IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Review On Uv Method Validation And Development

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ABSTRACT

UV method validation and development studies are important aspects for the pharmaceutical industry. It is utilized for the measurement of light radiation emission. This UV spectroscopy is useful for the development of novel drug formulation in pharmaceutical industries. In this study various spectroscopic instruments are utilized for the detection of wavelength of sample material by the use of radiation. The purpose of this validation is to show that processes involved in the development and manufacture of the drug, production and analytical testing can be performed in an effective and reproducible manner. This article provides the guideline on how to perform validation characteristics for the analytical methods which are utilized in pharmaceutical analysis.

KEYWORDS: UV method validation, spectroscopy, analytical method development, accuracy, precision, instrumentation.

INTRODUCTION

Until about 1945, the structure of a newly discovered organic compound was based almost entirely on its molecular formula and chemical reaction. The compound was converted into a known compound by a series of specific reactions, or it was synthesized from smaller known compound by specific reaction. This way of finding the structure of an organic compound was time consuming and not always 100% accurate. The conclusion was as reliable as the understanding of the reaction that was carried out.

The subfield of chemistry known as analytical chemistry is concerned with the identification of substances, samples, and mixtures' constituents quantitatively and qualitatively.It provides information regarding the content, stability, identity, purity, and starting materials of the additives, active pharmaceutical constituents, and excipients. Additionally, it ensures the efficacy, safety, and quality of pharmaceuticals utilized in therapeutic environments.

The use of spectroscopic method for analysis and structure determination. These methods have three major advantages over most chemical methods.

1) Spectroscopic methods are easier and faster to do than most chemical test or reactions.
2) Spectroscopic methods provide far information about molecular structure. Practically all functional group and structure feature can be detected with very small amount of sample.
3) Spectroscopic methods are non destructive and, if necessary the entire sample can be recovered.

There are four spectroscopic methods which are very widely used in organic chemistry. They are: ultraviolet visible, infrared, nuclear magnetic resonance, mass spectroscopy. The basis of these methods is electromagnetic radiations.

There are typically two main types of analytical procedures:

1) Instrumental Method
2) Non Instrumental Method

1) Instrumental Method

Measurement of physical properties of analyte such as conductivity, electrode potential, light absorption or emission, mass to charge ratio and fluorescence began to be employed for quantitative analysis of inorganic, organic, and biochemical analyte.
Instrumental method of analysis collectively named for newer method for separation and determination of chemical species.

Advantages

- High sensitivity
- The ability to perform trace analysis
- Generally large number of samples may be analyzed very quickly
- Less skill and training is usually required to perform instrumental analysis
- Less time consuming
- Small sample can be handled
- Reproducible

Disadvantages

- Not possible to separate the component in large scale.

2) Non-Instrumental Method

These approaches include chemical processes.These approaches are based on volume and weightmeasurements. These procedures are sometimesreferred to as the "classical analysis method.

Advantages

- It is not expensive method because cost of glassware is low
- Special training not required
- Calibration and standardization is not required
- Sensitivity and accuracy depends on expert person

INTRODUCTION OF SPECTROSCOPY:

Spectroscopy is the study of the interactions between matter and electromagnetic radiation. In these interactions, matter absorbs and emits energy in the form ofradiation. Two distinct types of spectroscopy exist: absorption and emission. Absorption spectroscopy (UV-visible, infrared, nuclear magnetic resonance, microwave, and radio wave spectroscopy) examines the spectra of electromagnetic radiation that is absorbed by the sample.

2.1 Ultraviolet-Visible Spectrophotometry:

UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraradiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the UV visible region are called Ultraviolet-Visible Spectrophotometers.
In ultraviolet visible spectroscopy, the 200 – 750 nm region of the ultraviolet spectrum is used. These includes both visible reasons (400 – 750 nm) and near ultraviolet region (200 – 400 nm) the radiation of these wavelength is sufficiently energetic to cause the promotion of loosly held electrons, such as nonbonding electrons or electrons involved in a π-bond to higher energy levels. For the absorption in this particular region of ultraviolet spectrum the molecule must contain conjugated double bonds. The ultraviolet visible spectrum is composed of only a few broad bands of absoption. The wavelength of maximum absorbance is reffered to as λmax.

