Method Validation

An Overview

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TEXAS A&M AGRILIFE RESEARCH

Assigned Reading

- Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis
- Validation of New Methods of Analysis. 2012. OTSC M0033

Presentation Outline

- Who
- What
- When
- Where
- Why
- How

Validation Definition

- 3.9 validation
  verification (3.8), where the specified requirements are adequate for an intended use

  3.8 verification
  provision of objective evidence that a given item fulfils specified requirements

  ISO 17025:2017

Validation of Methods - When

- 7.2.2 Validation of methods

  7.2.2.1 The laboratory shall validate non-standard methods, laboratory-developed methods and standard methods used outside their intended scope or otherwise modified. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application.

  ISO 17025:2017

Validation of Methods - When

- Harmonized guidelines for single-laboratory validation

  Validation applies to the defined protocol, for the determination of a specified analyte and range of concentrations in a particular type of test material, used for a specified purpose. In general, validation should check that the method performs adequately for the purpose throughout the range of analyte concentrations and test material to which it is applied. It follows that these features, together with a statement of any fitness-for-purpose criteria, should be completely specified before any validation takes place.

Role of Validation - Why

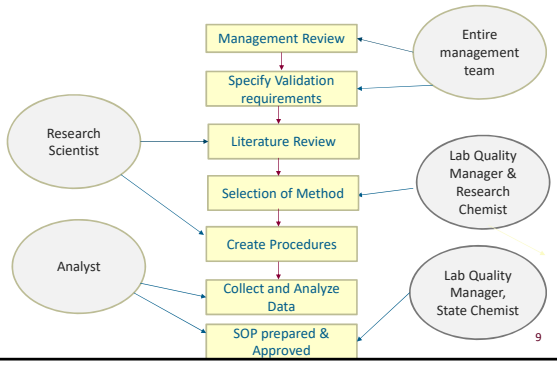
- Confirms the fitness-for-purpose of a particular analytical method.
- Analytical method validation forms the first level of quality assurance in the lab.
- To include all possible effects or factors of influence on the final result
- To make them traceable to stated references (reference methods, reference material etc)
- To know the uncertainties associated with each of these effects and with the references

Two Levels

- Single lab validation
- Inter-laboratory study

- Whether or not methods validated in a single laboratory will be acceptable for regulatory purposes depends on any guidelines covering the area of measurement concerned.

Flow of Method Validation



Method Selection

- Is based on sound underlying scientific principles
- Is applicable for routine analysis of samples
- Can detect analytes in the concentration range of interest
- Has sufficient specificity and sensitivity for intended use
- Can meet specific method performance criteria
- Has adequate QA/QC controls

Method Development and Optimization

- Method development and optimization is prior to method validation and is critical for method validation.
- Checklist for technical assistance for AgriLife Research.

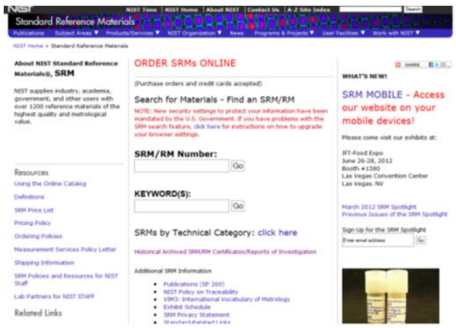
Minimum Validation Acceptance Criteria

- **Accuracy**
  - Trueness, close agreement between a test result and the accepted reference value of the property being measured.
  - CRM: certified reference material
  - Reference method
  - Spiking/ recovery

Minimum Validation Acceptance Criteria

- **Precision**
  - Closeness of agreement between independent test results obtained.
  - In terms of standard deviation or relative standard deviation

SRM from NIST



Minimum Validation Acceptance Criteria

- Typically for a single lab validation the Laboratory Analyst should perform *r* replicate analyses of *m* test portions over a period of *d* days for each sample type (matrix) *n*, where *r* is the number of replicates (2, 3,…), *m* is the number of test portions in each group, *d* is the number of days, and *n* is the number of different sample types.
- *r* x *m* should never be less than 10
- *n* should be at least 2 (preferably more)
- *d* should be at least 2

Reference Material and Internal Control

- Reference material production conforms to the same ISO 17034 criteria as CRM. Less rigorous documentation
- An internal control is developed by the lab and used to ensure the testing process is under control
- Manufacturer of an ingredient (e.g. drug)

Certified Reference Material

- A Reference Material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence.
- Whilst Certified Reference Materials are preferred, their availability is limited

Extent of Validation Studies

- Fully validated method
- Fully validate method, but a new matrix is to be used
- A well-established method, but not collaboratively studied
- Method published in scientific literature together with some analytical characteristics
- Method published in scientific literature
- Method is empirical
- The analysis is “ad hoc”
- Changes in staff and equipment

Method Performance Characteristics

- Applicability
- Selectivity
- Calibration and linearity
- Trueness
- Precision
- Recovery
- Range
- Detection limit
- Limit of quantification
- Sensitivity
- Ruggedness
- Fitness for purpose
- Matrix variation
- Measurement uncertainty

Appendix A Harmonize Protocol … 2002

Minimum Validation Acceptance Criteria

- Calibration and linearity
- Calibration (standard) curve - The relationship between instrument response and known concentrations of the analyte.
- For any quantitative method, it is necessary to determine the range of analyte concentrations or property values over which the method may be applied.

