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Modern Analytical Techniques in Pharmaceuticals

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ABSTRACT

Modern analytical techniques use a variety of techniques that change from easy qualitative chemical check to the employment of most subtle and costly laptop controlled instruments. This instrumentation provides the lower limit of detection needed to assure the security. In pharmaceutical business the chemical composition of raw material, intermediates and finished product quality got to be monitored to confirm satisfactory consistency. fashionable analytical techniques use a variety of techniques that change from easy qualitative chemical check to the employment of most subtle and costly laptop controlled instruments. The process of analytical method validation ought to demonstrate that the strategy is suitable its purpose. The validation ought to follow a thought that has the scope of the tactic, the tactic performance characteristics and acceptance limits.

Keywords: Robustness, Gravimetric, Ruggedness, Thermogravimetric.

Introduction:

The search for the reliable range of a method and continuous application of this knowledge is called validation. It can also be defined as the process of documenting that the method under consideration is suitable for its intended purpose. Method validation involves all the procedures required to demonstrate that a particular method for quantitative determination of the concentration of an analyte (or a series of analytes) in a particular samples is reliable for the intended application. Validation is also a proof of the repeatability, specificity and suitability of the method**.**

Regulatory Requirement

The International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) was initiated in 1990, as a forum for a constructive dialogue between regulatory authorities and industry, in order to harmonize the submission requirements for new pharmaceuticals between Europe, the United States of America and Japan. One of the first topics within the Quality section was analytical validation and the ICH was very helpful in harmonizing terms and definitions [1] as well as determining the basic requirements [2] the required validation parameters for the various types of analytical procedures are shown.

Table No.1: validation parameter

VALIDATION PARAMETERS	TYPES OF TESTS	ASSAY
Accuracy	**OK**	**Yes**
Precision(repeatability)	**OK**	**Yes**
Intermediate precision	**OK**	**Yes**
Reproducibility	**OK**	**Yes**
	OK	

Linearity	**OK**	**Yes**
Range	**OK**	**Yes**
Specificity	**OK**	**Yes**
LOD	**OK**	**No**
LOQ	**OK**	**No**
Stability solution	**OK**	**Yes**
Robustness	**OK**	**Yes**

A validation terminology that is also in agreement with the recommendations of important international organizations such as the ISO (International Organization for standardization), IUPAC (International Union of Pure and Applied Chemistry) and AOAC (Association of Official Analytical Chemists), since differences exist between their documents. For the validation of pharmaceutical drug formulations the discussion on a consensus terminology is relatively advanced. It is suggested to follow in general the proposal elaborated for the validation of drug formulation by the joint initiative of the pharmaceutical industry and the regulatory agencies of the three major regulatory authorities (the European Union, the USA and Japan), the International Conference on Harmonization (ICH). Validation of analytical methodologies is widely accepted as pivotal before they are put into routine use

- A method must be tested for effectiveness and must be appropriate for the particular analysis to be undertaken
- Method validation is defined as the process of proving, through scientific studies, that an analytical method is acceptable for its intended use and it self confidence that the method can generate test data of acceptable quality**.**

Recent guidelines for methods development and validation for new non-compendial test methods are provided by the FDA draft document, Analytical Procedures and Method

Validation

In recent years, a great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. The recent FDA methods validation draft guidance documents as well as the United States Pharmacopoeia (USP) both refer to the ICH guidelines**.**

The validation process may vary slightly between laboratories**.** However, a number of general tests are usually performed; recovery, accuracy, precision, reproducibility, linearity, specificity, limit of detection and quantitation and ruggedness. Therefore method development is complete only when the method has been stringently tested and shown to demonstrate acceptable analytical performance. It is important to realise that method validation is not a stand-alone process and is rather a part of an overall validation process, which includes the validation of the hardware and software being used (installation, operation and performance qualifications should be performed for all equipment being utilized) and the verification of system suitability and performance**.**

Analytical parameters used in assay validation as per ICH guidelines

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included.

Types of analytical procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

Identification Tests

Quantitative tests for impurities' content.

Limit tests for the control of impurities.

Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

A brief description of the types of tests considered in this document is provided below. Identification tests are intended to ensure the identity of an analyte in a sample this is normally achieved by comparison of a property of the sample (example spectrum, chromatographic behaviour, chemical reactivity etc.) to that of a reference standard.

Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.

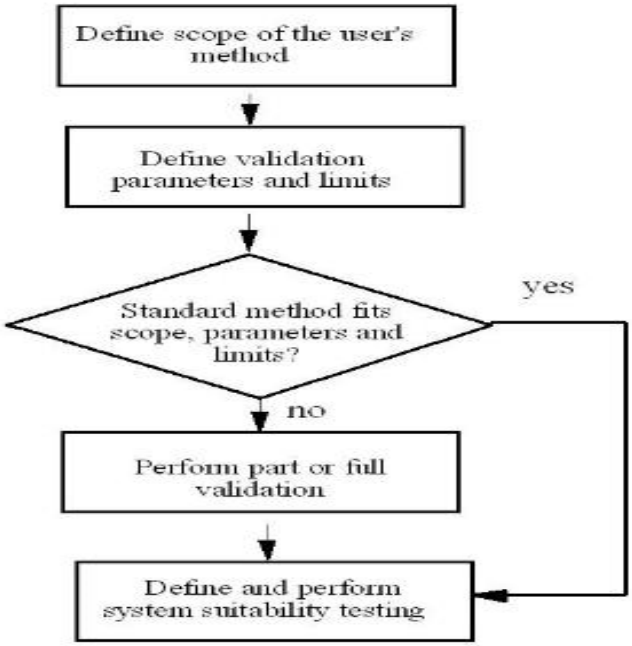

Fig no.21. Flow chart of Method Validation

Evaluation and Validation of Standard Methods

Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- **Accuracy**
- **Precision**
- **Range**
- **Specificity**
- **Linearity**
- **Detection Limit**
- **Quantization Limit**
- **Ruggedness**
- **Robustness**

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. Accuracy is represented and determined by recovery studies.