TERMS USED IN UV SPECTROSCOPY

A. Chromophore

- In Greek word : chromo means colour and phores means bearer
- A Chromophore is the part of molecules responsible for its colour. The colour that is seen by our eyes.
- Define as any isolated covalently bonded group that shows a characteristic absorption of electromagnetic radiation in the UV or visible region.
- A group that gives rise to absorption in visible and near ultra violet is called “chromophore”
- There is a faint absorption band between 200 and 300 nm for compounds with nonconjugated carbonyl groups.

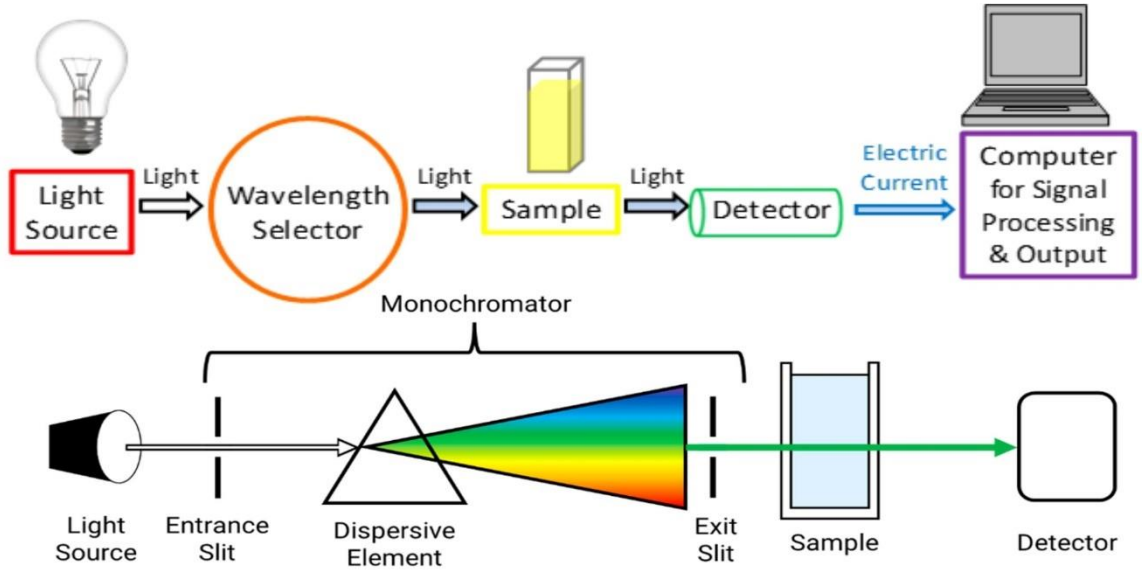
Example : R-CH=CH-R (Alkane), R-NH2 (Amine), R-C-OH (Carboxyl), R-NO2 (Nitro).

B. Auxochrome:

- In Greek : Auxo means increase and chromo means colour
- Auxochrome is defined as the any group of which does not itself act as a chromophore but whose presence brings about the shift of the absorption bands towards the red end of the spectrum. (longer wavelength).
- The effect is due to its ability to extent the conjugation of a chromophore by sharing the non bonding electrons.
- Take benzene, phenol, and aniline as examples; their λmax values are (255 nm), (270 nm), and (280 nm), respectively

INSTRUMENTATION OF UV VISIBLE SPECTROPHOTOMETRY

1. Source of radiation
2. Filters and Monochromators
3. Sample cell
4. Detectors
5. Recording Devices



4.0.1. Fig: Instrumentation of UV visible spectrometry

1. Source of radiation

The best source light is the one which is more stable, more intense and which gives the range of spectrum from 180-700 nm.

- **Hydrogen discharge lamp**
  In these lamps, hydrogen gas is stored under high pressure. When an electric discharge is passed through the lamp, excited hydrogen molecules will be produced which emit uv radiations.
- **Deuterium lamp**
  It is similar to hydrogen discharge lamp, but filled with deuterium(O2) in place of hydrogen.
  It offers 3 – 5 times more intensity than hydrogen lamp
- **Xenon discharge lamp**
  In this lamp, xenon at 10 – 30 atm pressure is filled it and it has two tungsten electrodes.
  Greater intensity than hydrogen lamp.
- **Mercury arc**
  This contain mercury vapours but not continuous so not widely used.

