VALIDATION CHARACTERISTICS AND STATISTICS IN ANALYTICAL METHOD DEVELOPMENT

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Abstract

Validation is asignificantprocessin the pharmaceutical industry and is utilized to ensure that quality is built into the processes supporting drug development and manufacture. Validation not only improves the process but also confirms that the process is properly developed. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The scope of developing and validating the method is to ensure a suitable strategy for a particular analyte that is more specific, accurate, and precise. Analytical method validation is done to make certain that dependable results are always obtained. Analytical methods require characterizing drug substances and drug products composition during all phases of pharmaceutical development. The present article includes information regarding the type of procedures to be validated, why it is required to be validated, how to perform validation and validation characteristics, and statistics ofanalytical method development. Generally, validation of an analytical method is intended to demonstrate that it is suitable for its intended use.

Keywords: Accuracy, Analytical method validation,Standard deviation,Theoretical plates, Resolution, Validation characteristics.

I.INTRODUCTION

The word validation is originated from the Latin word ‘validus’ meaning strong and suggests that something has been proved to be true, useful, and of an acceptable standard. Method validation can be defined as the process of proving that a particular developed analytical method is acceptable for its intended use. Analytical method validation is just one type of validation required during drug development and manufacturing to comply with the requirement of current Good Manufacturing Practices GMP [1-2].Method validation can be interpreted as the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with that the application required. The analytical methods developed may be different fromeach other in their way of scope, application, etc but the concept and postulates of validation remain the same despite the analytical method [3-8] or its applications.Development of methods [9-12] to achieve the final goal of ensuring the quality of drug products must be implemented in conjunction with an understanding of the physical and chemical behavior of drug substances.

Validation guidelines

- ICH Q2A Text on Validation of Analytical Procedures: definitions and terminology (March1995).
- ICH Q2B Validation of Analytical Procedures: Methodology (June 1997)
- FDA (Draft) Guidance for Industry: Analytical Procedures and Method Validation.
- Pharmacopoeias USP and European Pharmacopoeia.

Why is Analytical Method Validation Required?

Method validation is required for the following reasons:For assuring the quality of the product

- For achieving the acceptance of the product by the international agencies.
- It is a mandatory requirement for accreditation as per ISO 17025 guidelines
- A mandatory requirement for registration of any pharmaceutical product or pesticide formulation.

II. TYPES OF ANALYTICAL PROCEDURES TO BE VALIDATED

The discussion of the validation of analytical procedures is directed to the three most common types of analytical procedures [13-36]. A brief outlinethat explains the validation characteristics that should be applied for different analytical procedures is summarized in the table below.

Table 1: Validation characteristics recommended for different analytical tests

Validation Characteristics	Identification Tests	Quantitative Testsfor impurities	Limit Test	Assay Dissolution(measurement only), Content/potency	Specific Tests
Accuracy	-	✓	-	✓	✓ (4)
Precision					
Repeatability	-	✓	-	✓	✓ (4)
Intermediate precision	-	✓ (1)	-	✓ (1)	✓ (4)
Range	-	✓	-	✓	-
Specificity	✓ (2)	✓	✓	✓ (5)	✓ (4)
Linearity	-	✓	-	✓	-
Detection Limit	-	-(3)	✓	-	-
Quantization Limit	-	✓	-	-	-
Robustness	-	✓	-(3)	✓	✓ (4)

2.1 Accuracy

The closeness of agreement between the value, which are accepted either as a conventional true value or an accepted reference value, and the value found. Characterizing or identifying the accuracy will help us to find out how much effect the systematic errors (which are often due to inaccurate measurements, etc) impact the specific analytical process. Accuracy is represented and determined by recovery studies.

2.2 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions. A more comprehensive definition proposed by the ICH divides precision into three types:

2.2.1. Repeatability

It expresses the precision under the same operating conditions over a short period. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. The ICH requires repeatability to be tested from at least six replicates measured at 100 % test target concentration or from nine replications covering the complete specified range.

