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SUBJECT NAME: QUALITY ASSURANCE

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UNIT 05 (a): CALIBRATION AND VALIDATION

Content

Calibration and Validation: Introduction, definition and general principles of calibration, qualification and validation, importance and scope of validation, types of validation, validation master plan. Calibration of pH meter, Qualification of UV-Visible spectrophotometer, General principles of Analytical method Validation.

Introduction, definition and general principles of calibration, qualification and validation, importance and scope of validation

CALIBRATION:

Calibration is a demonstration that, a particular instrument or device produces results with in specified limits by comparisons with those produced by a reference or traceable standard over an appropriate range of measurements.

Calibration is responsible for defining the accuracy of any measurement and its quality that is recorded by any instrument. The instruments must be routinely calibrated to get the desired results.

"Calibration of an instrument is the process of determining its accuracy. The process involves obtaining a reading from the instrument and measuring its variation from the reading obtained from a standard instrument."

Calibration achieves 2 main objectives-

a) It checks the accuracy of an instrument
b) It determines the traceability of the measurement.

Need for Calibration:

- When the instrument is new
- When a specified time has been elapsed
- When operating hours has been elapsed
- When instrument has shock or vibration
- Sudden change in weather
- When observations are questionable

Scope of Calibration:

- To make sure that the readings of equipment or instruments are consistent with other measurements and display the correct readings every single time.
- To determine the accuracy, precision, reliability and deviation of the measurements produced by all the instruments.

- To establish the reliability of the instrument being used and whether it can be trusted to deliver repeatable results each time.
- To identify the 'drift' of the measuring device or equipment and make them accurate.

QULIFICATION AND VALIDATION

Validation: Validation is an integral part of quality assurance; it involves the systemic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified.

A validated process is one which has been demonstrated to provide a high degree of assurance that uniform batches will be produced that meet the required specifications and has therefore been formally approved.

Validation in itself does not improve processes but confirms that the processes have been properly developed and are under control. If a process is carried out with specified conditions, it should result in intended result.

According to ISO:

"Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled."

Validation is divided in to following:

1. Process validation
2. Analytical method validation
3. Cleaning validation
4. Computer system validation

Importance of Validation

1) Process parameters and controls are determined during the validation of any process or system.
2) It helps to determine the worst case and risks that may arise during the manufacturing of the quality products.
3) Validation helps to investigate the deviations caused during the process.
4) Deep study and understanding of the system and equipment are made possible due to validation.
5) The risk of the regulatory non-compliance is minimized after the validation.
6) A validated process required less process control and the finished product testing.
7) Batch to batch variation is minimized due to the validation of processes, systems and equipment.

8) Reduces the production cost of the product.

9) Increases the production of manufacturing facility due to minimized newwork and rejection.

10) Decreases the chances of the failure of the batches.

Scope of validation

- There should be an appropriate and sufficient system including organizational structure and documentation infrastructure, sufficient personnel and financial resources to perform validation tasks in a timely manner. Management and persons responsible for quality assurance should be involved.
- Personnel with appropriate qualifications and experience should be responsible for performing validation. They should represent different departments depending on the validation work to be performed.
- There should be proper preparation and planning before validation is performed. There should be a specific programme for validation activities.
- Validation should be performed in a structured way according to the documented procedures and protocols.
- Validation should be performed for new premises, equipment, utilities and systems, and processes and procedures at periodic intervals and when major changes have been made.
- Validation should be performed in accordance with written protocols. A written report on the outcome of the validation should be produced.
- Validation should be done over a period of time, e.g. at least three consecutive batches should be validated, to demonstrate consistency. Worst case situations should be considered.
- There should be a clear distinction between in-process controls and validation. In-process tests are performed during the manufacture of each batch according to specifications and methods devised during the development phase.

Qualification:

The act of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and comply with specified requirements. The process used to demonstrate the ability to fulfill specified requirements.

Qualification is part of validation, but the individual qualification steps alone do not constitute process validation.

