Regulatory Basis:
FDA Quality Systems Regulations

Reference: FDA CFR - Code of Federal Regulations Title 21

Purpose

The purpose of this guideline is to outline the content and approval process for analytical procedures and to describe those activities that should be carried out to demonstrate that analytical procedures used in GMP laboratories are suitable for their intended purpose.

Scope and Applicability

This guideline applies to qualitative or quantitative analytical procedures that are used to test finished drug product, in-process materials, excipients, raw materials, packaging materials and Active Pharmaceutical Ingredient (API), in support of regulatory registration documents and in cleaning validation.

Technology Transfer is outside the scope of this document.

Definitions

Analytical Procedure
A controlled document that describes in sufficient detail how a specific analysis is performed.

Analytical Procedure Validation
Confirmation that the performance characteristics of the analytical procedure meet the requirements for the intended application. This is usually established by laboratory studies.

Analytical Procedure Revalidation
Confirmation that the performance characteristics of the analytical procedure continue to meet the requirements of the intended application, following changes to the specific procedure or the synthetic route/method of manufacture of the test material. This is usually established by laboratory studies.

Validation Protocol
A validation protocol is written plan or protocol stating how validation, sampling and testing will be conducted, defining roles and responsibilities, and defining acceptance criteria. Analytical procedure validation protocols may be generic or specific and their content will depend on the phase of development or marketing.

Responsibilities

Analytical procedures should be developed, validated and approved by the originating laboratory. This may be within Analytical, Microbiology, Device or Packaging, or Quality Control functions.

Analytical procedure validation reports should be written and approved as part of the validation process. Analytical procedure revalidation is the responsibility of the department that will routinely use the revalidated procedure.

Quality Assurance will approve any local standard operating procedure that covers analytical method validation. QA will ensure that analytical procedure(s) and validation exists as required by

directly linked to the method's intended use.

Method Validation Summary Report:

It is recommended to analyse the experimental results and prepare a Method Validation Summary of the findings.

These method validation summaries may include but are not limited to:

- The performance results against criteria listed within this guideline, site SOP, or separate pre- approved protocol.
- For higher risk methods, at least two reviewer signatures are recommended to be obtained for the Method Validation Summary to be approved.

It is suggested that at a minimum one signatory should be a member of the Site Quality Team. This should be an independent reviewer, not involved in the validation activities for that test method, in order to avoid the potential for conflict of interest.

- The author should be responsible for determining that all data are accurately transcribed into the Method Validation Summary.
- The reviewing/ approvers should be responsible for
    - Technical correctness and completeness,
    - Regulatory Compliance – Practices,
    - Compliance – Registration,
    - Compliance with written site requirements, SOPs;
    - Authorization to implement.
    - Review of changes to methods and their impact on other quality systems (e.g. process validation, etc)
    - Review of deviations and failures during the method validation and their impact on the conclusions, if any.
    - Test Raw Data or Reference to Raw Data.
    - Reference to method development data (e.g robustness) if not referenced in a protocol.

Validation of Pharmacopoeial Methods:
Pharmacopoeial methods included in a specific official monograph are generally considered as validated. However, the suitability of compendial analytical procedures must be verified under actual conditions of use. It is recommended to demonstrate absence of interference with the compendial method, thus specificity (if applicable) should be assessed. Intermediate precision and stability of the sample solution should also be investigated using the compendial method for the specific API. Demonstration of the applicability of the method for use in the analysis of the specific product/material should be accomplished by the analysis of the material using the pharmacopoeial method. System suitability requirements of the method should be met, and for raw materials the results should conform to the expected result for that grade of material.

Methods may be grouped for this evaluation, such as in the above table to set prioritization for the team.

During this evaluation phase, a number of contributing factors tend to determine impact to quality. Downstream effects may also need to be considered. For example, once the material is isolated, tested, and discharged from the equipment, one may have to reprocess/ rework material because of potentially inaccurate data from the earlier IPC test method.

The use of what-if scenarios can assist in the risk analysis. For example, consider the following:

- *Stage in the API Process: Where does this test method's result lie within the overall quality 'control' or 'assurance' strategy in producing a quality API?*
- *Critical Quality Attribute: Can analytical data gleaned from this early stage of the step highlight where an impurity or its precursor is forming?*
- *Critical Quality Parameter: Could the process step be adjusted within allowable parameters to marginalize or purge unwanted impurities before isolation?*

Evaluation Conclusions and Corrective Actions:

For those test methods, which are identified as higher risk (e.g. See Table 1), one can review the existing validation documentation (if any) associated with the method.

