

Bioanalytical Method Validation Summary

Background:

In May 2001 the FDA promulgated its first official guidance for bioanalytical methods. This guideline is titled Guidance for Industry Bioanalytical Method Validation and represents the ultimate and last word in what is expected in bioanalytical methods supporting clinical and non-clinical studies. The following is a summary of what the FDA document says so that sponsors may better manage future and existing bioanalytical method validations. This summary does not address the specific guidance for microbiological and ligand-binding assays and sample analysis which are also covered in the FDA guidance. A copy of the actual FDA document can be obtained at www.fda.gov/cder/guidance/4252fnl.htm.

Overview:

A bioanalytical method used to support any bioequivalence, bioavailability, pharmacokinetic or non-human pharmacology/toxicology study must be validated. The principal elements of validation are:

- Specificity
- Range
- Linearity
- Precision
- Accuracy
- Recovery
- Stability

The method validation consists of the preparation and analysis of calibrators and QC on three separate days using three separate batches of matrix. A validation run will contain at least:

- Blank sample (matrix blank: no internal standard added)
- Zero sample (blank containing internal standard only)
- 6-8 calibration standards
- Five (5) replicates each of the following QC Samples:
 - Lower limit of Quantitation (LLOQ) QC at the method LLOQ (at the same concentration as the lowest non-zero standard)
 - Low QC $\leq 3X$ LLOQ
 - Mid QC midway between low and high QC concentrations
 - High QC at the upper boundary of the calibration range
- The preparation batch and analytical run will contain enough extracted samples (including blanks) to be equivalent to the projected batch size and total runtime for clinical samples.

Validation Acceptance Criteria

Each analytical run will be evaluated individually (intra-day) and as part of the sum of analytical runs (inter-day).

Specificity

Matrix blanks should show no interference at the LLOQ. LC-

MS/MS methods should be evaluated for potential matrix effects.

- 6 independent, unpooled matrix blanks (exceptions can be made for hyphenated MS methods)
- Blank (zero-level calibration standard) prepared with each curve
- The integrated area of the LLOQ calibration standard should be 5X the area of the blank for every analytical run

Range

- A calibration curve must be generated for each analyte
- The curve should consist of a sufficient number of calibration standards ($n \geq 6$)
- The curve should be constructed to cover the expected study range

Linearity

The LLOQ should be established by evaluating 5 non-calibration curve LLOQ standards. The calibration curve must be evaluated for each individual run and meet the following criteria:

- Standard curve fitting is determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting and statistical tests for goodness of fit.
- The ULOQ calibrator must back-calculate to within $\pm 15\%$ of the nominal concentration.
- At least four out of six non-zero standards should meet the back-calculation criteria, including the LLOQ and ULOQ standards.

Precision

The precision must be evaluated and meet acceptance criteria for each QC level for each validation run (intra-day) and for all runs taken as a group (inter-day). The acceptance criteria are:

- The mean precision for the LLOQ QC must be $\leq 20\%CV$.
- The mean precision for all other QC levels must be $\leq 15\%CV$, per level.

Accuracy

The accuracy must be evaluated and meet acceptance criteria for each QC level for each validation run (intra-day) and for all runs taken as a group (inter-day). The acceptance criteria are:

- The mean accuracy for the LLOQ QC must be within $\pm 20\%$ of the nominal concentration.
- The mean accuracy for all other QC levels must be within $\pm 15\%$ of the nominal concentration, per level.

Recovery

Absolute recovery is evaluated using low, medium, and high QC samples. No number of replicates at each level is specified. Recovery of the analyte need not be 100%, but the extent of the recovery of the analyte(s) and internal standard(s) should be consistent, precise, and reproducible.