Before validation studies are undertaken, it is imperative to ensure that equipment used for process validation functions well and functions within specified operational range. The equipment used

must achieve the intended object. This is done by qualifying the equipment. The qualification is the prerequisite of validation.

Qualification of analytical instrumentation is essential for accurate and precise measurement of analytical data. Qualification of instruments is not a single, but continuous process and includes results from many discrete activities. For convenience, these activities have been grouped into 4 phases of qualification.

- Design Qualification (DQ)
- Installation Qualification (IQ)
- Operational Qualification (OQ)
- Performance Qualification (PQ)

1. Design Qualification (DQ)

It is documented verification that the proposed design of the facilities, systems and equipment is suitable for the intended purpose.

DQ should be performed when new equipment is being purchased, or when existing equipment is being used for a new application. DQ serves as the precursor to defining the equipment installation Qualification (IQ) and OQ protocols.

The purpose is to ensure that all the requirements for the final systems have been clearly defined at the start.

DQ check items:

- GMP and regulatory requirements
- Performance Criteria
- Facility air flow; movement flow pressure regimens
- Reliability and efficiency
- Construct ability and installation of equipment
- Maintenance and access to critical equipment and instrumentation.
- Safety and environment impact.

Documentation:

Ideally in DQ the user i.e. pharmaceutical manufacturer should prepare user requirement document (URD) or user requirement specification (URS). This document should be shared & discussed with the manufacturer of equipment and utilities.

URS: Design of premises and equipment are influenced by the user's requirements. Therefore, it is always advisable to prepare user requirement document. Based on this document, a facility can be designed by the architect and based on the design the same can be constructed by the contractor.

Generally URS can contain a large number of requirements which is helpful in designing the defined feathers of that equipment.

Once the user requirement specification is documented, agreed and approved by pharmaceutical manufacturer, the engineers can then commence the preliminary design to establish exactly what functions are required for each of the items specified in the user requirement specification.

URS is also in the form of computer software according to GMP.

2. Installation Qualification (IQ)

IQ Should be performed on new or modified facilities system and equipment.

The purpose of the installation qualification is to demonstrate that all the critical components of process equipment and support installations have been installed appropriately and are installed to the respective manufacturer's or supplier's requirements.

IQ should include the following:

a) Installation of equipment, piping, services and instrumentation checked to current engineering drawings and specifications.
b) Collection and collation of supplier's operating and working instructions;
c) Calibration of requirements;
d) Verification of materials of construction.

Installation qualification requires a formal and systemic checking of all the newly installed or modified premises, utilities and equipment.

On the receipt equipment the following may be performed. The checklist will vary with the complexity of the equipment.

a) Inspection of delivery documents

b) Inspection of packaging to detect any evidence of visible physical damage.

c) Unpacking of equipment, assistance of vendor's representative should be taken in case of major equipment.

d) Inspection of equipment, supplies, accessories etc.

e) Installation of equipment – it may include:
   - Position in logical area;
   - Pug into dedicated circuits or emergency power, if necessary;
   - Installation according to manufacturer's instructions;
   - Proper start up and general operation.

f) Priority level – whether critical equipment or one of many.

g) Electrical testing.

h) Calibration may include the following:

- Confirmation of calibrating equipment with reference to national standards;
- Calibration of measuring devices related to installed equipment;
- Identification of calibration requirements for measuring devices for future use of the equipment, if necessary.
- Calibration instructions/manual.

i) Preventive maintenance schedule & manual.

j) Draft SOPs – At this stage SOPs for cleaning, maintenance, calibration, sterilization, if necessary can be prepared.

k) Documentation – documentation will include observations on all above mentioned points.

Documentation:

IQ is a documented process by which the physical components of a system are qualified and verified to have been installed.

The purpose of IQ protocol is to document installation of the specific equipment or system and to establish that adequate information is available in writing for the installation to assure its proper functioning. The contents of IQ protocol may include the following:

- Description
- Installation qualification
- General installation qualification acceptance criteria
- Specific equipment and system acceptance criteria
- Documentation
- Attachments (e.g. drawings, purchase order, specifications, instrument list, equipment manuals etc.)