Documentation content and accessibility of the raw data should be considered rather than specific validation documents when assessing legacy methods and their existing validation.

Sites should leverage existing documentation through remediation and reference (when possible), to address validation deficiencies.

System Suitability Testing

Per ICH Q7A, the degree of analytical validation performed should reflect the purpose of the analysis and the stage of the API production process. Material, In-Process Control and Early Intermediate Material Tests.

System Suitability is a predetermined set of tests and applied method requirements that are used to determine if an analytical method is performing within its validated parameters and is acceptable for its intended use. The method validation exercise may include statistical interpretation of data to provide adequate justification for reduced System Suitability Testing (SST) and numbers of standard and sample injections. Suggested System Suitability recommendations are established in **Appendix 1** for different types of API "In-Process testing".

Recommended SST Data:
System suitability criteria from compendial general chapter methods may be used for some test methods but should be evaluated against the intended use of the test method as to applicability.

- Resolution can be calculated between the major component and an internal standard, the major component and an expected impurity found in the reference standard, two impurity peaks in the reference standard (resolution material), or two peaks that are the most difficult to separate (often referred to as "the critical pair"). Since resolution is the primary criterion for specificity and robustness, it serves as a rigorous parameter for suitability. If there is no critical pair to establish a resolution criterion, a retention time window may be

Accuracy is important when a specific value is needed. For example, in the case when a method is used to monitor an impurity that is not reduced in downstream processing or if a minimum titer or amount is required (e.g. specific molar ratio) or if the assay is used to calculate the amount of catalyst needed to drive a reaction to completion.

Accuracy may be established across the specified range of the analytical procedure. Accuracy may be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations /3 replicates each of the total analytical procedure). Accuracy can be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

Accuracy may be established by one of the following:

- Application of the analytical procedure to an analyte of known purity (e.g., a reference material or stock standard) and demonstration that the expected true value is obtained. Accuracy should be determined across the range. This can be accomplished by spiking the analyte of interest with a known amount of concentration of the analyte material.

- In cases where specified impurities/degradation products are not available a surrogate material such as a compound with similar structure or API may be used to demonstrate accuracy. In these cases, a rationale for the use of a surrogate should be given.

- Known amounts of impurities or degradation products may be added to the process solution. The spiking procedure should include the high and low extremes of the range plus an intermediate value.

Recommended Accuracy Data:
Percent recovery is calculated for each reportable value as defined in the method. The average percent recovery may be calculated at each level and compared to the acceptance criteria.

Recommended Accuracy Criteria:
Several factors can be considered when selecting criteria: The intended purpose of the test and the expected specification range are important parameters. See Tables below for recommended acceptance criteria.

Statistical Basis for Acceptance Criteria for both Accuracy and Precision:
The following recommended criteria in the Tables are derived to insure that the method can support its intended purpose, which is release of product against specifications. For impurity methods, the accuracy of the impurity determination can be either determined concurrently with the method precision recovery of spiked impurities or by spiking the impurity into a sample at approximately the Quantitation Limit (QL), 100% and 120% of the specification limit. The overall % RSD of results from multiple occasions should meet the recommended criteria.

For impurities, the following tables may be used as guidance for setting acceptance criteria that is also based on the specification. These recommended acceptance criteria are based on what can typically be achieved by an impurities method, including those that are Area% methods.

Table 1: Recommended Criteria for Precision and Accuracy – Higher Risk Test Method Impurity Determinations

Impurity Spike Precision Accuracy

In some cases the instrument itself is the limiting factor for the analysis regardless of the sample.
An example of this is an LOD test using an analytical balance. In this case a discussion of the quantitation limit may be constructed in the validation documentation based on the calibration tolerance of the equipment rather than analysis of actual samples. The actual limit of quantitation would still be presented in numerical terms relevant to the assay method based on the discussion.

Another example of this may be for KF titration assays where the ability of the instrument to deliver a minimum amount of titrant would be the limiting factor. It is recommended that experiments to determine this minimum amount of sample should be conducted for the specific instrument model if this approach is taken. The experiment(s) could then be referred to in any validation that utilizes the same model of equipment.

