STANDARD OPERATING PROCEDURE
TEST METHOD VALIDATION

1. PURPOSE

This document presents a working description of validation of analytical test methods. Method validation confirms that an analytical procedure is suitable for its intended use. A successful method validation provides evidence of the accuracy, precision, sensitivity, selectivity, and reproducibility of the analytical data resulting from performance of the method. In accordance with industry standards and regulatory bodies, analytical methods must be evaluated for validation requirements before they can be adopted for routine use. Only validated methods should be used to acquire meaningful data, however, the validation scope can be reduced for non-routine methods.

2. SCOPE

This document applies to analytical test methods purchased from an external source, internally developed analytical methods, and standard analytical test methods*. Analytical test methods for use in the manufacture, QC testing, packaging, and labeling of products are within scope of this SOP. The depth of the validation is proportional to the level of potential risk to final product quality (and human safety) should the analytical method fail to meet its intended use.

**Standard analytical methods include those developed by organizations such as the EPA, American Society for Testing and Materials (ASTM), ISO, IEC, or the USP (United States Pharmacopeia).*

Note that a standard analytical method, though published by a reputable source, must be evaluated for validation requirements to provide evidence that the method's performance meets the intended use requirements in the intended environment. When the intended use requirements overlap those of the published standard method, the performance data resulting from the intended use must align with the published performance data.

Common analytical methods within the scope of these validation requirements include, but are not limited to, the following:

- Biological Assays: Functional molecular biological tests such as DNA or RNA amplification, DNA ligation, and DNA end-repair.
- Quantification Limits: Quantification of impurities, residual solvents, or other unintended materials. Quantity of target material.
- Potency Assays: Applied to raw materials or intermediates.
- Physical Characterization Assays
- Functional Characterization Assays
- Qualitative Assays: Tests to establish presence and identity of an analyte of interest
- Quantitative Assays: Tests to establish the concentration of an analyte of interest.

Reference note: ICH Guidelines on *Validation of Analytical Procedures: Text and Methodology Q2(R1)* Step 4 approved 27 October 1994 and November, 2005.

3. BASIC CONCEPTS

3.1 METHOD VALIDATION PHASES**:**

The validation of analytical test methods is divided into at least three distinct phases:

- Phase I: Pre-validation Characterization and Risk Analysis:

  Before formal validation, characterization of the analytical method is necessary to understand the factors that can impact the method's suitability for its intended use. During this phase, the method's design will develop until the suitability for its intended use is ensured. This is the final phase of the method's development; this phase provides a "sand box" for proof of concept of the method. Analytical performance characteristics (APCs) can be identified through the combination of risk analysis of the proposed method, regulatory requirements, and the intended use.

- Phase II: Validation

  Validation testing is based on the intended use specification, mitigations to risk, and the analytical performance characteristics (APC) requirements. Validation is performed using the intended processes, materials and equipment, by the intended end operators, in the intended use environment (or equivalents). If equivalents are used during validation, a documented justification is required.

- Phase III: Re-validation:

  Retesting may be required when there are changes to the analytical method that may invalidate the initial validation results. Changes to equipment, workflow, infrastructure, APCs requirements, or process may invalidate previous validation results.

Analytical method validation documentation (plans, protocols, results, and reports) are submitted to the change control and document revision system.

3.2 TEST METHODS EXEMPT FROM VALIDATION

Analytical test methods may be exempt from further validation if the following criteria are met:

- Methods published by recognized organizations; these are considered as validated.
- Traceable standards are used to establish system suitability.
- The sample is not modified except for dilution and the diluted sample is evaluated by direct detection using qualified equipment.

Based on the exemption criteria listed above, the following test methods are exempt from validation:

- pH determinations
- Conductivity measurements
- Gravimetric measurements
- Index of refractions
- Density determinations

3.3 TEST METHOD TYPES AND PERFORMANCE CHARACTERISTICS

Method types vary from highly analytical determinations to subjective evaluations of performance characteristics. In the two following tables there are lists of common analytical method types and their relationship to the performance characteristics, based on information from ICH, FDA, and USP.