2.Filters and Monochromators

Purpose of emplaying devices is to resolve wide band of polychromatic radiation into narrow band of monochromatic radiation.

➢ **Filter**

These are used for isolation of narrow band of radiant energy of desired spectral region. It allows transmission only limited wavelength region while absorbing most of radiation of other wavelength.

- **Types**
  - Glass filter
  - Gelatin filter
  - Interference filter

➢ **Prisms**

Prism made from glass, quartz or fused silica is employed as dispersing devices in spectrophotometer. Glass has dispersing power about 3 times that of quartz. It used in visible portion of spectrum.

Mechanism in dispersing polychromatic beam of radiation into small bands of wavelength by prism depends on variation of index of refraction with wavelength.

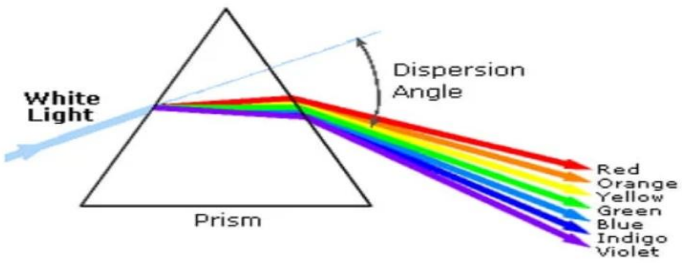


Fig : Prism

- **Types**
  - Cornue type
  - Littrow type

➢ **Diffraction Grating**

More refined nature of dispersion of light is obtained by means of a diffraction grating, these consist of large no. of parallel lines or graves about parallel lines or groves about 15,000 – 30,000 per inch are ruled on highly polished surface of aluminium.

The lines or grooves drawn on plate are scattering center for light beam impinging on it. The resolving power of grating depends on number of lines per inch on surface and increase with testing number of lines.

3. sample cell

Sample containers, which are usually called as cells or cuvettes, must have windows that are transparent in the spectral region of interest. Quartz or fused silica is required for the UV region (wavelengths less than 350nm) and may be used in the visible region. Silicate glass is ordinarily used for 375 – 2000 nm region because of its low cost compared to quartz, plastic cells are also used in the visible region.

The best cells have windows that are perpendicular to the direction of the beam in order to minimize reflection losses. The most common cell path length for studies in the UV and visible regions is of 1cm; matched, calibrated cells of this size are available from several commercial sources.