Under this element IQ program should be defined e.g.

i. Equipment list: The equipment list can be found out from purchase order and specification and the delivery challan. The items delivered can be verified. The format for IQ should provide space for recording equipment received, manufacturers' name, model number, machine/tag number, capacity and quantity.

ii. Drawings: All relevant drawings can be listed under this element.

iii. Lubricant List: If the equipment require lubrication for its operation, lubricants can be listed here.

iv. Equipment manual(s): All the manuals received with the equipment can be listed here.

v. Instrument list: The equipment might have attachment of instruments which may be critical or noncritical. In case of critical instruments calibration manuals will also be

required. A verification of instruments and calibration manuals wherever applicable will be required.

vi. Recommended spare parts list: A list of recommended spare parts should be obtained from the vendor or should be prepared in consultation with the representative of vendor.

3. Operation Qualification (OQ)

After the equipment has been installed at its final processing site and IQ has been completed, operation qualification should be performed.

The objective of the operational qualification is to define the specifications and controls for the process which will result in acceptable output. The operational qualification confirms that the process will operate within the defined specifications and control limits.

OQ should include but not limited to the following.

a) Tests that have been developed from knowledge of processes, system and equipment;
b) Tests to include a condition or conditions with lower & upper operating limits, sometimes referred to as 'worst case' conditions.

Documentation:

The objective of OQ protocol is to give adequate assurance that the equipment when operated according to the approved SOP performs within assigned limits. The OQ protocol should have atleast the following elements:

- Description;
- Operation qualification;
- General operation qualification acceptance criteria;
- Documentation;
- Attachments;
- Utilization list;
- Critical operating parameters;
- Test functions.

4. Performance Qualification (PQ)

After successful completion of IQ and OQ, the next qualification is PQ.

The purpose of PQ is to assure that equipment/system consistently perform in accordance with the design specifications. The objective of the performance qualification is to demonstrate that the process as operated under normal expected conditions will produce acceptable output.

PQ should include atleast the following:

a) Tests using production materials, qualified substitutes or simulated product that has been developed from the knowledge of process and facilities, system or equipment;

b) Tests to include a condition or conditions encompassing upper and lower limits.

The PQ protocol has the same elements as the OQ protocol. The test function in case of PQ is made on the processed material to assess that the process has resulted in the designed delivery.

PQ is described as a separate activity, it may, in some cases, be appropriate to perform it in conjunction with OQ.

The object of performance qualification is to provide rigorous testing to demonstrate the effectiveness and reproducibility of the process.

Each process should be defined and described with sufficient details and specificity so that employees understand what is required to be done.

Types of validation

The USFDA in the guidance document has defined process validation as under:

Process Validation: "Process validation is establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics."

FDA guidelines on general principles of process validation mention three options i.e prospective process validation, retrospective process validation and revalidation. In the event of significant changes in the premises, facilities, equipment or processes, revalidation has been recommended under the Indian and WHO GMP.

Following are the types of validation -

1. **Prospective validation**
2. **Retrospective validation**
3. **Concurrent validation**
4. **Revalidation**

1. Prospective validation

In prospective process validation, validation protocol is executed before process is put into commercial use means prospective validation should normally be completed prior to the distribution and sale of medicinal product. This type of process validation is usually carried out in connection with introduction of new drugs.

"Prospective validation is establishing documented evidence, prior to process implementation, that a system performs as is intended, based on pre-planned protocols."

This types of validation is performed during the product development stage.

Elements of the validation concept should be incorporated during each of the various stages of the product and process development. These steps are:

1) Formulation development;
2) Process development;
3) Development of manufacturing capability;
4) Full scale product/process development;
5) Defining experimental programs;
6) Experimental design and analysis;
7) Full scale production;

Prospective validation should be carried out only when the following operations and procedures have been completely satisfactorily:

- It should be ensured that facilities and equipment meet GMPs requirements.
- The person who will run validation batches should have understanding of the process and its requirement.
- Critical processing steps and process variables should be identified and provisional operational control limits for each critical test parameter should be provided using pilot laboratory batches.
- Detailed technical information on the product and the manufacturing process should be obtained.
- At least one qualification trial using pilot production batch should be made to show that there were no significant deviations from the expected performance of the process.