Table 1: Analytical Test Method Types

Analytical Test Method Type	Intended Use Description
Identification or Qualitative Test	Ensure the identity or presence of an analyte in a sample. This can be achieved by comparison of a property of the sample to that of a well defined reference standard. Examples include spectrum, chromatographic behavior, chemical reactivity, etc.
Quantitative Test	Measure the analyte of interest present in a sample, or measure the chemical compounds or physical entities which are unintentionally present in the sample. The test represents a quantitative measurement of the major component(s) in the sample.
Limit Test	A non-quantitative test that substantiates that the concentration of an analyte (or impurity) is above or below a specified value.
Assay Test	Quantitative measure of the biologically active component or analyte of interest in a sample. Assays may be used to measure concentration, potency, or specific activity of an analyte of interest. They are often used to assess a material or product for acceptability.

Table 2: Analytical Performance Characteristics

Analytical Performance Characteristic	Description	Identity (Qualification)	Limit	Quantitative	Assay
Specificity	The ability to unequivocally assess the analyte of interest in the presence of components which may be expected to be present. The method produces a response for a single analyte only. A ***selective*** method is one that responds to a number of chemical entities *but* can distinguish one from another. Because there are very few methods that respond to only a single analyte, some authorities use the term *selectivity* instead of *specificity*. The USP defines a selective method as one that accurately measures an analyte in the presence of interference such as synthetic precursors, excipients, enantiomers and known (or likely) degradation products in the sample matrix	YES	YES	YES	YES
Accuracy	The extent that an analytical method's result and the true value agree. Accuracy is a metric used to evaluate a result in comparison with the true value (or the mean of several results to the true value). Accuracy should be expressed as the difference between the mean and the accepted true value, together with the confidence intervals. The true value can be determined by: • Test method measurement of a certified reference material with its supplied true value. • Comparison of the test method results with the results of an established reference method. • Establishment of a standard curve by measurement of several samples of matrix of interest spiked with known concentrations of the analyte.	NO	NO	YES	YES
Precision	When the same entity is measured two or more times, the nearness of the measurements to each other is the precision of the measurements. Precision is independent of accuracy (the nearness of the measurement to the true value). ***Repeatability*** is a measure of the precision of a result under the same operating conditions over a short period of time, for example, within a batch.	NO	NO	YES	YES

Analytical Performance Characteristic	Description	Identity (Qualification)	Limit	Quantitative	Assay
	Intermediate Precision is the variability in the long-term within a single laboratory, such as different days, batches, operators, inconsistent work practice (thoroughness) of a single operator, standards for QC or reagents from disparate suppliers, and equipment (or a combination of these). Intermediate precision validation is performed to verify that in the same lab, the analytical method will provide the same results after the development phase is completed. ***Reproducibility*** is the precision between two or more laboratories under operational and environmental conditions that may differ but are within the validation specifications. Sources of variation include room temperature, humidity, experience and thoroughness of operators, equipment maintenance and age, consumables of different ages, and quality of materials. ICH changed the metric of "ruggedness" to reproducibility.				
Blank Limit (LoB)	The highest measurement likely to be observed for a blank sample within a specified confidence level. It is also the lowest value expected from a sample that contains a LoD, at a specified confidence level.				
Detection Limit (LoD)	The lowest amount of analyte in a sample which can be detected but not necessarily quantitified. It is often called the limit of detection (LOD) which is the lowest concentration that can be determined to be statistically different from a blank, at a specified level of confidence. Method detection limit (MDL) is the minimum concentration that can be measured with 99% confidence that the analyte concentration is greater than zero. It is determined from analysis of samples in a given matrix spiked with the analyte. Note that LoD is not a measure of sensitivity. Sensitivity of an analytical method is the capability of the method to discriminate small differences in the concentration or mass of the analyte of interest. It is the slope of the calibration curve obtained when method response is plotted against analyte concentration or	NO	YES	*	NO