- Based on the Standard Deviation of the Response and the Slope
  The quantitation limit (QL) may be expressed as: QL = 10 σ/ S where, σ= the deviation of the response; S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of σ is carried out in a variety of ways including:

  - Based on the Standard Deviation of the Blank:
    Analyzing an appropriate number of blank samples and calculating the standard deviation of these responses and perform measurement of the magnitude of analytical background response.

  - Based on the Calibration Curve:
    A specific calibration curve should be studied using samples containing an analyte in the range of the QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.
    In all cases, the quantitation limit can be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit or reporting level.

Two possible approaches include:

A)
Three replicate preparations of a spiked sample are prepared at the quantitation level or reporting level and analyzed. Calculate the % recovery. Calculate the average of the replicates and % RSD.

B)
Alternatively, accuracy or repeatability experiments at or near the quantitation limit or reporting level can be used for this determination.

Recommended QL Data:
The quantitation limit and the method used for determining the quantitation limit should be presented. For validation of the actual quantitation limit or reporting level:

For case (A) it is suggested to report the average of replicates, % recovery and RSD. For case (B) it is suggested to report the accuracy or repeatability of replicate experiments conducted at or near the reporting level.
The quantitation limit should be expressed as the amount actually measured, as well as the corresponding percentage of the target analyte concentration. If applicable, representative chromatograms can be presented at an expansion that allows visual inspection of the signal vs. noise and integrations that impact quantitation.

Figure: Parameters that can be used for test method robustness:

Parameters that can be used to test method robustness:
Suggested changes in parameters to prove robustness are given in the following table,

Attribute/Technique	Robustness Considerations
General	Different Analysts Test location environment (air conditioned lab vs non temperature controlled manufacturing area) Crude and purified products
Standard Solution Stability	Storage time Storage conditions (room temp, refrigerated, direct sunlight)
Sample Solution Stability	Storage time Storage conditions (room temp, refrigerated, direct sunlight), sample quenching (effect on stability)
HPLC	pH of mobile phase Mobile phase composition (e.g. % Organic) Different columns (lots or suppliers) Detection wavelength (typically +/- 5 nm Temperature Flow rate
GC	Different columns (different lots or suppliers) Temperature (injector, oven, detector) Flow rate (carrier, inlet, split)
AA (Atomic Absorption)	Gas flow Nebulization rate Slit width
TLC (Thin Layer Chromatography	Evaluation of plates (different brands, lots) Variation of solution (eluent conditions) Chamber saturation effects time vs. chromatography Elution time (separation , degradation of sample) Development time (e.g. spray with reagent then heat for "x" minutes) Degradation of sample (2d spots)
LOD (instrumental e.g. Denver or Computrac or oven)	Time vs. result (optimize required drying time) Result vs. temp (lack of temp. degradation)
UV	Detection wavelength Solution stability
IR	Effects of possible relevant variations, e.g. temperature (environment and sample), humidity, different position of the sample in the optical window, different sample presentation devices, probe depth. Effects of sample quantity (e.g. in a pellet).

Recommended Robustness Criteria:
Changes within the test range whether allowed explicitly or implicitly by the assay should not exceed the previously defined validation parameters for accuracy, precision or specificity. This may be accomplished by evaluation of system suitability parameters that are relevant to the change.

- For example, robustness for limits tests should confirm that variations still cause a pass/fail decision to be unaffected by the change. Multiple preparations below and above the specification can be used to demonstrate the ability of the method to reliably distinguish passing and failing results.

Increased robustness testing during development may provide additional support for an abbreviated System Suitability Testing (SST). If robustness testing is not adequately performed and documented during development and/ or validation, there is more of a reliance on detailed SST, which should be included during the run. A full SST at the beginning of a campaign could be performed, and then repeated periodically throughout the campaign. As a working practice, some sites allow 24 hours validity between SST and running a sample (provided no major changes in instrument operating conditions have been performed within the time period). This allows the laboratory to analyze a series of samples within that 24 hour period without repeating the SST and provides the advantage of allowing a quicker sample turn around in cases where analysis of many samples may be required over a short time period (e.g. when monitoring residual solvent by hourly sampling during drying). The choice of taking this approach should be carefully weighed with the risk of implicating a large amount of data if a system suitability failure were to occur.