Development and Validation of Analytical Instrumental Method for Pharmaceutical Products

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ABSTRACT

Development, and production of pharmaceutical products. The authorized test methods that result from HPLC, UV processes are used by quality control laboratories to make sure the Identity, characteristics, purity, potency, and performance of drug products. Analytical methods development ought to be validated to give reliable data for regulatory submissions. This review gives information regarding various stages involved in development and validation of analytical methods like UV, HPLC. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. As a result, analytical method development has developed into the basic activity of analysis. Recent development in analytical methods has been result from the advancement of analytical instruments.

Keywords : Keywords: High performance liquid chromatography (HPLC); (LOQ) UV-visible spectrophotometer. limit of detection (LOD); Limit of quantitation

INTRODUCTION

Analytical chemistry is concern with the determination of the chemical composition of matter, until recently, this was the main goal of analytical chemist, However, identification of substance, the illumination of modern analytical chemistry[1,2,3,4].

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It seeks ever improved means of measuring the chemical composition of natural and artificial materials

In recent years research in analytical chemistry has concerned mainly on the development and application of physical and physicochemical analytical methods, instrumental analysis, in which their speed and sensitivity have far surpassed the classical methods of gravimetric and volumetric analysis.

Almost all researchers have been supported by analytical techniques. Many Nobel prizes of chemistry and physics were given to the investigations of new method of analytical measurement. The goal of analytical measurement has always been to achieve high sensitivity and selectivity. Analytical chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

Currently instrumental analysis is based on comparison of the signal from the sample with that from standard. Analytical chemistry encompasses chemical analysis, separation chemistry and instrumental analysis, including analytical methods and techniques.

Factors Affecting the Choice of Analytical Method:[5]

Analytical techniques have different degrees of sensitivity and selectivity, sophistication, as well as, different cost and time requirements. An important task for the analyst is to select best procedure for a given determination this will require careful consideration of the following criteria:

a) The type of analysis required: elemental or molecular, routine or occasional.

b) Problem arise from the nature of the material to be investigated, e.g. radio-active substance, corrosive substance, substances affected by water.

c) Possible obstruction from components of the material other than those of interest.

d) The concentration range to be investigated.

e) The accuracy required.

f) The facilities available, particularly the instrument.

g) The time required to complete the analysis.

h) The number of analysis of similar type which have to be performed.

Selection of Analytical Method [6]

First stage in the selection or development of method is to create what is to be precise and how accurately it should measure. Unless one has series of methods at hand to assess quality of the product, validation programme may have limited validity. The selected method must have the following parameters:

1. As simple as possible,

2. Most definite

3. Most productive, economical and convenient,

4. As accurate and precise as required,

5. Multiple source of key component (reagents, columns, TLC plates) should be avoided,

6. To be fully optimized before transfer for validation of its characteristics such as accuracy, precision, sensitivity, ruggedness etc.

Classification of analytical methods: [7]

Analytical chemistry consists of qualitative analysis and quantitative analysis.

• **Qualitative analysis** deals with the identification of elements, ions or compounds present in the sample.

• **Quantitative analysis** deals with determination of how much of one or more constituent is present.

Qualitative analysis is subdivided in to following classical method.

A) Classical methods:

In the early years of chemistry, most analyses were carried out through classical methods by separating components of interest in a sample by precipitation, extraction, or distillation. For quantitative analysis, the separated components were then treated with reagents that yielded products that could be recognized by their colors, boiling points or melting points, their solubility in a series of solvents, their odors, their optical activities, or their measurable physical properties.

a) Volumetric methods:

It is also known as titrimetric method and is preferred over gravimetric due to speed and convenience. In volumetric methods, assay is based on measurement of volume of solution of

known strength that is required to react completely with the substance to be analyzed. Depending upon type of reaction involved this method is further classified as

a) Neutralization titration

 b) Non-aqueous titration

c) Precipitation titration

d) Complexo metric titration

e) Redox titration

b) Gravimetric methods:

Gravimetric analysis is quantitative analysis by weight and is process of isolating and weighing the compound of known composition.

c) Gasometric method:

This method involves measurement of volume of gas by gas burettes or nitrometers. They measure the volume of the gas liberated in the given chemical reaction and volume decreased of gas in present of adsorbing agent under standard condition of temperature and pressure.