Analytical Performance Characteristic	Description	Identity (Qualification)	Limit	Quantitative	Assay
	mass.				
Quantitation Limit (LoQ)	The level above which quantitative results may be determined with acceptable accuracy and precision. It is important to not only use pure standards as samples for this test, but also spiked matrix samples.	NO	NO	YES	NO
Linearity	The ability of the method to elicit results that are directly proportional to analyte concentration within a given range or proportional by means of a well-defined mathematical calculation. Often linearity is evaluated graphically. An example of a linear relationship of instrument output to analyte concentration is a spectrophotometric analysis of concentration – Beer's Law. Absorption at a characteristic wavelength is directly proportional to analyte concentration, in the absence of stray light.	NO	NO	YES	YES
Range	The interval between the upper and lower limits (including the upper and lower limits) for which it has been demonstrated that the analytical method has an acceptable level of accuracy, precision, and linearity.	NO	NO	YES	YES
Robustness (Reliability)	Evaluation of robustness provides an indication of the method's reliability over a realistic range of variation. Robustness is a measure of the effect of operational variances on the method's results. It is the measure of an analytical method's capacity to remain unaffected by small, but deliberate, variations in method parameters. If the influence of an operational variance does not cause the result to be out of the specified tolerance, the operational variance is evaluated as within the method's robustness range. A Design of Experiments methodology is used to evaluate robustness. Data used to evaluate ruggedness is the same data used to determine if a change to the method must trigger a re-validation cycle. Re-validation is not required when method changes are within a method's robustness range.	*	*	*	*

4. PROCEDURE

4.1 Pre-validation Characterization: Phase I

The required scope of pre-validation is dependent upon the required level of certainty that the proposed method is suitable for its intended use. These initial experiments provide information upon which decisions for changes to the method itself, the validation approach, or acceptance criteria are made. Method development and validation is an iterative process.

Establish a record in the change control and document revision system for the collection, storage, and protection of the documentation associated with the method characterization and its subsequent validation. All documentation supporting the validation of the test method should be referenced under one part number for facilitation of record retrieval.

4.1.1 **Team**: Identify and assemble a project team for the development and qualification of the analytical method. Assign and document the responsibilities.

4.1.2. **Specification**: Document a description of the proposed analytical method. The method's description is revised iteratively until it is a complete and unambiguous specification of the method. The formal validation is based on the completed method's specification. Include this information in the specification:

- **Intended use**. If it is important to understanding, include any unintended uses. Translate the intended use description into discrete, testable requirement statements.
- **Intended user(s)**: Include required skills or education. If practical, translate the description of the test method's user(s) into discrete requirements statements.
- **Use environment**: End-use physical environment.
- **Reference methods**: Other similar analytical tests which are validated. Document in detail the comparable attributes and the dissimilar attributes. (Similar structure, same matrix, shared elements of functional tests, etc.)
- **Test method type(s)**: The test method type is characterized by the nature of its output data (qualitative or quantitative). Reference Table 1 for terminology and definitions.
- **APCs**: Identify the Analytical Performance Characteristics that are associated with the method type and are relevant to the intended use (reference Table 2 for terminology and definitions). Identify critical characteristics.
    - Expected concentration levels, concentration ranges
    - Detection limits
    - Quantitation limits
    - Working range
    - Precision and accuracy targets for intermediates and final output
    - Robustness and stability targets
    - Standards required for QC, if any
- **Analytes**: Identify analyte(s) of interest.
- **Matrix**: Composition of the sample matrices.
- **Performance:** Detail the performance requirements.