2.2.2. Intermediate precision

It is the agreement of complete measurements when the same method is applied many times within the same laboratory. This can include full analysis on different days, instruments or analysts, but would involve multiple preparations of samples and standards. The main objective of intermediate precision is to verify that when an analytical procedure is performed in the same laboratory same results are produced once the development is completed.

2.2.3.Reproducibility

It examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments. Precision often is expressed by the standard deviation or relative standard deviation of the data set. The main objective of reproducibility is to verify same results are obtained from the same analytical procedure when performed at different laboratories.

2.3 Range

The range of a method can be defined as the upper and lower concentrations for which the analytical method has adequate accuracy, precision, and linearity. The ICH specifies the minimum specific range to be 80 – 120 % of the test concentration for assay procedures and to demonstrate an acceptable linear range it is suggested to prepare five different standard preparations from 50-150 % of the target sample or analyte concentration.

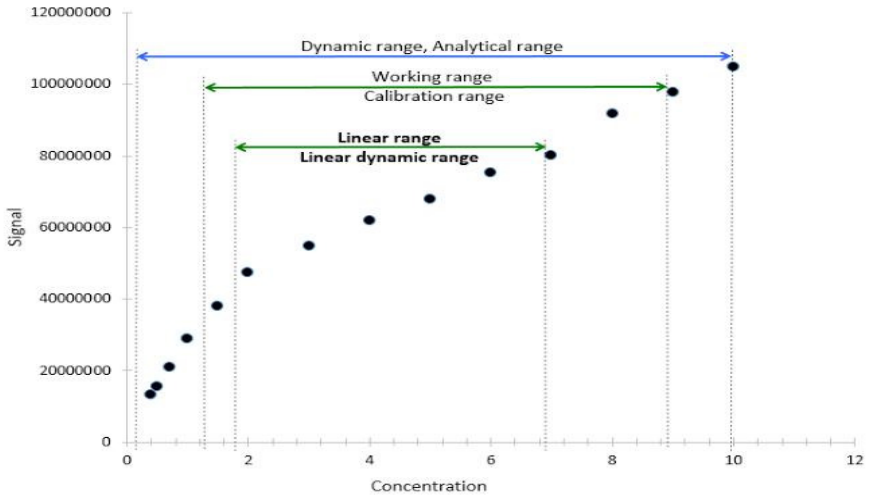

Fig. 1.Plot determining the range

2.4 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Assuring specificity is the first step in developing and validating a good method. If specificity is not assured, method accuracy, precision, and linearity all are seriously compromised. Method specificity should be reassessed continually during validation and subsequent use of the method.

2.5 Linearity [2]

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations or by separately weighing synthetic mixtures of the test product components. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods.

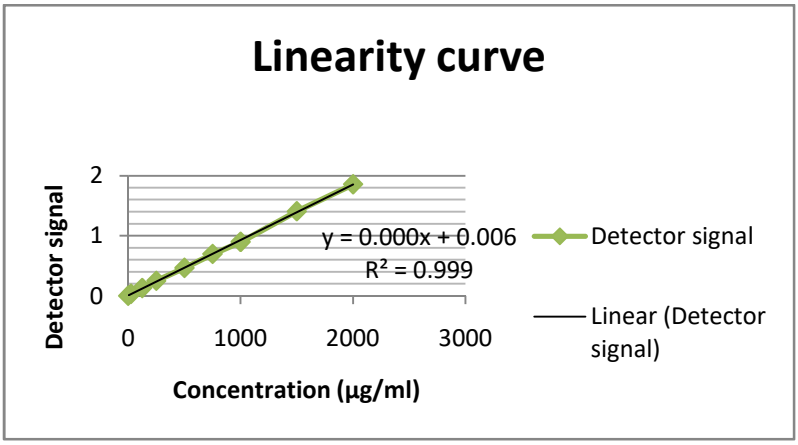

Fig. 2. Graph representing linearity

2.6 Detection Limit(LOD)

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualitated as an exact value. In chromatographic procedures, the detection limit is the injected volume of the sample that produces a peak with a height of at least 2 or 3 times as high as the baseline noise level. Apart from the signal to noise ratio method, there are three other methods described by ICH.

