How to Implement a Validated Method

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CPSA 2010
“A Practical Guide to Bioanalysis”
• Familiarize yourself with the method:
  – Review the method
  – Review the method validation report
  – Examine the stability claims
  – Build the “assets” required to run the method
  – Establish competency
• Reference standards
  – Identity
  – Purity
  – Storage condition
  – Expiration

• Sampling
  – Compare in-life protocol to method’s requirements
  – Matrix, species, anticoagulant
  – Preservatives, inhibitors, blocking agents
  – Timing, temperature
  – Containers
• Stability
  – Review the validation stability data:
    • Most windows will be for less than few weeks
    • Was validation data generated against a freshly prepared and extracted calibration curve?
  – Will you need to extend the stability data to protect the period from collection until analysis?
  – Is ongoing sample storage consistent with the stability data?
• Sample Analysis Plan
  – The “go to” agreement on how to run the study
  – Declares appropriate choices for: reference standards, regulatory level, calibration range, location of QC samples, acceptance criteria, etc.
  – Plan consolidates proprietary SOP content, but plan can also trump SOP.
  – Convenient for sponsor customization; prevents misunderstandings
• Sample prep preliminaries:
  – Make and compare stocks
  – Screen internal standard
  – Screen matrix
  – Calibration standards, QC samples
  – Reagents, mobile phases, system suitability sample

• Establish instrumental performance
  – Calibration still current?
  – Requalifications up to date?
  – Meeting system suitability
• Establishing Prestudy Competency
  – Manual methods
    • Analyst qualification
      – Prepare a core validation run
      – Linearity, precision, accuracy, specificity, carryover
  – Robotic methods
    • Method variation lessened by executing version-controlled files
    • Consistency from month to month
  – Audit trails- continuous **ON**
## Prestudy Qualification Run, 96 well plate

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• Inactivate any samples with known error or instrumental malfunction
• Integrate consistently with single parameter set
• Calculate:
  – Drop any Cal > 15% bias from intended; > 75% remain?
  – Blank, Blank + ISTD, Carryover < (20% *Cal 1)?
• QC performance:
  – ≤ 15% CV
  – ≤ ±15% mean bias from intended
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Method Sample Analysis Run
• Inactivate any samples with known error or instrumental malfunction
• Integrate consistently with single parameter set
• Calculate:
  – Drop any Cal > 15% bias from intended; > 75% remain?
  – Blank, Blank + ISTD, Carryover < (20% *Cal 1)?
• QC performance:
  – At least 4/6 within ±15% of nominal
  – No two samples can fail at the same concentration
• Are the sample concentrations well distributed throughout the range?
  – Pause after 2-3 runs are completed
    • High and middle QC’s should bracket range of $C_{\text{max}}$
    • Low QC anchors the low end at 3 x LLOQ
    • Range should cover 4-5 half-lives on the elimination phase
  – Special scrutiny for BE studies!

• Fixes
  – Relocate the curve and QC’s (single validation run)
  – Add more QC’s and revise acceptance rules
• Consistency of internal standard response:
  – Cals/QC’s vs. incurred samples
  – Between subgroups of incurred samples
• Divergence between the front and back sets of calibration standards?
• Drift in internal standard response
• Drift in system check sample response
- Pre-establish drop criteria in SOP (poor chrom, instrument malfunction, attributable error, etc.)
- Sample tracking integrity during sample storage and analysis
- Watch carryover-
  - Investigate affected pairs
  - Is CO factor responsible for more than 5%?
- Fractured, convoluted runs- just fail them!
• Avoid reprocessing; integrate, then accept consequences.

• Remedi ate any lapse in stability coverages

• Document any deviations or investigations and discuss their impact on study

• Carefully justify any repeat analysis requests

• Retain and report all runs performed