- **Interference**: Identity of potential interfering substances; possible sources of interference.
- **Stability**: Possible sources of instability (variation).
- **Samples:** Describe sample preparation requirement. Define sampling scheme.
- **Procedural Sequence**: Key processes and their proposed sequence; include justification for any processes determined to be exempt from validation. Define the functional assay.
- **Equipment**: Required equipment, specific or by type. Specify any operational environment requirements. Description of custom equipment, if any.
- **Prerequisites**: Preliminary set up instructions including those for sample preparation, instrumentation preparation, etc.
- **Unknowns**: Other unknowns to investigate, if any.
- **Maintenance QC**: Develop a plan for the monitoring of the analytical method. Identify relevant QC standards and their traceability to a recognized standard (if any), frequency of QC testing, and replicate sampling requirements. Include unambiguous acceptance criteria.

4.1.3 **Review**: Formally review the analytical method's description. When evaluated as acceptable (complete and unambiguous), submit the method description to the change control and document revision system.

4.1.4. **Protocol Development**: Develop a documented pre-validation and characterization protocol based on the approved method specification. Design the test method to:

- Ensure the method is suitable for its intended use.
- Evaluate precision, accuracy, specificity, stability, and repeatability.
- Evaluate the Analytical Performance Characteristics and their associated targets. Verify the proposed targets are achievable.
- Resolve unknowns.
- Evaluate robustness.
- Ensure full optimization of the method.

4.1.5 **Gap Analysis**: Using the analytical method's specification and the requirements of the pre-validation protocol, perform a resource gap analysis. The resource gap analysis and the development of the pre-validation protocol may be concurrent.

- Skills of personnel required to perform the pre-validation protocol (internal and 3rd party).
- Facilities and other infrastructure requirements.
- Equipment and technology requirements (purchase, lease, customize).
- Additional performance qualification for equipment already in use, but qualified for other applications.
    - Calibration or preventive maintenance requirements.
    - IOQ requirements.
- Suppliers of new materials and services.
- Identification of comparable test method validations, for reference.

4.1.6 **Test:** Perform the pre-validation protocol as documented. Additional tests may be developed "on-the-fly" to further investigate and characterize aspects of the proposed analytical method.

Document the pre-validation testing results. Include description and results of any "on-the-fly" testing.

Assemble the project team to evaluate the pre-validation results for acceptability.

4.1.9 **Iterate**: If appropriate, based on the results of the pre-validation testing, revise the analytical method's specification. Replace targets with requirements and their tolerances. Approve the revision in accordance with the change control and document revision procedure.

4.2 Risk Analysis

Perform a formal risk analysis on the test method as specified and approved.

Edit the analytical method description based on the results of the risk analysis; add requirements that are identified as risk mitigations, if appropriate.

4.3 Validation: Phase II

Based on the analytical method's specification, establish (define and document) a validation test plan and its required protocols.

4.3.1 **Validation Plan**: Ensure that these required elements are included in the validation plan:

- Identity of the component validation protocols. Identify each one by its:
  - Title
  - Test Method Type (See Table 1 above for definitions and terminology).
- Analytical Performance Characteristics to be validated (See Table 2 above for definitions and terminology).
- Traceability matrix. Document how each method requirement is validated; for each method requirement, identity the validation protocol used to provide objective evidence that the requirement is met. Generally traceability matrices are formatted as a table. Traceability may be a 1:1 comparison or a one-to-many comparison (one requirement to many validation tests).

  Include any required risk mitigations in the traceability matrix; document how each risk mitigation is verified for effectiveness.
- Description of the actual conditions of use. If the test method will not be validated under actual conditions of use, the equivalency of the simulation to the actual condition of use must be justified and the justification must be documented.
- Describe the method's end user. If the assigned validation tester is not an end user, a comparison of the end user's attributes and those of the validation tester must be made. The evaluation of equivalence must be justified, and the justification documented.
- If any critical or unique equipment is substituted, justify its evaluation of equivalence. Document the justification for the evaluation of equivalence.
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4.3.2 **Protocol(s**): Ensure these requirements are included in the validation protocol.