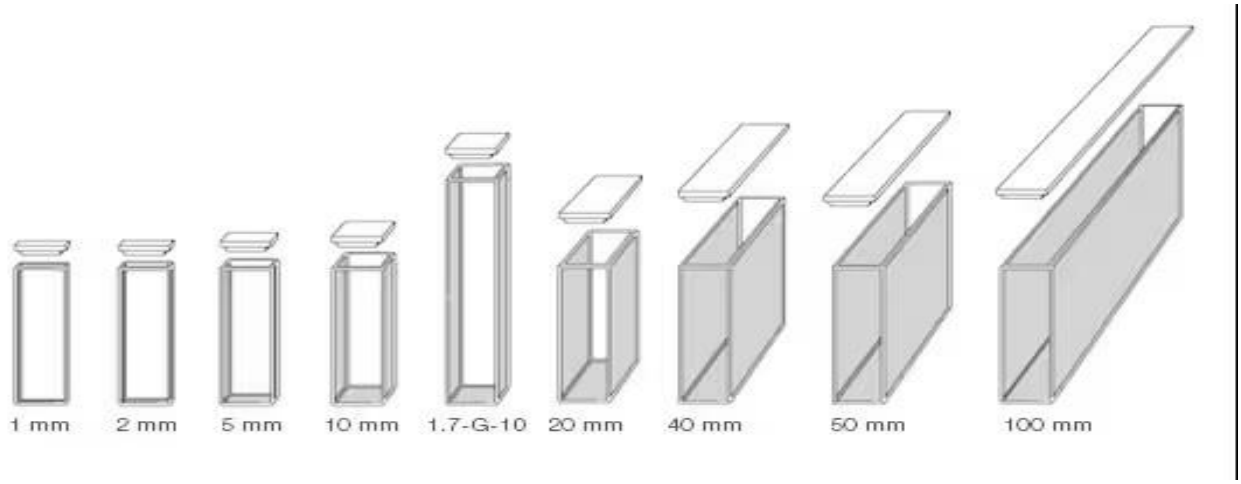


Fig : commercially available cells used in UV visible spectrophotometer

4. Detectors

Detectors used in UV-visible spectrophotometers can be called as photometric detectors. The most commonly used detectors are:

1. Barrier layer cell (photovoltaic cell)
2. Phototubes (photo-emissive tube)
3. Photo-multiplier tubes

1. Barrier layer cell (photovoltaic cell)

This cell is also known as photroniccell and operates without the use of a battery. It consist of a metal base plate (of iron or aluminium), that acts as an electrode. On its surface, a thin layer of a semiconductor metal (like selenium) is deposited. Then, the surface of selenium is covered by a very thin layer of silver or gold that acts as the second collector electrode.

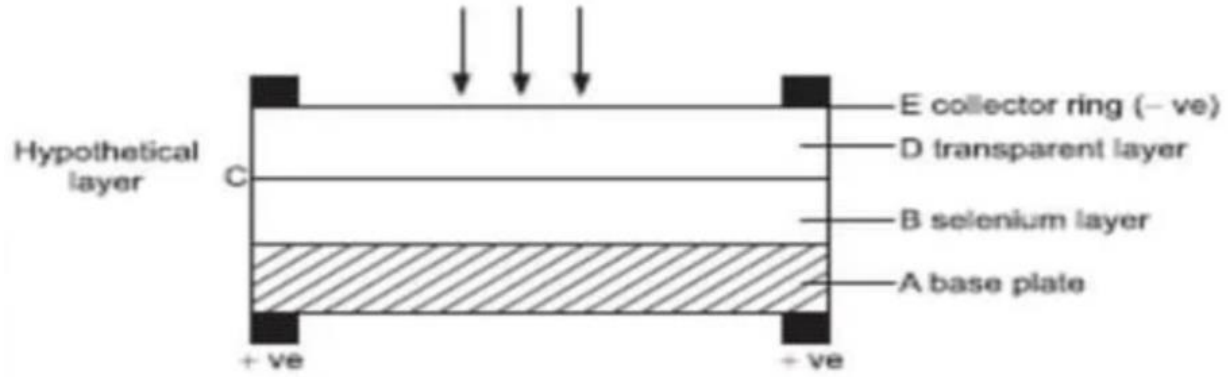


Fig: Barrier layer cell

2. Phototubes (photo – emissive tube)

This detector is composed of an evacuated glass tube, which consist of photo cathode and collector anode. The photo cathode is coated with elements of high atomic volume like (caesium, potassium, silver oxide), which can liberate electron when light radiation falls on it. This flow of electron towards anode produces a current proportional to the intensity of light radiation. Composite coatings like caesium / caesium oxide / silver oxide can also be used, which increases the sensitivity and range of wavelength in which the detector can be used (UV- region).

The signal from the detectors can also be amplified using an amplifier circuit. Phototubes have better sensitivity as compare to photovoltaic cell, and hence are more widely used.