The detection limit (LOD) may be expressed as

$$LOD = 3.3\frac{s}{S} \qquad (1)$$

s = the standard deviation of the response.
S = the slope of the calibration curve (of the analyte).

2.7 Quantitation Limit(LOQ) [3]

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.. Quantitation Limit may be expressed as

$$LOQ = 10\frac{s}{S} \qquad (2)$$

s = the standard deviation of the response.
S = the slope of the calibration curve of the analyte.

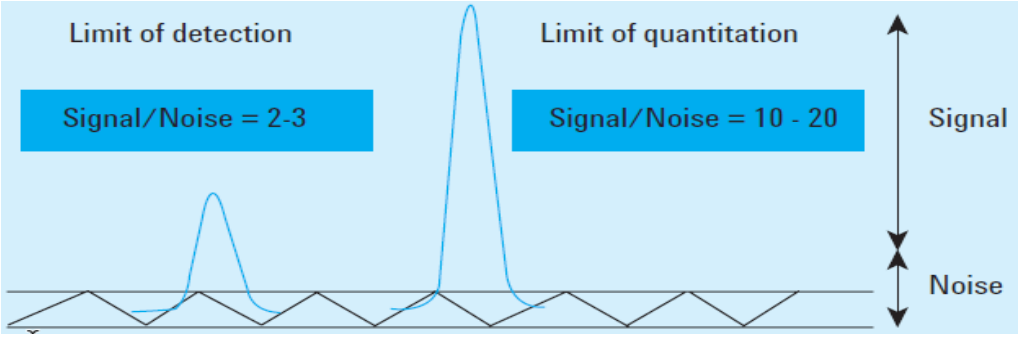

Fig.3. Signal to noise ratio method for the determination of LOD and LOQ

2.8 Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents, and so on.

2.9 Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". Acceptance criteria of various validation parameters for HPLC are shown in the table below:

Table 2: Acceptance criteria of validation parameters for HPLC

S. No	Parameter	Acceptance criteria
1	Accuracy	% Recovery 98 – 102 % %RSD of recovery concentrations must be < 2
2	Precision	RSD < 2%
3	Range	Concentration where data can be reliably detected(80 – 120%)
4	Specificity	No interference
5	Linearity	Correlation coefficient – NLT 0.999
6	Detection Limit	S/N > 2 or 3
7	Quantitation Limit	S/N > 10
8	Ruggedness	Should meet all system suitability parameters
9	Robustness	RSD < 2%

2.11 System suitability parameters:

2.11.1Retention factor (K)

The retention of a drug with given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length, and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_0}{V_0} \qquad (3)$$

V_A = Elution volume of A, V_0 = Elution volume of a non-retained compound (void volume)

2.11.2 Resolution (R_f) [4]

The resolution, R_f of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their

average peak width. For baseline separation, the ideal value of R_f is 2.0. It is calculated by using the formula,