2. Retrospective validation

The WHO GMP text states that the qualification and validation should not be considered as one-off exercises and that an on-going program and should follow their first implementation.

The Indian GMP states that processes and procedures shall be established on the basis of validation study and undergo periodic revalidation to ensure that they remain capable of achieving intended results.

In following cases retrospective validation can be used:

1. Therefore retrospective process validation can be used in those cases where prospective validation has been done and several batches have been manufactured thereafter to comply with above requirements.
2. The second situation is where prospective validation was not done by the manufacturer but has been manufacturing the products for years with satisfactory results.

Before a product can be selected for retrospective validation, it must satisfy certain criteria.. The product must have relatively stable process i.e. the method of manufacture of which has not been changed for a period of time.

Although, the ideal number of batches required for retrospective study will be the number which will allow all variables to come into play, but 20 batch rule is quite popular.

3. Concurrent validation

In-process monitoring of critical processing steps can show that, manufacturing process is in the state of control. Such a validation is called concurrent validation.

Concurrent validation is used to establish documented evidence that a facility and process will perform as they are intended, based on information generated during actual use of the process.

In-process tests that can be monitored in solid and liquid dosage forms are:

- Powder-blend uniformity
- Moisture content
- Particle/granule size distribution;
- Weight variation;
- Content uniformity;
- Disintegration time/dissolution time;
- Tablet hardness;
- PH value;
- Colour/clarity;
- Viscosity/density;
- Average unit potency.

It is not necessary that all the in-process tests will be required to demonstrate that the process is in the state of control. Selection of in-process test parameters should be made on the basis of the critical processing variables to be evaluated.

4. Revalidation

As per USFDA, WHO and GMP guidelines recommend for revalidation whenever there are changes in packaging, formulation, equipment or processes which could have impact on product effectiveness or product characteristic.

Conditions that require revalidation studies are listed below:

- Changes in critical component; (i.e. change in raw materials, change in primary packaging material should be taken care of at product-packaging material compatibility studies stage;)
- Changes in facility and/or plant;
- Change in equipment;
- Significant change (increase or decrease in batch size);
- Sequential batches that fail to conform product and process specifications.

Revalidation can also be done to demonstrate that the process has been stable and has been resulting in the intended results. Periodic review and trend analysis should be carried out at specific intervals. A decision not to perform revalidation should be fully justified.

Validation Master Plan (VMP)

The WHO GMP states that the Key elements of a qualification and validation program of a company should be clearly defined and documented in a validation master plan (VMP). These guidelines further state the VMP should contain data on at least the following.

- Validation policy;
- Organization structure of validation activities;
- Summary of facilities, systems, equipment and processes to be validated;
- Documentation format;
- Planning and scheduling;
- Change control;
- Reference to existing documents.

1) Validation policy:

The validation policy of the manufacturer should at least aim at the implementation of GMP requirement of the country where the plant is located and/or regional/international GMP. If the manufacturer is exporting his products, he should take into account the GMP requirement of that country.

The validation policy should reflect the scope of validation activities.

2) Organization structure of validation activities:

Under this element, responsibilities, at least for the following should be defined as to who would be responsible for:

- VMP;
- Validation protocols;
- Validation work;
- Document preparation and control
- Report;
- Approval of validation protocol and reports at various stages of validation;
- Training needs if any;

3) Summary of facilities, systems, equipment and processes to be validated:

This section suggests lists of systems (e.g. AHU, water system), processes (e.g. preblending, drying, and sterilization), products (e.g. ampicillin, injection, dexamethasone tablets) should be prepared, which should then be taken up for validation work as per planned schedule.