Quantitative analysis can be classified from different points of view based on the nature of material under examination, type of method employed, and the amount of desired constituent in sample material.

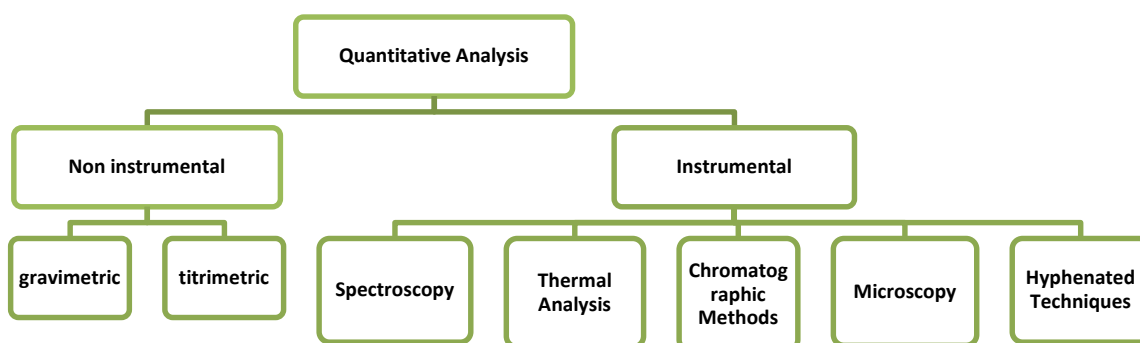Quantitative Analysis is further classified into:

Figure 1: Flow chart of classification of quantitative analysis.

➢ **B) Instrumental methods of chemical analysis**[8,9,10]

Instrumental method is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences . Analytical Instruments plays an important role in the production and evaluation of new products. This

instrumentation provides lower detection limits required to assure safe foods, drugs, water and air. Instrumental methods are widely used by Analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy Most instrumental techniques fit into one of the four principal areas, i.e., spectroscopy, electrochemistry, chromatography and miscellaneous techniques.

.1. Spectroscopy

- Ultraviolet & Visible spectrophotometry
- Fluorescence & Phosphorescence spectrophotometry
- Atomic spectrometry (emission and absorption)
- Infrared spectrophotometry
- Raman spectroscopy
- X-ray spectroscopy
- Radiochemical technique including activation analysis
- Nuclear magnetic resonance spectroscopy
- Mass spectrometry
- Electron Spin Resonance spectroscopy

2. Electrochemical techniques

- Potentiometry
- Voltametry
- Amperometric technique
- Coulometry
- Electrogravimetry
- Conductometry technique

3. Chromatographic techniques

- Gas chromatography
- High performance liquid chromatography (HPLC)
- High performance thin-layer chromatography (HPTLC)
- Electrophoresis
- Supercritical fluid chromatography (SFC)
- Ultra pressure liquid chromatography (UPLC)

4. Miscellaneous techniques

- Thermal analysis
- Kinetic technique

5. Microscopy

- Optical microscopy
- Electron microscopy
- Scanning probe microscopy

6. Hyphenated or combined techniques

- GC-MS (Gas Chromatography- Mass Spectrometry)
- LC-MS (Liquid Chromatography- Mass Spectrometry)
- ICP-MS (Inductively coupled Plasma Mass Spectrometry)
- GC-IR (Gas Chromatography-Infrared Spectroscopy.

UV-VISIBLE SPECTROPHOTOMETER [11-12]

It involves the measurements of the amount of ultraviolet (190-380) or visible radiation absorbed by a substance in solution. instruments which measure the ratio, of the intensity of the two beams of lights in the Ultraviolet-Visible region are called Ultra Violet –Visible Spectrophotometers. Absorption of light in both the ultra violet and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions.

The quantitative analysis by UV-Visible spectrophotometry is governed by the Beer-Lambert's law; it it states that, ''The intensity of beam of monochromatic light when Passed through transparent medium decreases exponentially as the thickness and concentration of absorbing media increases arithmetically''

$A = \log I_o / I_T = abc$

Where,

A=Absorbance of the solution at particular wavelength of the light beam,

I_o=Intensity of incident light beam.

I_T= Intensity of transmitted light beam

a=Absorptivity of molecule at the wavelength of beam.

b=path length of cell in cm.

c=concentration of solution in g/lit.

Application of UV-Spectrophotometry

1. Detection of impurities: UV absorption spectroscopy is one of the best methods for detecting impurities in organic compounds.