- Step-by-step sequential workflow instructions are required. The instructions must be complete and unambiguous. All experimental parameters must be documented to ensure that the validation can be precisely repeated.
- Complete and unambiguous acceptance criteria.
- Functional description of required equipment.
- Include tests to provide evidence that the risk mitigations are effective.

Review and approve the validation protocol. Submit the validation plan and protocol(s) to the change control and document revision system.

Conduct training of individual(s) responsible for performing the validation protocol, if required.

Run the analytical method validation protocol.

4.3.3 **Evaluate Results**: Evaluate the method validation results against the acceptance criteria.

- Investigate any nonconformances discovered during validation. Perform additional validation testing, if appropriate. If a root cause cannot be identified, determine if further pre-validation characterization is required. Analyze any nonconformance for risk.
- Document the validation. Include:
    - Description of any validation testing that was in addition to that originally planned and documented as protocols.
    - Test results.
    - Review discussions, decisions, and approvals.
    - Evaluation of suitability of analytical method for its intended use.
- Ensure that the validation description is detailed to the extent that someone, who is comparably competent as the original validation tester, can reproduce the validation.
- Submit the completed validation documentation to the change control and document revision system.

4.3.4 **Transfer Plan**: Develop and document the plan for the analytical method's transfer from development to routine analysis.

- Include these considerations in the transfer plan:
    - Identify at least two critical tests to qualify the routine use of the analytical method in the receiving environment. Include a documented rationale for the selection of the qualification tests. Specify sample type and replicates.
    - Training requirements for those identified as the routine users of the method. Include equipment use, critical parameters, and troubleshooting in the training plan.
    - Development/documentation of the method's user instructions (procedure).
    - Assigned responsibilities for qualification of transfer effectiveness, training of end users, development of use procedures, etc. Specify that a method is officially transferred after at least three samples are effectively analyzed.

- Implement the transfer plan.
    - Write the use procedure and submit it to the change control and document revision system
    - Document the user training and update the training records, if appropriate.
    - Verify and document the effectiveness of transfer of the method. Add this verification document to the validation documentation in the change control and document revision system. (Example is a successful performance of the test method on three batches of material.)

4.4 Re-Validation

Revalidation is required when a change to an analytical method:

- Causes the method to function outside of its validated range.
- Method changes are outside the method's robustness range. Proposed method changes can be evaluated as operational variances, thus the evaluation of robustness applies.

The scope of the re-validation is dependent upon the scope of the change and the potential for added risk to final product quality (or user safety). Examples of changes that may cause a requirement for partial or full re-validation include:

- **Tolerances**: Expanded operational tolerances or characterization tolerances
- **Intended Use**: Expanded intended use description
- **User Skillset:** New users with different skills
- **Environment**: Method transfer, a move to a different building or environment
- **Supply Chain**: New supplier of a custom material
- **Materials**: Properties of constituent materials such as composition, purity, concentration, stability, etc. New chemicals or reference standards
- **Equipment**: Equipment substitution, different functional characteristics
- **Technology**: New technology introduced, method "improvement"
- **Corrective Action**: Corrective action applied to the method to remove a root cause of non-conformance
- **Sample**: Sample matrix characterization; sample preparation procedure
- **Procedure**: Procedural step sequence or changes to duration/timing of processes
- **Re-activation**: Re-instatement of a retired analytical method

In addition to the documentation requirements for the initial validation, the re-validation plan and resulting documentation must include:

- Reason for the change
- Detailed description of the change
- Risk analysis data, updated to reflect the change
- Revalidation protocols: Identification of the initial validation protocols that must be repeated, any new protocols that must be performed, and rationale for any initial validation protocols that are removed from the re-validation plan
- Unambiguous acceptance criteria
- Evidence that the analytical method is suitable for its intended use

- Updated analytical method description/specification

REFERENCE DOCUMENTS

FDA - Guidance for Industry (draft) Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls and Documentation, 2000

ICH Q2B Validation of analytical procedure: Text and Methodology, Q2 (R1), November 2005

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