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Validation of Analytical Test Methods in Medical Device Manufacturing

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Date Prepared: January 2008

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1.0 Goals of Test Method Validation:

Test method validation goals can be broken into three parts:

- Show that the method is appropriate for the specification being assessed and suitable for its intended purpose.
- Demonstrate that the test method meets specified requirements for accuracy, precision, repeatability and operating range over repeated applications.
- Show method is sensitive and specific enough to distinguish borderline acceptable product from unacceptable product.

2.0 Background:

The validation principles and content of this document are distilled and adapted from guidance provided by the U.S. Food and Drug Administration's (FDA's) CDER (Center for Drug Evaluation and Research)[1] and by the ICH (International Conference on Harmonization)[2]. This document offers assistance in developing analytical method validation in the medical device manufacturing industry and provides general recommendations which can be adjusted or modified depending on the specific type of analytical method used.

Appropriately selective and sensitive analytical methods for quantitative evaluation are critical. Analytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given matrix is reliable and reproducible for the intended use.

Validation of a test method involves documenting, through the use of specific investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method.

Different types and levels of validation are appropriate based on the circumstances. Full validation is important when developing and implementing an analytical method for the first time. Partial validations may be considered when modifications are made to previously-validated analytical methods.

3.0 Definitions:

Accuracy: The degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed *trueness*.

Analyte: A specific chemical moiety being measured.

Analytical run (or batch): A complete set of analytical and study samples with appropriate number of standards and QCs for their validation. Several runs (or batches) may be completed in one day, or one run (or batch) may take several days to complete.

Matrix effect: The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample.

Precision: The closeness of agreement (*degree of scatter*) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Range: The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Repeatability: The precision of the method under the same operating conditions over a short period of time.

Sample: A generic term encompassing controls, blanks, unknowns, and processed samples, as described below:

> **Blank:** A matrix sample void of analytes that is used to assess the specificity of the analytical method.
>
> **Quality control sample (QC):** A spiked sample used to monitor the performance of an analytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.
>
> **Unknown:** A biological sample that is the subject of the analysis.

Selectivity: Also called ‘Specificity’, this is the ability of the analytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. Put another way, this is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lower limit of quantification (LLOQ): Also called ‘Sensitivity’, this is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Upper limit of quantification (ULOQ): The highest amount of an analyte in a sample that can be quantitatively determined with precision and accuracy.

4.0 Key Principles of Test Method Validation:

- The extent of validation should be commensurate with the risk associated with a measurement failure.

- The fundamental parameters to ensure the acceptability of the performance of an analytical method validation are (1) accuracy, (2) precision, (3) selectivity (4) sensitivity, and (5) range.

- A specific, detailed description of the analytical method should be written. This can be in the form of a protocol, study plan, report, and/or SOP.

- Each step in the method should be investigated to determine the extent to which environmental, matrix, material, or procedural variables can affect the estimation of analyte.

- It may be important to consider the variability of the matrix due to various factors.

- An analytical method should be validated for the intended use or application. All experiments used to make claims or draw conclusions about the validity of the method should be presented in a report (method validation report).

- For an analytical method to be considered valid, specific acceptance criteria should be set in advance and achieved for accuracy and precision for the validation of QC samples over the range of the standards.

- Achieve the Validation by Using Known Reference Standards: Analysis of samples in a matrix is carried out using calibration (reference) standards and quality control (QC) samples. An authenticated analytical reference standard of known identity and purity should be used. If possible, the reference standard should be identical to the analyte. Three types of reference standards are usually used: (1) certified reference standards (e.g., USP compendial standards); (2) commercially supplied reference standards obtained from a reputable commercial source; and/or (3) other materials of documented purity custom-synthesized by an analytical laboratory or other noncommercial establishment.

5.0 Validation Approach:

The fundamental parameters for an analytical method validation are accuracy, precision, selectivity, and sensitivity. Range may also be of value depending on the particular application.

5.1 Accuracy & Precision

The ***Accuracy*** of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of three to five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The mean value should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The deviation of the mean from the true value serves as the measure of accuracy. The ICH guideline states that accuracy should be reported as percent recovery…of known amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

The ***Precision*** of an analytical method describes the closeness of individual measurements in a series when the procedure is applied repeatedly to a sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between replicate measurements. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. The ICH guideline states that the standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

There are three levels of Precision that may be applicable depending on the situation:

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision

Intermediate precision expresses variations within the same laboratory: different days, different analysts, different equipment, etc.