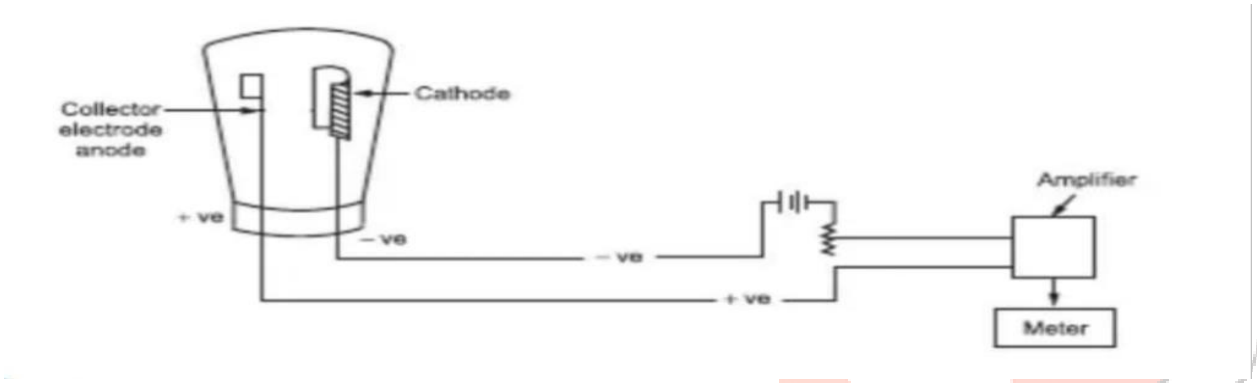


Fig : phototube (photo – emissive cell)

3. Photo-multiplier tubes

These tubes are incorporated in expensive instruments like spectrofluorimeter and its sensitivity is higher due to measuring weak intensity of light.

The principle employed in this detector that is multiplication of photo electrons by secondary emission of electrons. This is achieved by using a photo cathode and the series of anodes (dynodes) upto 10 dynodes are used. Each dynode is maintained at 75 – 100 V higher than the predicting ones.

Photo – multiplier tubes can detect very weak signals, even 200 times weaker than that could be done using photovoltaic cell. Hence it is useful in fluorescence measurements.

PMT should be shielded from stray lights in order to have accurate results.

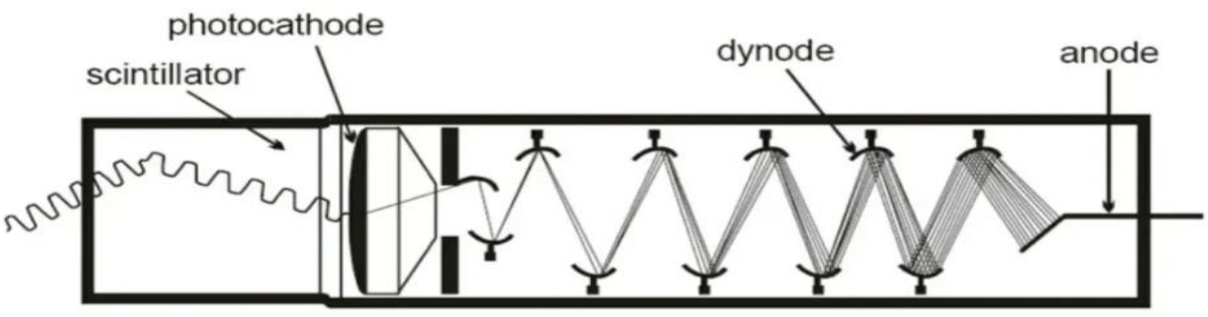


Fig : Photo - multiplier tube

5 . Recording devices

A pen recorder, which is frequently linked to a computer, is frequently connected to the amplifier. The computer generates the spectrum of the specified compound and stores all generated data.

ANALYTICAL METHOD DEVELOPMENT

New methods are being developed for analysis of novel products. To analyze the existing either pharmacopeial or non-pharmacopeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits.

The requirements for method development are as follows:

1) Qualified analysts

2) Instruments-qualified and calibrated

3) Documented methods

4) Reliable reference standards

5) Sample selection and integrity

6) Change control (Chauhan A, *et al.*, 2015)

Analytical method development is useful for:

1) New process and reactions

2) New molecule development

3) Active ingredients (Macro analysis

4) Residues (Micro analysis)

5) Impurity profiling

6) Degradation studies

7) Herbal products (Chauhan A, *et al.*, 2015)

5.0.1. Purpose of analytical method development

Drug analysis reveals the identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. During manufacturing process and drug development the main purpose of analytical methods is to provide information about potency (which can be directly related to the requirement of a known dose), impurity (related to safety profile of the drug), bioavailability (includes key drug characteristics such as crystal form, drug uniformity and drug release), stability (which indicates the degradation products), and effect of manufacturing parameters to ensure that the production of drug products is consistent.

