ASSAY METHOD VALIDATION AND BIOANALYSIS: WHAT DO WE REALLY NEED FOR BIOEQUIVALENCE?

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Maria Cruz Caturla, SFBC Anapharm Europe (Barcelona, Spain)

François Vallée, SFBC Anapharm (Québec, Canada)

Allan Xu, SFBC Analytical (North Wales, U.S.A.)

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Essential Parameters for a Bioanalytical Method Validation?

- Accuracy
- Dilution Integrity
- Linearity
- Matrix effect
- Precision
- Reproducibility
- Selectivity
- Sensitivity
- Stability

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When Should we Perform Full, Partial or Cross-Validation?

- When developing a method for the first time
- For a new drug entity
- If a metabolite is added to an existing method
- Method transfer between laboratories
- Method transfer between analysts
- Method inactive for “X” months/years
- Change in anticoagulant
- Change in matrix within species (human plasma-human urine)
- Change in species within matrix (human plasma-rat plasma)
- Rare matrices

When Should we Perform Full, Partial or Cross-Validation? (continued)

- Change in sample processing procedure
- Change in sample volume
- Change in concentration range
- Change of analytical instrument (GC/MS–LC/MS/MS)
- Change of detection systems (UV–MS/MS)
- Change in software platforms
- Administration of concomitant medication(s)
- Same analytical method used in more than one site or laboratory in a single study
- Different analytical methods used for a same compound in a single study or across different studies

Do you Perform Matrix Selectivity?

- In blank matrices
- In pre-dose study samples
- For rare matrices
- For gender
- For endogenous components
- For metabolites
- For decomposition/degradation products
- Concomitant medications

Calibration Curves

- Number of calibration standard levels
- Number of replicates per calibration standard level
- Establishment of concentration levels
- Procedure for endogenous drugs (matrix stripping, ion exchange, proxy matrices)
- Determination of curve ranges for each study design
- Regression model: linear, quadratic, cubic, other
- Weighting factor: statistical approach used
- Re-evaluation of regression model and weighting factor after partial validations
- Maximum of calibration standards which can be rejected

Between-Run Accuracy and Precision

- Number of concentration levels and replicates per level
- Number of runs
- Number of days
- Impact on column performance
- Impact on detector response

Sensitivity

- Methodology for assessment
- Acceptance criteria: accuracy, precision, selectivity, S/N ratio

Matrix Effect

- Major reasons: coeluting substances during sample extraction and/or analysis, late eluters, ion suppression/induction, slow/fast metabolizers, food effect, hemolysis
- Should we do it only for methods using mass spectrometers?
- Methodology for assessment
- Acceptance criteria

Recovery

- Number of concentration levels and replicates per level
- Separate evaluations for analytes and internal standards
- Methodology for assessment
- What do you consider acceptable: % recovery, %CV, difference of recovery between concentration levels, difference of recovery between analyte and internal standard?

Dilution integrity

- Performed when study samples are exceeding upper limit of quantitation or for partial sample volume
- Concentration(s) and dilution factors tested
- Acceptance criteria

Stability
What are you testing?

- Short-term stability of analyte(s) and internal standard(s) in solution
- Long-term stability of analyte(s) and internal standard(s) in solution
- Stability of analyte(s) following sample processing (post-preparative stability)
- Freeze and thaw stability
- Short-term stability of analyte(s) in matrix at room temperature
- Long-term stability of analyte(s) in spiked samples
- Long-term stability of analyte(s) in incurred samples

Stability
What are you testing? (continued)

- Methodology for assessment
- Acceptance criteria for solution and biological matrix
- Can I use stability results obtained from a previous method generated from the same laboratory or from another laboratory?
- Should I repeat stability assessments for another type of solution or type of matrix, change of concentration or container system or storage conditions?
- Recommend precautions for unstable drugs: lower storage temperature, add a stabilizer, change container system, protect from light.

Other Interesting Validation Tests

Pros and cons to validate the following parameters:

- Hemolysis effect
- Potentially interfering drugs
- Adsorption of analyte(s) onto collection devices
- Stability of analyte(s) during sample collection and handling
- Stability of sample load solution (for automated extraction procedures)
- Method ruggedness: test different analysts, columns, solutions, analytical instruments

Sample Processing

- Procedure used: protein precipitation, LLE, SPE, on-line extraction
- Manual or automated procedures
- With or without internal standard(s)
- Labeled internal standards are highly recommended
- Rejection criteria for internal standards
- How do you know if your sample is exceeding validated stability data (number of F/T cycles, time outside the freezer)?

Pre-Study Requirements

- Validated analytical method
- Approved analytical method standard operating procedure
- Qualified/calibrated analytical instruments/laboratory equipment
- Analyte(s) and internal standard(s) stock and working solutions meeting acceptance criteria
- Biological matrix meeting selectivity acceptance criteria
- Calibration standards and quality control samples meeting acceptance criteria
- Training and validation of analytical method users (method transfer from R&D to production)

Study Sample Analysis

- System suitability prior each run
- Carry-over evaluation
- Run acceptance criteria for blank matrix, zero standards, calibration standards, and QCs
- Sample dilution procedure
- Sample reinjection criteria
- Study sample rejection criteria
- Sample reintegration procedure
- Reporting of results

Sample Reassay

- Written procedure required
- Decision tree with acceptance criteria
- Major reasons: poor chromatography, equipment failure, sample processing error, inconsistent replicate analysis, sample value outside concentration range, rejected analytical run, rejected sample dilution, sample stability exceeding validated data, reassay requested by a client, unacceptable internal standard response, inconsistent pharmacokinetic data.
- Rationale for sample reassay must be clearly documented
- Reporting of reassay results

Thank you!

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