2. Quantitative analysis: UV absorption spectroscopy is generally used for quantitative determination of compound that absorb UV light. this determination is based on beer's law.

3. Dissociation constant of Acid and Bases.

Limitation:

1.Only moderately selective. The selectivity of the method depends on the chromophore of the individual drugs, e.g. a colored drug with an extended chromophore is more distinctive than a drug with a simple benzene ring chromophore.

2.Not radially applicable to the analysis of mixtures.

High Performance Liquid Chromatography [13]

Introduction to chromatography

Chromatography is an analytical technique base on the separation of molecules due to differences in their structure and/or composition. In general, Chromatography involves moving a sample through the system over a stationary phase. The molecules in the sample will have different affinities and interactions with the stationary support, leading to separation of molecules. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. Different compounds can be separated from each other as they move through the column.

Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography), volatile gases (gas chromatography), paper (paper chromatography) and liquids (liquid chromatography). High-performance liquid chromatography.

(HPLC) is a type of liquid chromatography used to separate and quantify compounds that have been dissolved in solution. HPLC is used to determine the amount of a specific compound in a solution.

For example, HPLC can be used to determine the amount of morphine in a compounded solution. In HPLC and liquid chromatography, where the sample solution is in contact with a second solid or liquid phase, the different solutes in the sample solution will interact with the stationary phase as described. The differences in interaction with the column can help separate different sample components from each other
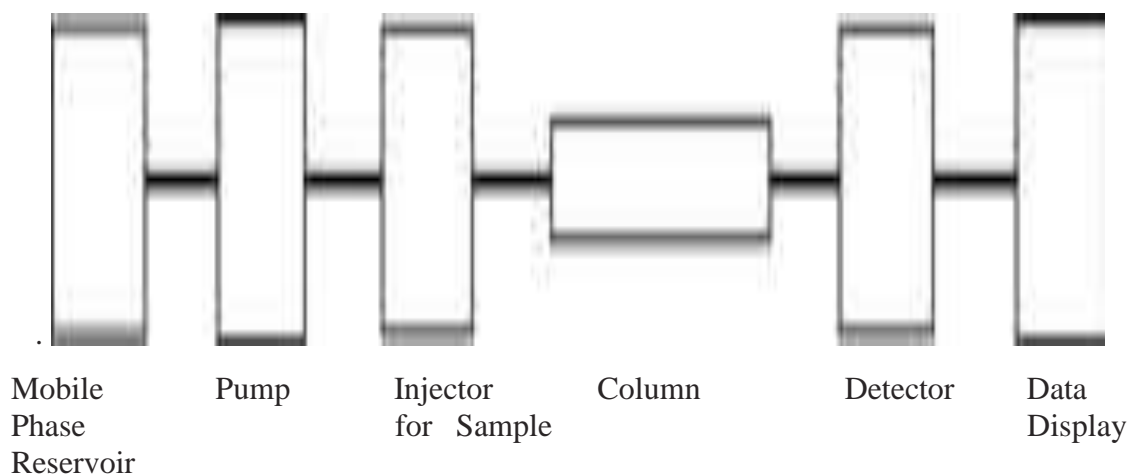
| Mobile Phase Reservoir | Pump | Injector for Sample | Column | Detector | Data Display |

Figure 2: Schematic of a High-Performance Liquid Chromatograph

Chromatographic Mechanisms

Systems used in chromatography are often categorized into one of four types based on the mechanism of action, adsorption, partition, ion-exchange and size exclusion. Adsorption chromatography arises from interactions between solutes and the surface of the solid stationary phase. Partition chromatography involves a liquid stationary phase that is immiscible with the eluent and is coated on an inert support.

Ion exchange chromatography has a stationary phase with an ionically charged surface that is different from the charge of the sample. The technique is based on the ionization of the sample. The stronger the charge of the sample, the stronger the attraction to the stationary phase; therefore, it will take longer to elute off the column.

Size exclusion is as simple as screening samples by molecular size. The stationary phase consists of material with precisely controlled pore size. Smaller particles get caught up in the column material and will elute later than larger particles. Several other types of chromatographic separation have been described, including ion-pair chromatography, which is used as an alternative to ion-exchange chromatography and chiral chromatography (to separate enantiomers)

Types of HPLC methods [14]

1. Reverse Phase HPLC

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution. Uses water-organic as mobile phase, columns may be C18 (ODS), C8,

phenyl, Trimethyl Silane (TMS), cyano as a stationary phase. It is first choice for most samples especially neutral or non ionized compounds, that dissolve in water organic mixtures.