Providing simple and analytical process for various complex formulations is a subject matter of utmost importance. Rapid increase in pharmaceutical industries and constant production of drug in various parts of the world has brought a quick rise in demand for new analytical techniques in the pharmaceutical industries as a consequence, analytical method development has become the basic activity of analysis in a quality control laboratory.

The reasons for the development of novel methods of drug analysis are:

a) When there is no official drug or drug combination available in the pharmacopoeias
b) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.
c) Analytical methods for the quantitation of the analyte in biological fluids are found to be unavailable.
d) The existing analytical procedures may need costly reagents and solvents. It may also involve burdensome extraction and separation procedures.

5.0.2. Steps for the development of the method

Development procedure follows with the proper documentation. All data relating to these studies must be recorded either in laboratory notebook or in an electronic database.

1) Analyte standard characterization

a) All known important information about the analyte and its structure that is to say physico-chemical properties like solubility, optical isomerism etc., is collected.

b) The standard analyte (≈100 % purity) is obtained. Necessary arrangement is to be made for the perfect storage (refrigerator, desiccators, and freezer).

c) In the sample matrix when multiple components are to be analyzed, the number of components is noted duly presenting the data and the accessibility of standards is estimated.

d) Methods like spectroscopic, HPLC, GC, MS etc., are considered when matched with the sample stability.

2) Method requirements

The requirements of the analytical method need to develop the analytical figures of merit such as linearity, selectivity, range, accuracy, precision, detection limits etc., shall be defined.

3)Literature search and prior methodology

All the information of literature connected with the drug is reviewed for physico-chemical properties, synthesis, solubility and appropriate analytical methods with reference to relevant books, journals, USP/NF, AOAC and ASTM publications and it is highly convenient to search Chemical Abstracts Service automated computerized literature.

4) Choosing a method

a) Duly utilizing the information available from the literature, methodology is evolved since the methods are changed wherever required. Occasionally it is imperative to get additional instrumentation to develop, modify or reproduce and validate existing procedures for analytes and samples.

b) If there are no past suitable methods available to analyze the analyte to be examined.

5)Instrumental setup and initial studies

Installation, operational and performance qualification of instrumentation with reference to laboratory standard operating procedures is verified by setting up appropriate instrumentation.

6) Optimization

While performing optimization, one parameter is changed at a time and a set of conditions are isolated, before utilizing trial and error approach. The said work need to be accomplished basing on a systematic methodical plan duly observing all steps and documented with regard to dead ends.

7) Documentation of analytical figures of merit

The actual decided analytical figures of merit like Limit of quantitation, Limit of detection, linearity, time taken for analysis, cost, preparation of samples etc. are also documented.

8) Evaluation of development method with real samples

The sample solution should lead to unequivocal, total identification of the peak interest of the drug apart from all other matrix components.

9) Estimation of percent recovery of real samples and demonstration of quantitative sample analysis

Percent recovery of spiked, genuine standard drug into a sample matrix which contains no analyte is estimated. Optimization to reproducibility of recovery (average ± standard deviation) from sample to sample has to be showed. It is not necessary to get 100% recovery so far as the results are reproducible to recognize with a high degree of certainty.

METHOD VALIDATION

Method validation is a "process of establishing documented evidence" that provides a high level of guarantee that the product (equipment) will meet the requirements of the desired analytical applications (La-vanya G, *et al.*, 2013).

Analytical methodologies are absolutely essential for the development of new medications, formulation and preformulation research, stability studies, and quality control inspections. This strategy must be uncomplicated, precise, accurate, cost-effective, and user-friendly. It is necessary to conduct approach validation both during development and in use. Analytical validation refers to the process of assessing and substantiating that an analytical method successfully accomplishes its designated objective.

Importance of validation

- Assurance of quality
- Minimal batch failure
- Reduction in rejections
- Improved efficiency and productivity
- Increased output
- Reduced testing in process and in finished goods (Lavanya G, *et al.*, 2013).