It is advisable to compile them in matrix form. The matrix may provide:

- List of items;
- Extent of validation required (i.e. IQ, OQ and or PQ);
- Validation option (i.e. prospective, concurrent or retrospective)
- Revalidation frequency;
- General statement on key acceptance criteria.

4) Documentation format:

Under this element, formats to be used to be described or alternatively a reference can be made.

5) Planning and scheduling:

It is almost impossible to validate all the products of a company at a time because of resource limitations. It is obvious that the products with higher profitability or the products which account for major sales should be given priority.

Based on priority a tentative schedule for validation activities can be prepared under this element.

6) Change control:

Under this element pharmaceutical manufacturers' commitment to control critical changes to materials, facilities, equipment or processes (including analytical methods) should be included.

7) Reference to existing documents:

As the title of this element suggests reference to existing documents which may be required in validation work should be made in the VMP. Apart from this, list of relevant SOPs should be presented in this document.

VMP is a summary document and therefore, it should be brief, concise and clear. If an information has already appeared in it, it should not be repeated, a reference should be made. Reference to the existing document like policy documents, SOPs etc. should be made.

Importance of VMP document:

- VMP helps in several ways. It helps management to understand its necessity and its implication in respect of time, manpower and money.
- It helps validation team members to know their jobs and responsibilities.
- It helps GMP auditors to understand pharmaceutical manufacturers' approach to validation and organization of validation activities.

Calibration of pH meter & Qualification of UV-visible Spectrophotometer

Calibration of pH meter:

- Before starting the calibration make sure that the correct measurement mode is selected.
- Wash the electrode thoroughly with de-ionized water or a rise solution. Do not wipe the electrode; this causes a build-up of electrostatic charge on the glass surface.
- Maintain the temperature of the buffers to 25 °C ± 2 °C, unless otherwise specified in the individual monograph and dip the electrode along with the temperature sensor into the buffers.
- Perform the 3 point calibration using standard buffers of pH 4.0, pH 7.0 and pH 9.2.
- Deep the electrode into the calibration buffer. The end of the electrode must completely immersed into the sample. Stir the electrode gently to create a homogenous sample.
- Press CAL/MENS key to enter pH calibration mode.
- Wait for the measured pH value to stabilize in pH 7.0 buffer at least 1 or 2 minutes, the meter should automatically recognize the buffer and display its value. If the meter does not automatically recognize the buffer, set the value of its pH by using knob. Then change knob at standby mode.
- Rinse the pH electrode first with deionized water and then in the pH 4.01 buffer rinse beaker. Make sure to rinse the electrode with deionized water over a waste beaker to prevent contamination of the buffers. The electrode should never be rinsed in the same buffer beaker that will be used for calibration.
- Place the electrode into the pH 4.0 buffer calibration beaker, so the electrode tip and junction are fully immersed in the buffer, and stir the buffer at a moderate, uniform rate.
- Wait for the measured pH value to stabilize in pH 4.0 buffer at least 1 or 2 minutes, the meter should automatically recognize the buffer and display its value. If the meter does not automatically recognize the buffer, set the value of its pH by using knob. Keep knob at standby knob.
- Follow same procedure at pH 9.2 and set the value.
- Rinse the pH electrode with deionized water and store the electrode in pH electrode storage solution. Use this electrode for measurement of solution of any pH value which will give correct value.

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Qualification of UV Visible Spectrophotometer:

UV-Visible spectroscopy is concerned with ultra violet and visible regions which ranges from 200-780 nm.

DESIGN QUALIFICATIONS:

DQ should be performed before the purchase of any new model of UV Spectrophotometer.

a. **Examples of functional and operational specifications:**
   Optics: Double beam/Single beam
   Measurable range: e.g. 190-1100 nm
   Wavelength Accuracy: e.g. ± 0.5 nm or better

b. **Criteria for selection of the vendor:**
   Warranty and maintenance support
   Discount
   Cost of annual Maintenance Contract (AMC) after expiry of standard warranty

INSTALLATION QUALIFICATION:

While the UV instrument was shipped after the precise adjustment and inspection at the factory, it is recommended to install according to the following procedures so as to provide its optimum performance and to meet the user's demands.