2. Normal Phase HPLC

In this the mixtures of organic solvents for mobile phase and columns i.e. cyano, diol and amino silica can be used as stationary phase. It is first choice for mixtures of isomers and for preparative scale HPLC and second choice for lipophilic samples that cannot dissolve well in water-organic mixture.

Instrumentation [15]

HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs. Since the stationary phase may be composed of micron-sized porous particles, a high-pressure pump is required to move the mobile phase through the column. The chromatographic process begins by injecting the solute into the injector at the end of the column. Separation of components occurs as the analytes and mobile phase are pumped through the column. Eventually, each component elutes from the column as a peak on the data display. Detection of the eluting components is important, and the method used for detection is dependent upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram. To collect, store and analyze the chromatographic data, integrators and other data-processing equipment are frequently used.

Mobile Phase and Reservoir

The type and composition of the mobile phase affects the separation of the components. Different solvents are used for different types of HPLC. For normal-phase HPLC, the solvent is usually nonpolar, and, in reverse-phase HPLC, the solvent is normally a mixture of water and a polar organic solvent. The purity of solvents and inorganic salts used to make the mobile phase is paramount. A general rule of thumb is to use the highest purity of solvent that is available and practical depending on the particular application. The most common solvent reservoirs are as simple as glass bottles with tubing connecting them to the pump inlet.

Pumps

High-pressure pumps are needed to push the mobile phase through the packed stationary phase. A steady pump pressure (usually about 1000–2000 psi) is needed to ensure reproducibility and accuracy. Pumps are typically known to be robust, but adequate maintenance must be performed to maintain that characteristic. Inability to build pressure, high pressures or leakage could indicate that the pump is not functioning correctly.

Injectors

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi). For liquid chromatography, liquid samples can be directly injected and solid samples need only to be diluted in the appropriate solvent.

Detectors

There are many different types of detectors that can be used for HPLC. The detector is used to sense the presence of a compound passing through and to provide an electronic signal to a data-acquisition device. The main types of detectors used in HPLC are refractive index (RI), ultraviolet (UV-Vis) and fluorescence, but there are also diode array, electrochemical and conductivity detectors. Each detector has its assets, limitations and sample types for which it is most effective. Most applications in drug analysis use detectors that respond to the absorption of ultraviolet radiation (or visible light) by the solute as it passes through the flow-cell inside the detector. The recent development of the so-called hyphenated techniques has improved the ability to separate and identify multiple entities within a mixture. These techniques include liquid chromatography-mass spectrometry (LC-MS), liquid chromatography- mass spectrometry-mass spectrometry (LC-MSMS), liquid chromatography-infrared spectroscopy (LC-IR) and liquid chromatography-nuclear magnetic resonance (LCNMR). These techniques usually involve chromatographic separation followed by peak identification with a traditional detector such as UV, combined with further identification of the compound with the MS, IR or NMR.

Data Acquisition/Display Systems

Since the detector signal is electronic, use of modern data acquisition techniques can aid in the signal analysis. The data acquisition system of most HPLC systems is a computer. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret. Other more advanced features can also be applied to a chromatographic system. These features include computer-controlled automatic injectors, multi-pump gradient controllers and sample fraction collectors.

Columns

The column or stationary phase is the core of any chromatographic system. Columns are commercially available in different lengths, bore sizes and packing materials. The use of the correct combination of length and packing material in correlation with the appropriate mobile phase can assist in the most effective separation of a sample compound. A variety of column

dimensions are available including preparative, normal- bore, micro- and mini-bore and capillary columns. Different column dimensions can be used for different types of separations and can utilize different packing materials and flow rates. The most widely used packing materials for HPLC separations are silica-based. The most popular material is octadecyl- silica (ODS-silica), which contains C18 coating, but materials with C1, C2, C4, C6, C8 and C22 coatings are also available. Miscellaneous chemical moieties bound to silica, as well as polymeric packing, are designed for purification of specific compounds. Other types of column packing materials include zirconia, polymer-based and monolithic columns. Theoretical plates relate chromatographic separation to the theory of distillation and are a measure of column efficiency. The number of theoretical plates (n) can be determined by the following equation.

$$n=16\left(\frac{tR_1}{w}\right)^2$$

where tR1 is the total retention time and W is the band width of the peak.