Types of validation

There are four types of validation:

1) Equipment validation

a. Design Qualification
b. Installation Qualification
c. Operational Qualification
d. Performance Qualification

2) Process validation

a. Prospective validation
b. Retrospective validation
c. Concurrent validation
d. Revalidation

3) Analytical method validation

4) Cleaning validation (Lavanya G, *et al.*, 2013)

6.0.1. PARAMETERS (COMPONENTS) OF METHOD:

1. Accuracy
2. Precision
3. Linearity
4. Limit of detection
5. Limit of quantitation
6. Specificity
7. Range
8. Robustness

1) Accuracy: Accuracy is defined as the closeness of the test results to the true value.

2)Precision: Precision is defined as the measurement of closeness of agreement for multiple measurements on the same sample. The precision is expressed as the relative standard deviation. %RSD = Standard deviation/Mean ×100

3) Linearity: Linearity is the ability of analytical procedure to obtain a response that is directly proportional to concentration (amount) of analyte in the sample. Linearity is expressed as the confidence limit around the slope of the regression line.

4) Limit Of Detection (LOD): LOD is defined as lowest amount (con-centration) of analyte in a sample that can be detected or identified, not quantified. LOD is expressed as a concentration at a specified signal: noise ratio, usually 3:1. LOD = 3.3 × S/ SD

5) Limit Of Quantitation (LOQ): LOQ is defined as lowest amount (concentration) of analyte is a sample that can be quantified. For LOQ, ICH has recommended a signal: noise ratio 10:1. LOQ = 10 × S/SD

6) Specificity: Specificity is defined as the ability of an analytical meth-od to measure the analyte clearly in the presence of other components. This definition has following implications: a. Identification
b. Purity tests
c. Assay

7) Range: The range of the method is the interval between upper level and lower level of analyte that have been determined with acceptable accuracy, precision and linearity. It is determined on either a linear or nonlinear response curve and expressed in the same unit as the test results are expressed

8) Robustness: Robustness is defined as the measurement of capacity ofanalytical procedure to remain unaffected by small variations in method parameters (Vidushi Y and Meenakshi B, 2017).

ELEMENTS OF VALIDATION:

7.0.1. Design Qualification (DQ):

In this certification, GMP conformance for the design should be demonstrated. The apparatus design principles ought to ensure that the objectives of the GMP are fulfilled. It is essential to review the mechanical drawings and design elements provided by the equipment manufacturer.

7.0.2. Installation Qualification (IQ):

Installation qualification is an essential process that must be undertaken on newly constructed or modified equipment, systems, and structures. The certification for installation should encompass the subsequent critical domains. Conducting an examination of the installations of instrumentation, infrastructure, services, and equipment while collecting the provider's operational guidelines, maintenance specifications, and calibration requirements. Verification of construction materials, maintenance sources, and spare parts.

7.0.3.Operational Qualification (OQ):

This stage ought to follow IQ and encompass the subsequent: assessments developed on the basis of knowledge of the processes, systems, and instruments that establish minimum and maximum operational thresholds. An occasional usage of the term "worst case conditions" is to describe that.

7.0.4. Performance Qualification (PQ):

Following the conclusion of IQ and OQ, the subsequent qualification that must be accomplished is PQ. The PQ should comprise the subsequent: Investigations involving prototypes,substitutes, or modelled products. Developing knowledge of the facilities, systems, apparatus, and processes can yield these outcomes. Include tests that possess upper and lower bound criteria.

CONCLUSION

This research study provides an idea how to perform validation process to prove that the method is a part for its intended purpose and to assure the capabilities of the test method.
UV Visible spectroscopy plays a crucial role in analyzing the optical characteristics of polymer nanocomposites, shedding light on the relationship between matrices and nanofillers, thereby enhancing the properties of these materials for various technological applications. By providing a quantitative measure of light absorption, UV-Visible spectroscopy enables researchers to determine the identity, strength, quality, and purity of compounds, essential aspects in pharmaceutical development and manufacturing. Validation is an important procedure in the pharmaceutical industry and it is utilized to ensure that quality is built in to the processes supporting drug development and manufacture.

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