- Checklist for IQ:

– Delivered equipment is according to DQ?
– All parts received?
– All accessories undamaged?
– The serial numbers of software, hardware and all accessories are correct and according to DQ?
– What are the tests required for calibration, maintenance and performance tests by the user and supplier/manufacturer?
– Details of calibration intervals and services provided by suppliers/manufacturer.
– All necessary contacts for maintenance/ calibration/ any other service e.g. requirement of any new spare parts provided by supplier or manufacturer?
– Availability of required utilities (e.g. electricity supply, space etc) are available in the premises?
– Working environment given in specifications matching the environment provided (e.g. air conditioning)
– After the successful check, all parts assembled together and installed, software also installed in UV spectrophotometer and initial checks ensures installation.

ACCEPTANCE PROCEDURES:

ITEM TO BE CHECKED	SPECIFICATION

Appearance	No defect
Number of parts	No missing parts
ROM check	Latest version
Linearity of Absorbance	Bent: ±0.002Abs (Shock noise: ±0.004 Abs)
Noise level	Noise width: ±0.002Abs (Shock noise: ±0.004 Abs)
Accuracy of wavelength	±0.5nm
Repeatability of wavelength	±0.1nm

OPERATIONAL QUALIFICATION:

Wavelength accuracy

- Deviations in wavelength may cause significant error in analysis by UV Spectrophotometer.
- Thus, this is essential to check the accuracy and reproducibility of wavelength by UV spectrophotometer.
- This test is performed to understand deviation of the wavelength reading from the known wavelength of the band.

There are many methods to estimate the wavelength accuracy e.g. wavelength standards such as a deuterium lamp, mercury vapor lamp and holmium oxide filter, holmium oxide solution and Didymium filter.

Acceptance:

± 1 nm in UV range (200-380 nm) and

± 2 nm in visible range (380-800 nm)

Three repeated scan of the same peak should be with in ±0.5 nm

Stray light:

Stray light is any light reaching the detector without passing through the sample is outside the band width of the wavelength selected and may decrease the absorbance and reduces the linear range of the instrument.

This may be due to poor design or faulty monochromator or may be because of operator, usually not problem in new instrument but increases with age of optics and its degradation.

Stray light will cause apparent negative deviations from Beer's law and thus effects the quantitative analysis of analyte.

The test is performed by using some selected material for different wavelengths.

Spectral range (nm)	**Liquid or solution**

190-205	Aqueous potassium chloride (12 g/L)
210-295	Aqueous sodium iodide or potassium iodide (10 g/L)
250-320	Acetone
300-385	Aqueous sodium nitrite (50 g/L)

Acceptance: the transmittance of the solution in a 1 cm cell should be less than 0.01 or the absorbance value should be greater than 2.

Resolution power:

The resolution of the UV-VIS spectrometer is related to its spectral band width. Insufficient resolution in any spectrophotometer may loose some features of fine spectrum or unable to show difference of absorbance values between two close wavelengths.

The smaller the band width the finer the resolution. Resolution of the spectrophotometer is determined by using the following procedure.

Measure the ratio of the absorbance of a 0.020 % (v/v) solution of toluene in hexane (UV grade) at the maximum and minimum at about 269 and 266 nm, respectively, using hexane as the reference.

Acceptance: The ratio of the absorbance at 269 nm and absorbance at 266 nm should be greater than 1.5.

Noise:

Noise originating from light source ad electronic systems in UV spectrophotometer and effects the measurement of absorbance in both lower and higher wavelengths.

Inaccurate absorbance at low wavelength is related to noise originating from random fluctuations of photon of light and electronic components at higher wavelength.

Noise also effects the prescription and instrument will respond only at particular concentration of analyte or in other words making it insensitive with increase in limit of detection value.

To estimate the noise of spectrophotometer air is scanned (no sample) for 10 minutes in absorption mode and Root Mean Square (RMS) is calculated recorded at 500 nm.