In general, LC columns are fairly durable with a long service life unless they are used in some manner that is intrinsically destructive for example, with highly acidic or basic eluents or with continual injections of "dirty" biological or crude samples. Column degradation is inevitable, but column life can be prolonged with proper maintenance. Flushing a column with mobile phase of high elution strength following sample runs is essential. When a column is not in use, it is capped to prevent it from drying out. Particulate samples need to be filtered and when possible a guard column should be utilized. Column regeneration could instill some life into a column, but preventive maintenance is the key to preventing premature degradation.

Steps for HPLC Method Development

Step 1 - Selection of the HPLC method and initial system

When developing an HPLC method, the first step is always to consult the literature to ascertain whether the separation has been previously performed and if so, under what conditions - this will save time doing unnecessary experimental work. When selecting an HPLC system, it must have a high probability of actually being able to analyse the sample; for example, if the sample includes polar analytes then reverse phase HPLC would offer both adequate retention and resolution, whereas normal phase HPLC would be much less feasible.

Step 2 - Selection of initial conditions

This step determines the optimum conditions to adequately retain all analytes; that is, ensures no analyte has a capacity factor of less than 0.5 (poor retention could result in peak overlapping)

and no analyte has a capacity factor greater than 10– 15 (excessive retention leads to long analysis time and broad peaks with poor detectability).

Step 3 - Method optimization

The experimental conditions should be optimized to get desired separations and sensitivity after getting appropriate separations. Stability indicating assay experimental conditions will be achieved through planned/systemic examination on parameters including pH (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, Injection volume and diluents solvent type.

Step 4 - Validation of method

Validation of an analytical method is the process which is established by laboratory studies to evaluate the performance uniqueness of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with planned and systematic collection by the applicant of the validation data to support analytical procedures.

Basic Terminologies in HPLC: [16]

Chromatography is described and measured in terms of four major concepts: capacity, efficiency, selectivity and resolution.

Dead time (t_0):

It is the time required by an inert compound to migrate from column inlet to column end without any retardation by the stationary phase. Consequently, the dead time is identical with the residence time of the sample compound in the mobile phase.

Net retention time (t_{R1} or t_{R2}):

It is the difference between total retention time and dead time. That is the time the sample component remains in the stationary phase.

$$t'_{R_1} = t_{R_1} - t_0 \qquad t'_{R_2} = t_{R_2} - t_0$$

Capacity factor (k'_R):

For effective liquid chromatographic separations, a column must have the capacity to retain samples and the ability to separate sample components efficiently. The capacity factor, (k'_R), of a column is a direct measure of the strength of the interaction of the sample with the packing material and is defined by the expression-

$$k'_R = \frac{t_R - t_0}{t_0} = \frac{V_R - V_0}{V_0}$$

Where t_R is the time taken for a specific solute to reach the detector and t_o is the time taken for no retained species to reach the detector. V_R is the volume of solution that is pumped through the detector before a specific peak is eluted, and V_0 is the volume of solvent pumped through the detector between the time of injection and the appearance of the no retained species.

The capacity factor of a column is mostly a function of the packing material but can be manipulated to a degree by varying the solvent strength. The higher the capacity factor of the column, the greater is its ability to retain solutes.

Selectivity (α):

The selectivity of the chromatographic system is a measure of the difference in retention times between two given peaks and describes how effectively a chromatographic system can separate two compounds. Selectivity is usually defined in terms of α, where

$$\alpha = \frac{t_2 - t_0}{t_1 - t_0} = \frac{V_2 - V_0}{V_1 - V_0} = \frac{k'_2}{k'_1}$$

Where, V_0 = the void volume of the column,

V_1 & V_2 = the retention volumes of the second and the first peak respectively.

The selectivity of a column is primarily a function of the packing material, although the analyst has some control using the mobile phase or temperature.

Resolution (R):

Resolution is a term used to describe the degree of separation between neighboring solute bands or peaks. Resolution is calculated as 2.0 times the retention time between two adjacent peaks divided by the sum of the width of the peaks. Theoretically, the peak width used in the resolution formula should be the width of the peak at baseline. Resolution is never measured for the first peak in a chromatogram because there is no preceding peak to use in calculation.

