thawed without warming at room temperature.

Short-term Matrix Stability: Room Temperature

The analyte(s) must be demonstrated to be stable in matrix at ambient temperature for the duration of time equivalent to the time required for thawing and preparation of samples in the proposed batch size, usually 4-24 hours.

Long-term Matrix Stability: Storage

The analyte(s) must be demonstrated to be stable in matrix at the proposed storage condition for the duration of time equivalent to the time samples are expected to remain stored waiting for preparation during a typical study sequence, usually 1-3 months. Every effort should be made to extend this stability.

Stock and Spiking Standard Stability

Stock and spiking standards must be documented to be stable for the duration of use during the execution of the method validation. Every effort should be made to extend this stability. This includes evaluating stability of these solutions at room temperature over the duration of expected use for preparing standards and spiking solutions.

Extract Stability

The analyte(s) must be demonstrated to be stable after preparation for analysis for the duration of time equivalent to the time samples are expected to wait, in storage, for analysis (or potential re-analysis) during a typical study sequence. Every effort should be made to extend this stability.

Autosampler Stability

The analyte(s) must be demonstrated to be stable after preparation for analysis for the duration of time equivalent to the time samples are expected to sit on an autosampler based upon the proposed batch size.

Additional Elements: Dilution QC Accuracy and Precision

When the method calibration range fails to cover the expected range of the analyte in the matrix, then the method should be validated for the planned dilution scheme. Validation should include the dilution of QC samples into the analytical calibration range calibration range. These dilution QC samples should be diluted in the same manner as incurred samples and should cover all possible dilution scenarios (i.e. 5X, 10X, 20X). The accuracy and precision of these diluted QC samples should be evaluated for at least on batch, for intraday accuracy and precision, and should meet the normal intraday accuracy and precision requirements.

Additional Elements: System Suitability

A challenge to the system should be established that when executed before the beginning of each analytical run would provide confirmation that the system is performing optimally. The form of the system suitability will be method-dependant.

Additional Elements: Reference Standard

A reference standard of known origin and identity must be used for the preparation of all standards (calibration and QC). Three sources are acceptable:

- Certified reference standards (NIST, USP)
- Commercially available from a reputable source
- Custom synthesized supplied with complete documentation as to structure and purity

Additional Elements: Method SOP or Protocol

A specific, detailed description of the bioanalytical method should be written. This can be in the form of a protocol, study plan, report, or SOP.

What a Sponsor Should Do

The sponsor is ultimately responsible for the bioanalytical method used to support their studies, regardless of where the method was validated. Every sponsor should take action to ensure that their bioanalytical methods now conform to the FDA guidelines. Here are some of the things that should be controlled or addressed:

- Establish a policy for deciding weighting and modeling of curves
- Evaluate and report possible weighting schemes using standard residuals
- Establish a minimum correlation coefficient for linear and transformed-linear calibration curves
- Establish acceptance criteria for how many calibration standards must meet back-calculation criteria when more than 6 standards are used in a curve
- Ensure that at least six batches of unpooled matrix are evaluated for specificity
- Evaluate all possible co-medications and add to the list as clinical studies progress
- Establish what "...consistent, precise, and reproducible..." will be for recovery experiments
- Ensure that all calibration standards and QA sample results are reported and included in the statistics for the method validation
- Ensure that the method SOP or protocol captures all of the specific details and parameters established during the validation and as things like stability are extended, that that information is correctly reflected