FIT-FOR-PURPOSE BIOANALYTICAL VALIDATION AND SAMPLE PROCESSING

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Goals

- When a study is GLP
- Bioanalytical guidances
- Bioanalytical batch
  - Value of a system suitability process
  - Requirement of standards
  - Relationship between standards and quality control samples
  - Various types of regression parameters used to define a batch
  - The importance and timing of proper chromatographic integration
  - Batch acceptance criteria
  - Dilution integrity
- Incurred sample reproducibility
- How to report sample concentrations
GLP Cornerstones

- Say what you’re going to do
- Do what you said you would do
- Document it
GLP Cornerstones

- Say what you’re going to do
  - Write a study plan that is based upon your SOPs
  - SOPs must be based upon a detailed understanding of international bioanalytical guidances

- Do what you said you would do

- Document it
  - Who, when and with what equipment
  - Data change control – who made the change? when did it occur? what was the original value? why was the change made?
  - QC reviewed
  - QA audited
Bioanalytical Guidance’s – Assay must be:

- Selective
- Accurate
- Precise
- Stable

Nicotine selectivity vs. contamination:
Selectivity also means that sources of contamination are minimized!

Every surface (transfer tubes, pipette tips, injection vials, etc.) must be rinsed with methanol.

- Sample collection and handling
- Freezer (-20°C or -80°C)
- Freeze/Thaw
- UV light sensitivity
- Benchtop
  - Pre-extraction
  - Post-extraction
Bioanalytical Batch History

1\textsuperscript{st} Crystal City Meeting in 1990 – Bioanalytical foundation was created

- GMP guidelines existed – verifying a known concentration
- Clinical chemistry guidelines existed – normal or abnormal
- Bioanalytical chemistry needed accuracy and precision over a broad concentration range for PK analysis

Bioanalytical Batch!

- Standards & Quality Control (QC) Samples in every batch
- If the standards and the QCs passed acceptance criteria then all of the systems, equipment and personnel used to generate that batch were working successfully that day
Bioanalytical Batch

- Stand alone entity
  - No data from a different experiment can be used as supportive data
  - Standards, QC's and study samples are all processed at the same time in the same experiment by the same analyst using the same equipment
    - What about hydrolyzing samples for glucuronides & aglycones?
  - Standards and QC's must be prepared in matrix that has been shown to be free of interferences
  - In contrast - some clinical chemistry assays use one standard curve/week
Bioanalytical Batch on LC-MS/MS

- LC-MS/MS System Suitability
  - Mass spectrometers have a tendency to drift over time
  - Process must be developed to assure the instrument response is stable during a batch

- The following MS parameters must be consistent between method development and sample processing:
  - Smoothing factor
  - Dwell time
Bioanalytical Batch – Standards & QCs

- Each batch must contain its own standard calibration curve
  - Minimum of six different calibrator concentrations
  - Range of the standard curve should reflect the expected range of the study sample concentrations
Bioanalytical Batch – Standards & QCs

- Each batch must contain its own standard calibration curve
  - Minimum of six different calibrator concentrations
  - Range of the standard curve should reflect the expected range of the study sample concentrations
- Each batch must contain a minimum of 3 QC concentrations in duplicate (low, middle and high)
- What’s the difference between standards and QCs?
  - Philosophically – standards create the calibration curve and QCs demonstrate that non-standards can be accurately measured – so they must be different
  - Different weighings – getting standards and QCs to match each other is sometimes very difficult
  - QCs are stored with study samples to verify sample integrity
Bioanalytical Batch – Regression Parameters

- Regression curve model can be linear or quadratic for chromatographic assays and a 4-parameter logistic (4PL) non-linear model for ELISA type assays.

- The regression model can be weighted by no-weight, 1/x or 1/x^2 where x = concentration.
- The simplest regression model and weighting must be used and it is selected during method validation.
Bioanalytical Batch
Chromatographic Integration

- Big Deal – the vast majority of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria.
Bioanalytical Batch – Chromatographic Integration

- **Big Deal** – The vast majority of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria.

- **Best Practice** – Review all chromatographic integration and manually redraw baselines then “lock-it-down” prior to performing regression analysis. It should be very difficult to modify integration following regression analysis.
Bioanalytical Batch
Batch Acceptance Criteria

- Review
  - All standards, QC's and study samples were analyzed on the same day by the same analyst(s) using the same equipment
  - For LC-MS/MS methods, the system suitability process proved that the instrument response was stable during injection of the entire batch
  - All chromatographic baseline integration has been reviewed, was consistent and “locked-down”

Now you are ready to review and accept the batch
Bioanalytical Batch
Batch Acceptance Criteria

- **Standard Curve Acceptance**
  - Must use the same regression model and weighting option that was chosen during method validation
  - Must have an SOP in place that describes how to reject standard(s)
  - Only sample concentrations that are within the range of your accepted standards can be reported

- **QC Acceptance**
  - The predicted concentration of 2/3 of the QC samples must be within 15% of their nominal concentration (20% for ligand binding assays)
  - At least 50% of the QCs must be accepted at each QC level
Bioanalytical Batch
Batch Acceptance Criteria

- Blank (no internal standard) and standard zero samples
  - Prepared with matrix that was free of interferences
  - At least 50% of these samples must have a response that is <20% of the response of the lowest standard – reject low standard

- Sample Dilution
  - Can only report sample concentrations that fall within the range of the standard curve
  - Sample concentrations that exceed the concentration of the highest standard must be diluted
  - Must prepare dilution QCs and dilute them using the same respective dilution (for example, 1/10 dilution)
  - Dilution QCs are used for acceptance of the diluted samples
  - 50% of dilution QCs must be within 15% of their nominal concentration
Incurred Sample Reproducibility (ISR)

- Purpose is to demonstrate that re-assaying study samples is reproducible.
- Recommended by FDA and EMA
- Generally accepted practice:
  - minimum 20 samples
  - If n < 1000 samples then reanalyze 10%
  - If n >1000 samples then reanalyze 100 samples + 5% of samples beyond 1000
  - Select more subjects with fewer samples per subject
  - Acceptance Criteria: The difference between the repeat value and the original value < 20% (< 30% for ligand binding assays) for 2/3 of the samples
How to Report Sample Concentrations

- Demonstrate the sample analysis plan was followed
- Must have an SOP to select samples for re-analysis
- Must show that the acceptance criteria for each batch was met
- Demonstrate inter-batch reproducibility: List all of the QCs by batch and perform statistical analysis (% mean deviation and %CV) on each QC level for the study
- Report sample concentrations that were < the concentration of the lowest standard as < LLOQ
- Report sample concentrations that were > the concentration of the highest standard as > ULOQ
- Prepare a table by batch as either passed or failed
Conclusion

- It’s easy to say that you follow GLP. It’s challenging to have GLP processes in place that remain current.
  - Selective, accurate, precise, stable and documented!
  - Bioanalysis is all about compliance!

- Sample processing revolves around the “BATCH”
  - Stand alone experiment – uses the same analysts(s) and stable equipment on the same day
  - Must have SOPs that minimize data “interpretation”
  - Strict acceptance criteria for standards, QCs and blanks

- ISR analysis is a requirement

- Reminder – Bioanalysis is all about compliance!