$R = 2.0 \, (t_{R\,2} - t_{R\,1}) / (W_2 + W_1)$

Where,

R = Resolution

t_R = Retention time

$(W_1 + W_2)$ = sum of width at tangent values using tangent lines.

Relative resolution:

Relative resolution is measured between the named in the calibration table and their   referenced peaks R is a measure, of how well two peaks are separated. For reliable quantitation, well-separated peaks are essential for quantitation. This is a very useful parameter if potential

interference peak (s) may be concern. The closest potential eluting peak to the analyte should be selected. R is minimally influenced by the ratio of the two compounds being measured.

Tailing factor (T):

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peaks ends and hence the calculation of the area for the peak of interest. If the integrator is unable to determine exactly when an up slope or down slope occurs, accuracy drops.

Tailing factor establishes the maximum permissible asymmetry of the peak.

$T = W / 2 * F$

Where:

T = Tailing factor

W = Peak width at 5% of peak height

F = time from width start point at of peak height to retention time (t_R)

Efficiency (N):

Column efficiency refers to the performance of the stationary phase to accomplish particular separations. This entails how well the column is packed and its kinetic performance. The efficiency of a column (N) is a number that describes peak broadening as a function of retention and is dependent on the entire chromatographic system.

$$n = 16\left(\frac{t_{R_1}}{w}\right)^2 \quad \text{or} \quad n = 5.54\left(\frac{t_{R_1}}{w_{1/2}}\right)^2$$

Height equivalent of a theoretical plate (h):

HETP is the length, in which the chromatographic equilibrium between mobile and stationary phase is established. Since a large number of theoretical plate is desired, h should be as small as possible.

$$h = \frac{L}{n}$$

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.

Method Development by HPLC:

Components of Method Validation [17,18]

The method validation /evaluation imply the process of documenting or providing that analytical method provides analytical data for the intended use . Validation analytical method require the following

- Assuring quality,
- Achieving acceptance of products by the international agencies,
- Mandatory requirement for registration of any pharmaceutical product or pesticide formulation,
- Validation methods are only acceptable for under taking proficiency testing,
- Validated/Evaluated method undergoes quality control procedures for further evaluation.

The following are typical analytical performance characteristics which may be tested during methods validation:

1. Specificity

2. Accuracy

3. Precision

3.1 Repeatability

3.2 Intermediate precision

3.3 Reproducibility

4. Limits of detection and Quantitation

5. Linearity

6. Range

7. Robustness

8. Ruggedness

1. Specificity

Specificity is the ability to assess un equivocally, the analyte in the presence of components which may be expected to be present typically these might include impurities, degradants and matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: To ensure the identity of an analyte.

Purity Tests: To ensure that, all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (Content or Potency): To provide an exact result, that allows an accurate statement on the content or potency of the analyte in the sample.

2. Accuracy

Accuracy is the nearness of a measured value to the true or accepted value indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analyzed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements which is obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample, it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability

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

Intermediate Precision

Intermediate precision expresses within laboratories variations: different days, different analysts and different equipment, etc.

Reproducibility

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

4.Limits of detection and Quantitation

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It is expressed as a concentration at a specified signal: noise ratio, usually 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The ICH has recommended a signal: noise ratio

10:1. LOD and LOQ may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve(s) at levels approximating the LOD according to the formulae: LOD53.3(SD/S) and LOQ510(SD/S) .

5. Linearity

Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line.

6. Range

Range is defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

8.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 conditions, such as different laboratories, analyst, instruments and lots of regents, elapsed assay times, assay temperature or days. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst.

CONCLUSION:

Analytical methodology provide to an analyst the essential data for a given analytical problem, accuracy, precision, sensitivity, range of analysis, Repeatability, Linearity, Reproducibility, LOD and LOQ i.e. the minimum requirements which are essential for are the specifications of the method for the intended purpose to be able to analyse the desired analyte in different matrices with surety and certainty. The main activities involved in the analytical development of a method are separation and characterization of impurities as well as analytical investigations, studies for identification and finally setting up of parameters optimization to specific requirements. The mean of this article is to provide a simple way to use proper scientific background to improve the

quality of the method development and validation. Applications of analytical method in routine drug analysis are also taken into consideration in this article. Method development involves a series of simple steps. All the conditions are optimized as essential for the purpose of the separation.

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