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

- **Repeatability**
- **Intermediate Precision**
- **Reproducibility**

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

Intermediate precision 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.

2.3.3.2.2.3. Reproducibility 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.

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 range of concentrations examined will depend on the type of method and its use.

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.

Linearity

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. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods.

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Detection Limit

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualtitated as an exact value.

The detection limit (LOD) may be expressed as:

$$LOD = \frac{3.3\sigma}{S}$$

Where σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Quantification Limit

The Quantification limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantification Limit (LOQ) may be expressed as:

$$LOQ = \frac{10\sigma}{S}$$

Where σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

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. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

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". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters.(ICH guidelines,1996)

System Suitability Testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Retention (K_A)
- Resolution (R_s)
- Capacity factor (k')
- Selectivity (α)

- Column efficiency (N) and
- Peak asymmetry factor (A_s)

Retention (K_A)

The retention of a drug with a 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

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0} \text{ ----------->equation 1}$$

Where,

V_A = Elution volume of A

V_0 = Elution volume of a non retained compound (void volume)

At constant flow rate, retention times (t_A and t_o) can be used instead of retention volumes.

Retention data is sometimes expressed, relative to a known internal standard (B). The ratio of retention times (t_A/t_B) can be used, but the ratio of adjusted retention times $\left(\frac{t_A - t_o}{t_B - t_o}\right)$ is better when data need to be transferred between different chromatographs.

Resolution (R_s)

The resolution, R_s 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_s is 1.5. It is calculated by using the formula,

$$Rs = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$ ----------->equation 2

Where,

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.

Capacity Factor (k')

Capacity factor, k'is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column or TLC plate during an isocratic separation. The ideal value of k' ranges from 2-10. Capacity factor can be determined by using the formula,

$$k' = \frac{V_1 - V_0}{V_0}$$ ---------->equation 3

Where, V_1 = retention volume at the apex of the peak (solute) and

V_0 = void volume of the system.

The values of k' of individual bands increase or decrease with changes in solvent strength. In reversed phase HPLC, solvent strength increases with the increase in the volume of organic phase in the water / organic mobile phase. Typically an increase in percentage of the organic phase by 10 % by volume will decrease k' of the bands by a factor of 2-3.

Selectivity (α)

The selectivity (or separation factor), α, 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, $\alpha = \frac{V_2 - V_0}{V_1 - V_0}$ ---------->equation 4

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 peak respectively.

2.3.3.2.10.5. Column Efficiency

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. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{w^2} \qquad \text{----------->equation 5}$$

Where, Rt is the retention time and w is the peak width.

Peak Asymmetry Factor (A_s)

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.

$$A_s = \frac{b}{a}$$

Statistical Parameters

Regression Equation:

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

$$Y = mx + c \qquad \text{----------->equation 6}$$

It is used to describe the dependence of one characteristic (Y) up on the other characterstic (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 known as regressive coefficients which shows change expected in Y for unit change in X, it is dependence of Y & X; b is the regressive coefficient of Y& X.

The regressive coefficient of b is estimated,

$$b = \frac{\Sigma\left(x - \bar{x}\right)\left(y - \bar{y}\right)}{\Sigma\left(x - \bar{x}\right)^2}$$ ----------->equation 7

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.

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.

Pearson's Correlation:

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

Where the value of Correlation coefficient is +1 – positive

Correlation coefficient is -1 – negative

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{N}}{\sqrt{(\sum X^2 - \frac{(\sum X)^2}{N})(\sum Y^2 - \frac{(\sum Y)^2}{N})}}$$ ---------->equation 8

Where,

X – Value of one character

Y - Value of another character

Standard Deviation:

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 denoted by

$$\text{Standard Déviation} = \frac{\Sigma(x - X)^2}{n - 1}$$ ---------->equation 9

$$\text{R.S.D (\%)} = \frac{S.D}{\bar{x}} \times 100$$ ---------->equation 10

Where Σ = Sum of observations

$\bar{x}$ = Mean or arithmetic average ($\Sigma x / n$)

x = Individual observed value

$x - \bar{x}$ = Deviation of a value from the mean

n = Number of observations

Standard Error of 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.

$$S.E. = \frac{S.D}{\sqrt{N}} \quad \text{----------->equation 11}$$

Where,

S.D = Standard deviation

n = no. of observations

(Michael W.Dong, 2006)

Conclusion

Analytical methods are necessary for development and validation department of pharmaceuticals. it requires rapid development of validation. It is important for quantification of various development process. these are critical part for safe drug. It is necessary for method development of pharmaceutical dosage form. Method validation is a continuous development process. It is necessary for safer pharmaceutical dosage

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