$$R = \frac{Rt_2 - Rt_1}{0.5(W_1 + W_2)} \quad (4)$$

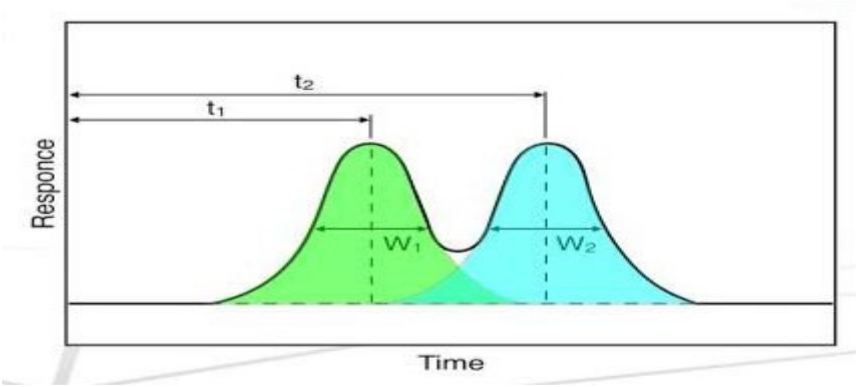

Fig. 4. Determination of resolution

Rt_1 and Rt_2 are the retention times of components 1 and 2; W_1 and W_2 are peak widths of components 1 and 2

2.11.3 Selectivity

The selectivity (or separation factor) a, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0} \quad (5)$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peaks respectively.

2.11.4Column efficiency [5]

The efficiency N of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. The smaller the band spread, the higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 1, 00,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{W^2} \quad (6)$$

R_t or t_r is the retention time and W is the peak width.

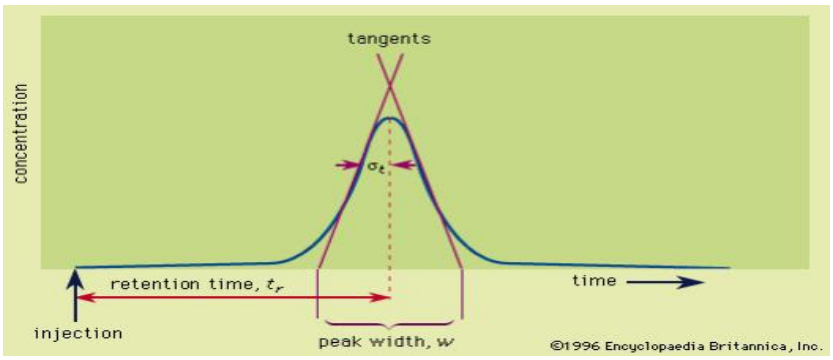

Fig.5. Determination of column efficiency

Table 3: Different equations for calculating the number of theoretical plates

S. No	Equation	Use
1	$N = 16\frac{Rt^2}{W^2}$	Commonly used equation with time units
2	$N = 16\frac{{V_r}^2}{{W_v}^2}$	Used when retention volume is required(W_v= peak width in units of volume)
3	$N = 16\frac{{d_r}^2}{{W_d}^2}$	Used with planar or open column chromatography(W_d= peak width in units of length)
4	$N = 5.54\frac{Rt^2}{{W_{1/2}}^2}$	Used for asymmetric, skewed, or partially resolved peaks ($W_{1/2}$= W measured at one-half peak height)
5	$N = 4\frac{Rt^2}{{W_{2\sigma}}^2}$	Alternative to the above third equation ($W_{2\sigma}$= peak width at 2σin time units)

2.11.5 Peak asymmetry factor (A_s) [6]

Peak asymmetry factor A_s can be used as a criterion of column performance. The peak half-width 'b' of a peak at 10 % of the peak height divided by the corresponding front half-width 'a' gives the asymmetry factor.Acceptance criteria for system suitability parameters are shown in table 5.