Reproducibility
Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Let's restate accuracy and precision testing in a way that is a good bridge into the post-validation monitoring discussed later in this document: The accuracy and precision with which known concentrations of analyte in matrix can be determined should be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations ("QC samples") from an equivalent matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within 3x the lower limit of quantification (LLOQ) ("low QC sample"), one near the center ("middle QC"), and one near the upper boundary of the standard curve ("high QC").

5.2 Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components (potentially interfering substances) in the sample. For selectivity, analyses of blank samples of the appropriate matrix should be obtained (from at least six sources if possible). Each blank sample should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ). Matrix effects should be considered to ensure that precision, selectivity, and sensitivity will not be compromised.

5.3 Sensitivity (Lower limit of quantification - LLOQ)

Testing should be devised that will demonstrate the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

5.4 Range

Testing should be devised that will demonstrate the range of the analytical method if this is needed to show the test method is appropriate for its intended use.

Reported method validation data and the determination of accuracy and precision should include all outliers; however, calculations of accuracy and precision excluding values that are statistically determined as outliers can also be reported.

6.0 Validation Documentation:

The validity of an analytical method should be established and verified by laboratory studies, and documentation of successful completion of such studies should be provided in the assay validation report. General and specific SOPs and good record keeping are an essential part of a validated analytical method. The data generated for analytical method establishment and the QCs should be documented and available for data audit and inspection.

7.0 Monitoring the Method After Validation:

Once the analytical method has been validated for routine use, its accuracy and precision should be monitored regularly to ensure that the method continues to perform satisfactorily. To achieve this objective, a number of "QC samples" and, where appropriate, blanks prepared separately should be analyzed along with processed test samples at appropriate intervals (typically based on the total number of samples).

The QC samples in duplicate at three concentrations (one near the LLOQ (i.e., 3x LLOQ), one in midrange, and one close to the high end of the range) should be incorporated in each assay run. The number of QC samples (in multiples of three) will depend on the total number of samples in the run. The results of the QC samples provide the basis of accepting or rejecting the run. At least four of every six QC samples should be within 15% of their respective nominal value. Two of the six QC samples may be outside the 15% of their respective nominal value, but not both at the same concentration.

- ***The following recommendations should be noted when monitoring the analytical method during routine use:***

  - QC samples and where appropriate, blanks, should be used to accept or reject test runs.

  - Based on the analyte and technique, a specific SOP (or sample definition) should be identified that will be used in ongoing fashion to ensure the test method continues to operate as planned.

  - It is important to establish an SOP or guideline for repeat analysis and acceptance criteria. This SOP or guideline should explain the reasons for repeating sample analysis. Reasons for repeat analyses could include repeat analysis for regulatory purposes, inconsistent replicate analysis, samples outside of the assay range, sample processing errors, or equipment failure. Re-assays should be done in triplicate if sample volume allows. The rationale for the repeat analysis and the reporting of the repeat analysis should be clearly documented.

- ***The following acceptance criteria should be considered for accepting the analytical runs during routine processing:***

    - Samples, blanks, QCs, and study samples can be arranged as considered appropriate within the run.

    - Placement of standards and QC samples within a run should be designed to detect assay drift over the run.

    - Matrix-based standard calibration samples: 75%, or a minimum of six standards, when back-calculated (including ULOQ) should fall within ±15%, except for LLOQ, when it should be ±20% of the nominal value. Values falling outside these limits can be discarded, provided they do not change the established model.

    - Acceptance criteria for accuracy and precision as outlined in the Validation Approach section of this document should be provided

    - Quality Control Samples: Quality control samples replicated (at least once) at a minimum of three concentrations [one within 3x of the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end of the range (high QC)] should be incorporated into each run. The results of the QC samples provide the basis of accepting or rejecting the run. At least 67% (four out of six) of the QC samples should be within 15% of their respective nominal (theoretical) values; 33% of the QC samples (not all replicates at the same concentration) can be outside the ±15% of the nominal value. A confidence interval approach yielding comparable accuracy and precision is an appropriate alternative.

Note: The minimum number of QC samples (in multiples of three) should be at least 5% of the number of unknown samples or six total QCs, whichever is greater.

REFERENCES

1. FDA/CDER, *Guidance for Industry, Bioanalytical Method Validation* (May 2001)
2. ICH, *Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1)* (Current *Step 4* Version, Parent Guideline dated 27 October 1994)

About the Author

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