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Validation and Analytical Method: A Review

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HUMAN

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ABSTRACT

Analytical chemistry is useful for the study of the separation, identification, and qualification of chemical components obtained from natural and artificial materials. There are different analytical methods used like spectral method, electroanalytical method, chromatographic method, the spectrophotometric method which include UV spectroscopy. After developing any process, technique, or formula next step is to validate these with the help of pre-determined criteria to ensure the procedure is as accurate as per standard. There are the different parameter of validation which ensures the accuracy of any specific process like specificity, linearity, accuracy (percentage recovery), precision, repeatability, and limit of detection, the limit of qualification, robustness, and system suitability testing. Effective analytical method development and its validation can provide significant improvements in precision and a reduction in bias errors. It can further help to avoid costly and time-consuming exercises.

INTRODUCTION:

Analytical chemistry is a useful study of the separation, identification, and qualification of chemical components obtained from natural and artificial materials. Qualitative analysis gives us information about chemical species in the sample and qualitative study gives us information on quantity of any component in given substances. There is two types of the analytical method (1) Classical method (2) Instrumental method. Precipitation, extraction, and distillation have come under these categories. Quantitative analysis like odor, colour, and melting point also come in the classical method.

Classical quantitative analysis is done by measurement of weight or volume of product Instrumental methods use an apparatus to measure physical quantities of the analyte such as light absorption, fluoresces, or conductivity. The separation of material is accomplished using chromatography, electrophoresis, or field flow fractionation method. (**1-4)**

Validation: Validation is a documented process that helps to ensure that any process or equipment is accurate as per pre-determined criteria or as compared with standard. There are different types of validation

1. Equipment validation
2. Facilities validation
3. HVAC system validation
4. Cleaning validation
5. Process validation
6. Analytical method validation
7. Computer system validation

Similarly, the activity of qualifying system and equipment is divided into several subsections including the following

1. Design qualification (DQ)
2. Component qualification (CQ)
3. Installation qualification (IQ)

4. Operation qualification (OQ)

5. Performance qualification (PQ)

Design qualification (DQ): Demonstration that the purposed design will be accurate as per user requirement specification (URS). Satisfactory execution of the DQ is a mandatory requirement before the construction of the new design can be authorized.

Installation qualification (IQ): Demonstrates that the process or equipment meets all specifications is installed correctly and all require components and documentation needed for continued operation are installed and in place.

Operational qualification (OQ): Ensure that all the data of the process or equipment are operating correctly as per standard.

Performance qualification (PQ): Ensure that the process or equipment performs as intended consistently over time.

Component qualification (CQ): CQ is a new process that developed recently in 2005. This term refers to the manufacturing of auxiliary components to ensure that they are manufacturing to the correct design criteria. This could include packaging components such as folding cartons, shipping cases, labels, or even phase change material.

There is an instance when it is more expedient and efficient to transfer some tests or inspections from the IQ to the OQ or from the OQ to PQ. This is allowed for in the regulation, provided that a clear and approved justification is documented in the validation plan (VP). The validity of the method can be insuring by laboratories studies. Validation work is done by a pre-planned document that called validation protocol that ensures that all process that defined as per standard.

Reason for validation: Validation is a documented process that assured that process or equipment is accurate as per predetermined criteria or as per compared with standard.

Validation master plan

A validation plan is a document that describes how the validation program will be executed in reality. It includes all validation criteria like process validation, facility and utility qualification and validation, equipment qualification, cleaning, and computer qualification.

Importance of analytical methods

The analytical method helps to identify, characterization and determination of drugs in different dosages form and biological fluid.

Their various reasons by which new analytical methods are developed.

1. There is no information on the drug or its combination in the official pharmacopeia.
2. No literature information is available about the drug or its combination.
3. It is not available any analytical method for estimation of the drug.
4. There is not a suitable analytical method available for the estimation of a combination of the drug.

Other reasons:-

1. Required expensive instruments or reagents or solvents.
2. Having a long process of analysis which is time-consuming.
3. The process is not sensitive, rapid, or reliable**. (5-8)**

Various types of analytical methods

1. Spectral methods

Spectral methods include measure electromagnetic radiation, which may be absorbed or emitted like UV visible spectroscopy, I.R spectroscopy, NMR spectroscopy, flame photometry, etc.

2. Electroanalytical methods: These methods are used for the measurement of current, voltage, or resistance. E.g. Conductometry, amperometry, etc.

3. Chromatographic methods: Chromatographic methods are those methods which use to identification and separation of different components. There are different chromatographic methods like

a. GLC: - The full form of GLC is gas-liquid chromatography. It is based on the principle of partition. Gas is used as a mobile phase and liquid is used as a stationary phase. Separation is mainly based upon the partition coefficient that is component which is less soluble in

stationary phase are run fast and eluted out first and component which is more soluble in stationary phase are eluted later.

