Quality Controls

By:

Prof Amer Jamil
Department of Biochemistry
University of Agriculture
Faisalabad

Validation

Documentation

Performance characteristics

Acceptability of data – criteria

When modified - should be validated for Suitable performance

Research laboratory

- **Good Laboratory Practices (GLPs)**
- **Sound principles of quality assurance**
- **Standard Operating Procedures (SOPs)**
  QC & Assurance

Process of Analytical method

Developed, validated and used

- Reference standard preparation
- Method development - assay procedure
- Routine analysis and acceptance criteria for analytical run and/or batch (In Study validation)

- **Well defined & fully validated Method**
- **Quantitative measurement**

  Reliable and reproducible

Method validation

Reliable and reproducible.

Fundamental parameters

- **Accuracy**
- **Precision**
- **Selectivity**
- **Sensitivity**
- **Linearity**
- **Stability**

SOPs

- **Record keeping, security and chain of sample custody**
- **Sample preparation**
- **Analytical tools**
  - **Methods**
  - **Reagents**
  - **Equipment**
  - **Instrumentation**
  - **Procedures for quality control**
  - **Verification of results**

Specificity/ Selectivity

Ability to assess analyte in the presence of endogenous compounds, degradation products and metabolites

Reject Blank with significant interference
> 10 % blank show interference
additional blanks, > 10 % still show interference
Modify method to eliminate interference

Calibration/Standard Curve

Instrument response & concentrations

- **Each analyte, Same biological matrix**
- **A blank sample**
- **A zero sample**
- **5 minimum non-zero samples (including LLOQ.)**

Expected concentration range in the study

Standard or Calibration Curve

Concentration-Response

- **Simplest model, Concentration-response relationship**
- **Selection of weighting and use of a complex Regression equation should be justified**

Sensitivity/Lower limit of detection (LOD)

Smallest conc. distinguishable from noise level
Detected only, not quantified

Lower Limit of Quantification (LLOQ)

- **Twice the response of LOD**
- **Lowest standard on the Calibration curve**
- **5 times the response compared to blank response**
- **Identifiable, discrete, and reproducible with**
- **a precision of 20% and accuracy of 80-120%**

Accuracy, Precision

Determines the error

Primary criteria for Quality

Precision

Closeness of individual measures of an analyte procedure is applied repeatedly to multiple aliquots

- **3 QCs concentrations in calibration range.**
- **3 determinations per QC concentration.**
- **Should not exceed 15% of CV**

Precision

- **Within-run**

intra-batch precision or repeatability

- **Between-run**

inter-batch precision or repeatability

time, different analysts, equipment ,

reagents, and laboratories.

Accuracy *(Trueness)*

Closeness of test results to the true value

- **3 QCs concentrations in range of calibration curve**
- **3 determinations per concentration of QCs**
- **Deviation within 15% of the actual value**
- **Should not deviate by > 20% at LLOQ**

Recovery

Detector response about analyte added to and extracted from the biological matrix

Compared to the true concentration of standard.

- **Extraction efficiency need not be 100%**
- **Extent of recovery of an analyte and IS**

  Consistent, precise and reproducible
- **Compare 3 conc. (low, medium, and high QCs) with unextracted standards**

For quantitation.

- External standards
- internal standards

External standards

- Analyzed on a separate chromatogram from the sample

- comparison of the peak area/height (HPLC or GC) or spot intensity (TLC) of the sample to that of a reference standard of the analyte of interest.

Internal standard

- Known purity
- No interference in the analysis
- Added to the sample mixture.

Response ratio of

Compound of interest to IS vs reference standard (HPLC or GC).

1. Complex sample preparation procedures, (multiple extractions)

2. Low concentration sample (sensitivity)

3. Wide range of concentrations expected

Validation

- **Full Validation**
- **Partial Validation**
- **Cross-Validation**
- **Pre-study Validation**
- **In-study Validation**

METHOD VALIDATION

Generation of data

Well defined & fully validated

Method does- intended to do

Quantitative measurement

Reliable and reproducible

Pre-study Validation

Analytical method development and documentation

Each Biological Matrix and Chemical species

- **Selectivity**
- **Calibration curve & Linearity**
- **Accuracy, Precision, Recovery**
- **Stability of analyte**
- **Acceptance criteria**
- **Documentation**

In Study Validation

Application of validated method for routine analysis

Accuracy & precision should be monitored

Method works satisfactorily

QC sample in duplicate at 3 concentrations
Low , Medium & High QCs
Should be incorporated in each assay run

Basis for acceptance or rejection of run

4/6 within ± 20 %
2/ 6 outside ± of 20 %
But not both at same concentration

Cross-Validation

Comparison of validation parameters
two or more analytical methods within the same study or across different studies.

Inter-laboratory reliability
within a single study more than one site or lab, spiked matrix standards and subject samples

Ruggedness

Studying the eventual effect of different sets of conditions on the method (cross validation)

Robustness

A measure of the analytical procedure's capability to remain unaffected by small but deliberate variations

Should be performed during development of the analytical procedure

Lab Safety

- **Precautions**: Never do any work until precautions are known
- **Supervision**: Always get supervision of experienced
- **Lab coat**
- **Gloves**
- **Goggles**: Wear appropriate goggles e.g., UV
- **Shoes**: Do not wear open shoes
- **Make-up**: Do not wear
- **Water splash**: Must have in lab
- **First-aid kit**: Must have
- **Mask**: Wear if chances of inhalation
- **Food/drink**: Never in lab., separate refrigerator/oven etc for
- **Fire extinguisher**: Must have in lab
- **Radioactivity**: Special requirements

Lab Safety…cont.

- **Waste disposal**: Separate waste disposals for different types of wastes
- **Labels**: Be aware of safety and chemical labels
- **Lab. Levels** (from lowest to highest):
  - BSL-1 (Biosafety Level 1) Lowest level
  - BSL-2 (Biosafety Level 2) Working with pathogens
  - BSL-3 (Biosafety Level 3) Working with pathogens with aerosols
  - BSL-4 (Biosafety Level 4) Working with pathogens having no cure


THANKS