Recovery

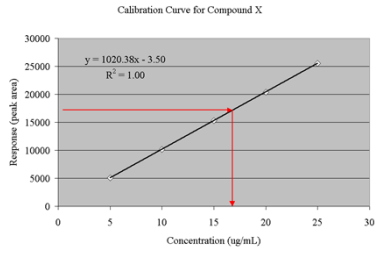
- Analytical methods do not always measure all of the analyte of interest present in the sample.
- Analytes may be present in a variety of forms in samples not all of interest to the analyst.
- The method may thus be deliberately designed to determine only a particular form of the analyte.
- It is necessary to assess the efficiency of the method in detecting all of the analyte present

Calibration Curve

- Within the working range there may exist a linear response range.
- Regression calculations on their own are insufficient to establish linearity. To do this a visual inspection of the line and residuals may be sufficient; objective tests, such as 'goodness-of-fit' tests, are still better.

Use of Spiking and Recovery

- In the absence of reference material, bias can be investigated by spiking and recovery study.
- The material was spiked with the analyte of interest and analyzed by the method under validation
- Spiking and recovery studies are strongly subject to the observation that the good recovery is not a guarantee of trueness.



Minimum Validation Acceptance Criteria

Limits of detection and limits of qualification

- Where measurements are made at low analyte or property levels, e.g. in trace analysis, it is important to know what is the lowest concentration of the analyte or property value that can be confidently detected by the method.
- The 'limit of quantitation' (LOQ) is strictly the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness.
- Not generally accepted.

LOD and LOQ continued

- LOD must be demonstrated. Typically this is defined as the analyte concentration at 3 times the signal produced by analyzing a blank. For many laboratories the LOD is not as significant as the LOQ. Typically this is defined as the analyte concentration at 10 times the signal produced by analyzing a blank.
- Note that neither LOD nor LOQ represent levels at which quantitation is impossible. It is simply that the size of the associated uncertainties approach comparability with the actual result in the region of the LOD.

Minimum Validation Acceptance Criteria

Accuracy and precision target limits

Concentration	Repeatability (%)	Recovery (%)
100 %	1.3	98 – 102
10 %	1.9	98 – 102
1 %	2.7	97 – 103
0.1 %	3.7	95 – 105
0.01 %	5.3	90 – 107
0.001 %	7.3	80 – 110
1 ppm	11	80 – 110
100 ppb	15	80 – 110
10 ppb	21	60 – 115
1 ppb	30	40 – 120

OTSC SOP M0033

Important Parameters

- Confirmation of identity and selectivity/specificity
- The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced (those thought likely to be present in samples).

Collaborative studies

- Minimum of 8 laboratories
- Only in special cases a minimum of 5 laboratories is allowed
- The optimum number of labs is between 8-10
- For qualitative analyses, a minimum of 10 laboratories is needed
- Collaborative study must be designed to include 2 analyte levels per matrix, 6 test samples per level, and 6 negative controls per matrix.

Analysts

- Most designs require only 1 analyst per laboratory
- 2 analysts from the same laboratory cannot be substituted for different laboratories

Test Materials

- Homogeneous
- Concentration range

Statistical Analysis

- Major discrepancies : displaced means, unduly spread replicates, outlying values, differences between methods, consistently high or low laboratory rankings
- Invalid data may result
  - (*1*) the method is not followed;
  - (*2*) a nonlinear calibration curve is found although a linear curve is expected;
  - (*3*) system suitability specifications were not met;
  - (*4*) resolution is inadequate;
  - (*5*) distorted absorption curves arise;
  - (*6*) unexpected reactions occur;
  - (*7*) other atypical phenomena materialize.

Outliers

- Inherent
- *Cochran test:* Compute the within-laboratory variance for each laboratory and divide the largest of these by the sum of all of these variances.
- *Grubbs test*: removal of laboratories with extreme averages.

Precision

- Two circumstances of replication: within laboratory and among laboratory
- Repeatability
- Reproducibility

HORRAT Value

- HORRAT value is the ratio of the reproducibility relative standard deviation to the predicted reproducibility relative standard deviation

  $RSD_R = 2^{(1-0.5\log C)}$

  C is the concentration of the analyte
- HORRAT Value= Experimental RSD/RSD_R
- HORRAT values between 0.5 to 1.5 may be taken to indicate that the performance value for the method corresponds to historical performance. The limits for performance acceptability are 0.5–2.

HORRAT Value

- HORRAT <0.5.Method reproducibility may be in question due to lack of study independence, unreported averaging, or consultations.
- 0.5 < HORRAT < 1.5.Method reproducibility as normally would be expected.
- HORRAT > 1.5.Method reproducibility higher than normally expected:
- HORRAT > 2.0.Method reproducibility is problematic.

Validation of Qualitative Method

- Method of analysis whose response is either the presence or absence of the analyte, detected either directly or indirectly in a certain amount of a sample
- Terms such as screening systems, test kits, field tests or immunoassays are traditionally used when referring to qualitative methods, they could also be used when dealing with quantitative and semi-quantitative methods.

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