Advantages of GLC

1. Shorter run time
2. Greater sample throughput
3. Cheaper columns
4. Higher signal to noise ration

Disadvantages of GLC

1) Easy to overload phase
2) More awareness need for split-less injection.

b. HPLC: The full form of HPLC is high-performance liquid chromatography is a technique in which a pump is used to produce the pressure. Each component is interact slightly with absorbent material, having a different flow rate.

Advantages of HPLC

1. It is an automated and quick process.

2. Having high resolution and easy to read.

Disadvantages of HPLC

1. Higher cost

2. Difficult to identified those components having the same elution. **(16)**

Spectrophotometric method

1. UV – Visible spectroscopy

UV spectroscopy is an instrumental scanning method that analyzes the absorption of light within the UV spectrum. It is used for measuring absorption, emission, and transmission of UV and visible wavelength by matters. It is also known as the phenomenon of the interaction of molecules with UV light. Ultraviolet-visible spectrometry (UV-VIS) refers to absorption

spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared) ranges. In the visible range absorption or reflectance is direct affects the perceived colour of the chemical involved. In this region of the electromagnetic spectrum, molecule electronic transitions occur. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with the transition from the excited state to the ground state, while absorption measures transition from the ground state to the excited state. **(16)**

2. Principle of UV spectroscopy

Molecules containing bonding and non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (If HOMO and LUMO have lower energy gap then longer wavelength of light can absorb). More the number of molecules capable of absorbing light of a given wavelength the greater the extent of absorption. Furthermore, the more effectively a molecule absorbs light of a given wavelength, the greater the extent of light absorption. For these guiding ideas, the following empirical expression, known as the Beer-Lambert.

2.1. Beer's law

According to this law when a beam of colored radiation is passed from a given solution of an absorbing substance, the rate of decrease of intensity of radiation with a thickness of absorbing solution is proportional to the intensity of incident radiation as well as the concentration of the solution. In another word, absorbance is proportional to the concentration.

$$-\frac{dI}{dX} = \text{k IC}$$

Where c= concentration of the solution in moles per litter and k= molar absorption coefficient and its value depends upon the nature of the absorbing substance. –dI/dx = rate of decrease of intensity of radiation with a thickness of absorbing medium.

2.2. Lambert's law

It states that when a beam of monochromatic radiation passes through a solution of the homogeneous absorbing medium. The rate of decrease of intensity of radiation with the thickness of the absorbing medium is proportional to the intensity of incident radiation.

$$-\frac{dI}{dX} = kI$$

Where - dI/dx= rate of decrease of intensity of radiation with a thickness of absorbing medium and I is the intensity of radiation after passing through a thickness x, of the medium, k is proportionality constant.

Beer-Lambert law is a combination of these two laws.

Beer-Lambert law

When a beam of light is passed from a solution of an absorbing substance, a decrease in the intensity of the light may occur. Mathematically, Beer-Lambert law is expressed as

$$\text{Log } I/I = €.c.\ I = A$$

Where A= absorbance or optical density

Io = Intensity of incident light

I = Intensity of transmitted light

I = path length of radiation through the sample (cm)

C = concentration of solute in solution

€ = molar extinction coefficient

It helps in the qualification of medicinal substance by spectrophotometer by preparing the solution and measures its suitable wavelength. The assay of the single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of the three principle procedures. These help in find standard absorptive value, calibration graph, and single or double point standardization. In the standard absorptive value method, the use of standard A (1%, 1 cm) or E value is used to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the

reference substance. In the calibration graph method, the absorbances of some standard solutions of the reference substance at concentration encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The concentration of the substance in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

$$C\ test = (A\ test \times C\ std)/A\ std$$

Where C test and C std are the concentration in the sample and the standard solution respectively and A test and A std are the absorbances of the sample and standard solution respectively. For assay of substance/s in multicomponent samples by spectrophotometer; the following methods are being used routinely, which includes,

1. Simultaneous equation method

2. Derivation spectrophotometer method

3. Absorbance ratio method (Q-Absorbance method)

Different spectrophotometry

Region	**Wavelength**
Far (or vacuum) Ultraviolet	100-200nm
Near ultraviolet	200-400nm
Visible	400-750nm
Near IR	0.75-2.2µm
Mid IR	2.5-50µm
Far IR	50-1000µm

The instrument used in UV-visible spectroscopy is called a UV/Vis spectrophotometer. It measures the intensity of light passing through a sample and compresses it to the intensity of light before it passes through the sample cell. The ratio is called transmittance and is usually expressed as a percentage (%T). The absorbance is based on transmittance. The UV-visible also use to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample cell and compares it to the intensity of light reflected from

reference material (such as a white tile). The ratio is called the reflectance and is usually expressed as a percentage (%R).