Acceptance: The RMS noise should be less than 0.001 AU

Baseline flatness

The flat baseline test demonstrates that the ability of the instrument to normalize the light intensity measurement and the spectral output at different wavelength throughout the spectral range.

Baseline is used to nullify the effect of environment in measurement by removing background noise to get true absorption profile of analyte.

In double beam UV spectrophotometer baseline correction is usually performed by keeping solvent in one cuvette and test solution in other. This will automatically correct the baseline and subtract the background noise by solvent.

The test is performed by scanning air in the absorbance mode in UV region and highest and lowest deflections in the absorbance unit are recorded.

Acceptance: The measurement is typically less than 0.01 AU

Stability:

The lamp intensity is a function of the lamp age, temperature fluctuation and wavelength of the measurement. These changes can lead to errors in the value of the measurements, over an extended period of time, which causes instability of response by UV spectrophotometer resulting error in reading.

The error caused by this factor may be positive or negative (Positive: more than actual observed; Negative: less than actual response observed.)

For checking stability air is for 60 minutes in absorbance mode at particular wavelength (generally 340 nm) and deflections in the absorbance are recorded.

- **Acceptance:** The deflection is less than 0.002 AU/ hr

Photometric accuracy:

Photometric accuracy is determined by comparing the difference between the measured absorbance of the reference material and the established value.

- **Acceptance:** Six replicate measurements of the 0.006% w/v of the potassium dichromate solution at 235, 257, 313 and 350 nm should be less than 0.5% RSD.

Linearity:

Ideally, the absorbance should increase with increase in the concentration of analyte in the solution.

For this various dilutions of potassium dichromate are prepared ranging from 20, 40, 60, 80, and 100 mg/L in 0.005 M sulfuric acid and scanned by keeping 0.005 M sulphuric acid in reference cuvette.

The calibration curve is prepared by measuring absorbance of each solution and correlation coefficient is calculated.

The acidic solution of potassium dichromate gives peaks at 235, 257, 313, and 350 nm.

The accuracy of the quantification of the sample depends on the precision and linearity of the measurements.

- **Acceptance:** Correlation coefficient $r > 0.999$

PERFORMANCE QUALIFICATION:

The purpose of PQ is to determine that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement and to ensure that it will function properly over extended periods of time.

The purpose of the PQ is to provide evidence that instrument is fit for its routine use.

Performance of instrument may change gradually over time because of normal wear of parts, failure or change of its components.

Generally, performance of spectrophotometer measurements are performed under identical conditions for the test specimen and the reference substance (e.g. USP Reference Standard)

The initial PQ tests may include comparison of results obtained by instrument with other qualified instruments of the laboratory under identical conditions and samples.

Sufficient system suitability tests are performed for the clear understanding of the threshold outside which instrument performance is not appropriate.

For example absorption linearity should be established and coefficient of variance (CV) is calculated before routine analysis.

The general acceptable value of CV is less than or equal to 1.

Analytical Method Validation

All instruments shall be calibrated and testing procedures validated before these are adopted for routine testing. Periodic calibration of instruments and validation of procedures shall be carried out.

Validation studies shall be an essential part of good manufacturing practices and shall be conducted as per the predefined protocols. These shall include validation of processing, testing and cleaning procedures.

Validation of analytical methods is of critical importance as it is the cornerstone of process validation. If there is no way to judge whether a process has performed the way it was intended, no validation can be done.

Generally USFDA and WHO have laid down guidelines on validation of analytical procedures used in examination of pharmaceutical materials. According to WHO guidelines, purpose of analytical validation is to ensure that a selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose.

USP parameters of method validation:

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

ICH parameters of method validation:

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

Accuracy:

Accuracy is the closeness of a measured value to its true value. Usually it refers to the difference between the mean of the set-of-results and the value accepted as true value for the quantity measured.

Generally results of analysis of unknown are compared with the results obtained from the analysis of standards or reference substances (RS).

In the assay of a drug formulation, accuracy may be determined by application of the analytical method to synthetic mixtures of the API and excipients used where known amount of API have been added within the range of the method.