$$A_s = \frac{b}{a} \qquad (7)$$

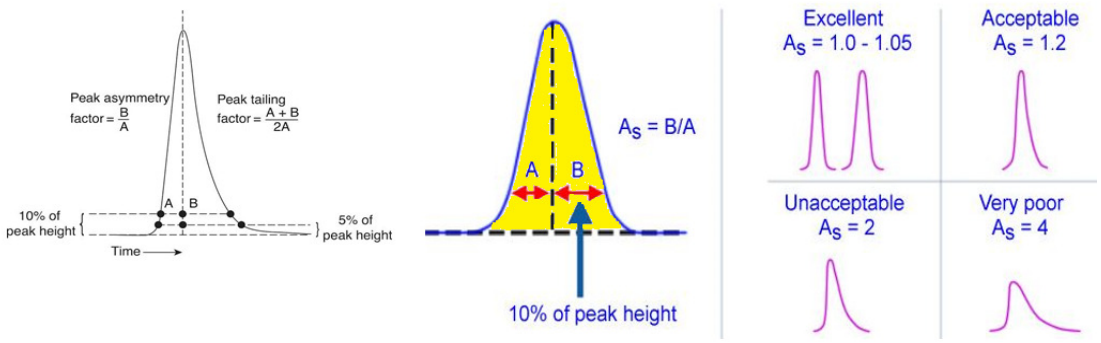

Fig. 6. Determination of peak asymmetry factor and peak tailing factor

Table 5: Acceptance criteria for system suitability parameters

S. No	Parameter	Acceptance criteria
1	Capacity factor (K)	< 1
2	Resolution (Rs)	>1.5
3	Separation factor (α)	>1
4	Column efficiency (N)	>2000
5	Peak asymmetric or tailing factor (T)	< 2

2.12 Statistical parameters

2.12.1 Regression equation

The linear relationship is characterized by the tendency of the points of the scattered diagram to cluster along a straight line known as the regression line.

$$Y=a + bX \quad (8)$$

It is used to describe the dependence of one characteristic (Y) up on the other characteristic (X), both X,Y represent values of two characters a, b are two constants it will be evident that two regression lines can be computed for every set of data-one each to describe the dependence of one character to another. 'b' is a regressive coefficient which shows change expected in Y for a unit change in X, it is the dependence of Y & X;. The regressive coefficient of b is estimated.

$$b = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2} \quad (9)$$

b = the slope of the regression line and is calculated by this formula

x= an arbitrarily chosen value of the predictor variable for which the corresponding value of the criterion variable is desired**.**

2.12.2 Correlation Coefficient

A measure of the strength of the relationship between two variables is provided by the coefficient of correlation denoted by r, if the relationship between the two variables is of the linear form. It is also called the coefficient of linear correlation.

2.12.3 Pearson's correlation

The correlation coefficient calculation for data values should be +1 or -1,

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2}\sqrt{\sum(y-\bar{y})^2}} \quad (10)$$

r= Karl Pearson coefficient of correlation, x and y are the variables

For, r=-1 indicates perfect negative correlation

r=+1 indicates perfect positive correlation

r =0 indicates no correlation and a value of r=0.95 indicates excellent correlation

2.12.4 Standard Deviation

The standard deviation of a set of values is the measure of the spread of the values in the given dataset. It is the square root of the average of the squared deviations of the observations. From the arithmetic mean, it is used for measures of dispersion. It is calculated by

$$SD = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}} \quad (11)$$

$$\%RSD = \frac{SD}{\bar{x}} * 100 (12)$$

$\sum$ = sum of observations, $\bar{x}$ = mean value, x= Individual observed value, n = number of observations.

2.12.5 Standard error of the mean (S.E)

The population of standard deviation is not given, but the size of s is large. So the sample standard deviation is representing the population of standard deviation.

$$S.E = \frac{SD}{\sqrt{n}} (13)$$

SD = Standard Deviation,n = number of observations

The equation implies that sampling error decreases as sample size increases, which is important as it informs that if sampling error is to be reduced to a great extent we need to use a large sample size possible.

III. CONCLUSION

It is internationally recognized that a developed method should necessarily be validated as these validation methods also show the qualification and competency of the analytical laboratory. Newer analytical procedures are being developed and introduced at an exponential rate these days and the importance of quality attachment to proportional to it. For this purpose, validation has made its impact and became a crucial step to impart quality at each step in every analytical method. This article provides information regarding method validation in detail, including various approaches in which it is performed, various validation characteristics along with statistical parameters are defined along with their procedure for performing, methods for calculating, and their acceptance criteria. Thus validation provides the quality for the system which cannot be attained by normal testing alone.

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