Application

Transition metals ions, highly conjugated organic compounds, and biological macromolecules are quantitatively determined by UV/Vis spectroscopy. Solutions of transition metal ions can be colored (i.e., absorb visible light) because electrons within the metal atoms can be excited from one electronic state to another.

- Identification of an unknown compound.
- Detection of the functional group.
- Detection of the strength of hydrogen bond. **(10, 11)**

Validation parameter

The main objective of validation of an analytical procedure is to ensure that the procedure is as accurate as per standard or as per predetermined criteria. Analytical procedures for biological and biotechnological products in some cases may be different. Well-characterized reference material, with documented purity, should be used throughout the validation study.

1. Specificity

Specificity should be check during the validation of identification tests, the determination of impurities, and the assay. The procedure used to demonstrate specificity will depend on the intended objective of the analytical procedure. There are possibilities that the given analytical procedure is not always specific. In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

2. Linearity

Various methods are used for the identification of linear relationships by the range of analytical procedures. Visual inspection of a plot of the signal as a function of analyte concentration or content is used to check linearity. Some analytical procedures, such as immunoassay, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an appropriate function of the concentration of an

analyte in a sample. For the establishment of linearity, a minimum of five concentrations is recommended.

3. Range

Linearity studies and depends on the analytical procedure provide an acceptable degree of linearity, accuracy, and precision when used for a sample having the quantity of analyte within or at a high specified range of the analytical procedure provide a specific range.

4. Accuracy (Percentage recovery)

Accuracy can be defined as percentage recovery by the assay of known, added amounts of analyte. Accuracy should be reported as percent recovery by the assay of the known added amount of analyte in the sample. (**3-9)**

5. Precision: Validation is done for calculating precision.

5.1. Repeatability

Repeatability is defined as the closeness of agreement between independent test results, obtained with the same method, on the same test material, in the same laboratory, by the same operator, and using the same types of equipment with the short intervals of time.

5.2. Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstance under which the procedure is intended to be used. The applicant should establish the effect of random events on the precision of the analytical procedure. Typical variations to be studied include days, analytes, equipment, etc.

5.3. Reproducibility

Reproducibility is assessed utilizing an inter-laboratory trial. Reproducibility should be considered in the case of the standardization of analytical procedures, for instance, for the inclusion of procedures in pharmacopeias.

6. Limit of detection (LOD)

6.1. Based on visual evaluation

The detection limit can be determined by establishing the minimum level at which the analyte can be reliably detected and analysis of sample done with the known concentration of the analyte.

6.2. Based on signal-to-noise

Based on signal –to noise is only applicable for that analytical procedure having exhibit baseline noise. With knows low concentration of analyte comparing measured signals from the sample with those of blank sample and establishing the minimum concentration at which the analyte can be reliably detected using for Determination of the signal-to-noise ratio. 3:1 or 2:1 is generally considered acceptable for a signal-to-noise ratio for estimating the detection limit.

6.3. Based on the standard deviation of the response and the slope

$$LOD = 3.3 (SD/S)$$

Where, SD=Standard deviation of the response

S= Slope of the calibration curve **(2-9)**

7. Limit of quantification (LOQ)

7.1. Based on visual evaluation

Visual evaluation is applicable for non-instrumental methods, but may also be used with instrumental methods. Analysis of sample with the known concentration of analyte is used to determine the limit of qualification and establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

7.2. Based on signal-to-noise

This approach can only be applied to the analytical procedure that exhibits baseline noise. Determination of the signal-to-noise ratio is performed by comparing the measured signal from samples with known low concentrations of analyte with those of blank sample and by

establishing the minimum concentration at which the analyte can be reliably quantified, a typical signal-to-noise ratio.

7.3. Based on the standard deviation of the response and the slope

The limit of quantification may be expressed as:

$$LOQ = 10 (SD/S)$$

Where, SD= Standard deviation of response

S= Slope of the calibration curve

8. Robustness

It should show the reliability of analysis concerning deliberate variation in method parameters. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- Stability of analytical solution
- Mobile phase composition
- Extraction time
- Influence of variation of pH
- Temperature etc.

9. 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 operation, and sample to be analyzed constitute an integral system that can be evaluated as such, system suitability test parameter to be established for a particular procedure depend on the type of procedure being validated. **(1-11)**

CONCLUSION:

The analytical method helps to identify, characterization and determination of drugs in different dosages form and biological fluid. Their various reason by which new analytical methods are developed like there is no information of drug or its combination in the official pharmacopeia, no literature information available about drug or its combination, there is not available of any analytical method for estimation of the drug, there is not a suitable analytical method available for estimation of a combination of the drug. It's necessary to validate the analytical method because the main objective of validation of an analytical procedure is to ensure that the procedure is as accurate as per standard or as per predetermined criteria. Different validation methods ensure the accuracy of the analytical method.

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