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ADVANTAGES OF ANALYTICAL VALIDATION METHOD

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ABSTRACT
The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user. Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.The Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

Method validation is required when

- A new method has been developed
- Revision of established method
- When established methods are used in different laboratories and different analysts etc.
- Comparison of methods required
- When quality control indicates method changes.

Type of Analytical Method of Validation

It is important for one to understand the parameters or characteristics involved in the validation process. [26]. The various performance parameters, which are addressed in a validation exercise, are grouped as follows.

A) Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value.

The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

B) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances. [27]

The Repeatability involves analysis of replicates by the analyst using the same equipment and method

and conducting the precision study over short period of time while reproducibility involves precision study at

- Different occasions
- Different laboratories
- Different batch of reagent
- Different analysts
- Different equipments.

1) Determination of Repeatability

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time.

It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value.

The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 2% for assays in finished product).

The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration)

2) Determination of Reproducibility

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

C) Linearity and Range

The linearity of an analytical method is its ability to draw out test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range.

The Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. [28].

The linear range of detect ability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression.

The Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y= ax + b together with the correlation coefficient of determination. For the method to be linear the r value should be close to1.

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and

linearity using the method as written.

D) Limit of Detection and Limit ofQuantitation

1) Limit of Detection

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions.

The limit test thus merely substantiates that the analyte concentration is above or below a certain level. The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa) which may be related to LOD and the slope of the calibration curve, b, by **LOD = 3.3 Sa / b**

2) Limit of Quantitation

Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable, Where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

E) Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective.

On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analytes.

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other

components, compared to the results obtained on the same analyte without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analyte with and without the addition of the potentially interfering materials.
When such components are either unidentified or unavailable, a measure of selectivity can often be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other components. [29].

F) Robustness and Ruggedness

1) Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

2) Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay.
The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.
For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

G) Stability and System Suitability Tests

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.
System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results.
The System suitability test are run every time a method is used either before or during analysis.
The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion.
The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments

Analytical Performance Characteristics	Assay Category I	Assay Category II		Assay Category III	Assay Category IV
		Quantitative Tests	Limit Tests		
Accuracy	X	X	May be	May be	
Precision	X	X		X	
Specificity	X	X	X	May be	X
Limit of Detection			X	May be	
Limit of Quantitation		X		May be	
Linearity	X	X		May be	
Range	X	X	May be	May be	

Table1.1 Showing Data elements required for assay validation Where, X indicates the tests to be performed

Category I: Analytical methods for quantitation of major components of bulk drug substances or active ingredients including preservatives in finished pharmaceutical products.

Category II: Analytical methods for determination of impurities in bulk drugs or for determination of degradation compounds in finished pharmaceutical products.

Category III: Analytical methods for determination of performance characteristics (e.g. dissolution, drug release).

Category IV: Identification tests.

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