Usually six samples of drug in matrix spanning 80 to 120 percent or even 50 to 150 percent of the expected content are prepared and each of them is assayed. The acceptance criterion in the accuracy test is expressed in terms of standard deviation of the method.

Precision:

Precision is reproducibility of measurement within a set of independent replicate measurements of the same property. It is the degree of agreement among individual test results.

Generally six replicates of a representative composite sample containing 18-24 times the amount of the drug needed for one assay are taken for determination of the precision. The measure of system precision is calculated from the data obtained on repeatedly analyzing aliquots of a single standard solution.

Specificity:
Specificity is an important characteristic. If analysis of one component interferes with other components of mixture, the analytical method is nonspecific for that component. When the method is specific, concentration of the component can be measured completely regardless of presence of other compounds in the sample.

In assay methods, demonstration of specificity requires that the procedure is not affected by the presence of impurities and excipients. This can be demonstrated by spiking the drug substance or drug formulation with appropriate levels of impurities or excipients. The results should not be affected by these materials for demonstration of specificity.

Limit of Detection:
The limit of detection is the lowest level of analyte which can be detected. However, it is not necessary that it can be determined quantitatively, under the stated conditions of the experiment. The detection limit is, therefore, a characteristic limit test.

The detection limit is usually expressed in terms of percentage, parts per million (ppm) in the sample.

In case of non-instrumental methods detection limit can be determined by analyzing samples of different known concentration levels (very low concentration) and by establishing the minimum level which can be readily detected.

The same approach could be used for instrumental methods also. However, notice has to be taken of the background response.

Limit of Quantitation:
The limit of quantitation is the lowest concentration of analyte in a sample that may be determined quantitatively with acceptable accuracy and precision under the stated conditions of experiment.

The quantitation limit is expressed in terms of percentage, parts per million (ppm) in the sample.

For determination of quantitation limit, approach is the same as in case of detection limit. It is usually determined by analyzing samples with known concentrations of analyte and by establishing the minimum concentration which can be determined with acceptable accuracy and precision.

In case of instrumental methods, the ICH approach is to compare measured signals from samples of known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can be quantitatively determined reliably is established.

Linearity and Range:
Linearity of an analytical method can be defined as its ability to produce results which are directly proportional to the concentration of analyte in the sample.

Range of analytical method can be defined as an expression of the lowest and highest levels of anlyte which have been shown to be determinable with acceptable accuracy, precision and linearity. Usually the range is expressed in the same units as the test results (e.g. percentage, ppm).

A minimum of five standard solutions should be used and spanned 80 to 120 percent or even 50 to 150 percent of the expected working concentration range. Concentration versus response can then be plotted.

Ruggedness:

The other term which is used for this characteristic is robustness. Ruggedness or robustness of an analytical method is the ability of the procedure to yield results of acceptable accuracy, and precision under a variety of conditions.

In other words, robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. These includes:

- Source and age of reagents,
- Concentration and stability of solution;
- Heating rate;
- Column temperature;
- Humidity;
- Voltage fluctuation;
- Variation of columns;
- Variation of analysts, etc.

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots in different laboratories, by different analysis using different operational and environmental conditions. However, the difference in operational and environmental conditions.

System Suitability:

System suitability tests indicate how good and reliable is the performance of a given analytical system on a given day. System suitability testing has been recommended by USP in HPLC procedures.

In system suitability tests, system's precision measurement and system's powers of resolution measurement to check the performance of the analytical system on the given day are checked.

System suitability testing is different than method validation testing. Generally method validation studies are initiated at the method development stage and by these studies it is demonstrated that method is scientifically sound and technically suitable for a particular drug. Thus validation is done generally once while system suitability testing should be done on continuing basis.

Measurement of system precision is done by employing replicate aliquots of the same solution. Signal responses such as peak height, peak area and response ratio derived from these aliquots are determined. From this data relative standard deviation is calculated to indicate system precision as compared to historical data.