Name of Unit:	Calibration and Validation as Per ICH And USFDA Guidelines Calibration of Some Instruments
Course/Subject Name	Advanced Instrumentation Techniques
Course/Subject Code	BP811ET
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Learning Outcome of Module-III

LO	Learning Outcome (LO)	Course Outcome Code
LO1	To study the Calibration and Validation as Per ICH And USFDA Guidelines	BP811.4
LO2	To study the Calibration of Instruments	BP811.4

MODULE CONTENT TABLE

Topic
• Types of Analytical Procedures to be Validated
• Validation of Analytical Procedures: Methodology
• Calibration of Weighing balance
• Calibration of UV-Visible spectrophotometer
• Calibration of HPLC
• Calibration of Gas Chromatograph

TYPES OF ANALYTICAL PROCEDURES TO BE VALIDATED

The validation of analytical procedures is directed to the four mostcommon types of analytical procedures:

- Identification tests.
- Quantitative tests for impurities' content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures are equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below.

- Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc.) to that of a reference standard.
- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.
- Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

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

  - Accuracy
  - Precision
  - Repeatability

- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore, revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changesmay require validation as well.

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by othersupporting 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 substancestest, heavy metals,

Assay (content or potency):

to provide an exact result which allows an accurate statement on thecontent or potency of the analyte in a sample.

3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

4. PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements 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, standarddeviation or coefficient of variation of a series of measurements.

a. Repeatability

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

b. Intermediate precision

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

c. Reproducibility

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

5. DETECTION LIMIT

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

6. QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

7. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

8. RANGE

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

9. 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.

VALIDATION OF ANALYTICAL PROCEDURES: METHODOLOGY

This document is complementary to the parent document, which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity), the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data that should be presented ina new drug application.

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.

Approaches other than those set forth in this guidance may be applicable and acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product. However, it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document. Well-characterized reference materials, with documented purity, should be used throughout thevalidation study. The degree of purity necessary depends on the intended use.

In accordance with the parent document, and for the sake of clarity, this document considers the various validation characteristics in distinct sections. The arrangement of these sections reflectsthe process by which an analytical procedure may be developed and evaluated.

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: Specificity, linearity, range, accuracy, and precision.

Part II of this guidance was developed within the Expert Working Group (Quality) of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, November 1996. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions. This guidance is applicable to drug and biological products.

I. SPECIFICITY

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities, and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

A. Identification

Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sensible scientific judgment with a consideration of the interferences that could occur.

B. Assay and Impurity Test(s)

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity, and individual components should be appropriately labeled. Similar considerations should be given to other separation techniques. Critical separations in chromatography should be investigated at an appropriate level. For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other. In cases where a nonspecific assay is used, other supporting analytical

procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used.

The approach is similar for both assay and impurity tests:

1. Impurities are available

For the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples). For the impurity test, the discrimination may be established by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or fromother components in the sample matrix.

2. Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure, e.g., pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: Light, heat, humidity, acid/base hydrolysis, and oxidation.

- For the assay, the two results should be compared.
- For the impurity tests, the impurity profiles should be compared.

Peak purity tests may be useful to show that the analyte chromatographic peak is not attributableto more than one component (e.g., diode array, mass spectrometry).

I. LINEARITY

A linear relationship should be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method

of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample.

For the establishment of linearity, a minimum of five concentrations is recommended. Other approaches should be justified.

II. RANGE

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy, and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analyticalprocedure.

The following minimum specified ranges should be considered.

- For the assay of a drug substance or a finished (drug) product: Normally from 80 to 120percent of the test concentration;
- For content uniformity: Covering a minimum of 70 to 130 percent of the test concentration, unless a wider, more appropriate range, based on the nature of the dosageform (e.g., metered dose inhalers), is justified;
- For dissolution testing: +/-20 percent over the specified range; e.g., if the specifications for a controlled released product cover a region from 20 percent, after 1 hour, up to 90 percent, after 24 hours, the validated range would be 0-110 percent of the label claim;
- For the determination of an impurity: From the reporting level of an impurity to 120 percent of the specification;
- For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate

withthe level at which the impurities must be controlled.

Note: For validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit;

- If assay and purity are performed together as one test and only a 100 percent standard is used, linearity should cover the range from the reporting level of the impurities to 120 percent of the assay specification.

III. ACCURACY

Accuracy should be established across the specified range of the analytical procedure.

A. Assay

1. *Drug substance*

Several methods of determining accuracy are available.

(a) Application of an analytical procedure to an analyte of known purity (e.g., referencematerial);

(b) Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined. Accuracy may be inferred once precision, linearity, and specificity have been established.

2. *Drug product*

Several methods for determining accuracy are available.

(a) Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analyzed have beenadded;

(b) In cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well-characterized procedure, the accuracyof which is stated and/or defined;

(c) Accuracy may be inferred once precision, linearity, and specificity have been established.

B. Impurities (Quantitation)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities. In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure The response factor of the drug substance can be used. It should be clear how the individual or total impurities are to be determined, e.g., weight/weight or area

percent, in all cases with respect to the major analyte.

C. Recommended Data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with theconfidence intervals.

IV. PRECISION

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

Repeatability

Repeatability should be assessed using:

(1) A minimum of 9 determinations covering the specified range for the procedure (e.g., 3concentrations/3 replicates each); or

(2) A minimum of 6 determinations at 100 percent of the test concentration.

Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to study these effects individually. Theuse of an experimental design (matrix) is encouraged.

Reproducibility

Reproducibility is assessed by means of an interlaboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

Recommended Data

The standard deviation, relative standard deviation (coefficient of variation), and confidence interval should be reported for each type of precision investigated.

V. DETECTION LIMIT

Several approaches for determining the detection limit are possible, depending on whether the procedure is non instrumental or instrumental. Approaches other than those listed below may beacceptable.

Based on Visual Evaluation

Visual evaluation may be used for non instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

Based on the Standard Deviation of the Response and the Slope

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may becarried out in a variety of ways, for example:

1. Based on the standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

2. Based on the calibration curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines may be used as the standard deviation.

Recommended Data

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal-to-noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification. In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

VI. QUANTITATION LIMIT

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is noninstrumental or instrumental. Approaches other than those listed below

may be acceptable.

Based on Visual Evaluation

Visual evaluation may be used for noninstrumental methods but may also be used with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can bequantified with acceptable accuracy and precision.

Based on Signal-to-Noise

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typicalsignal-to-noise ratio is 10:1.

Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

1. Based on standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

2. Based on the calibration curve

A specific calibration curve should be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines may be used as the standard deviation. The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

VIII ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. 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 solutions
- Extraction time

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

In the case of gas-chromatography, examples of typical variations are:

- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

IX SYSTEM SUITABILITY TESTING

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

WEIGHING BALANCE CALIBRATION

In the Pharmaceutical industry, calibration of weighing Balance is done on a monthly basis to check the accuracy of Balance. While performing Weighing balance calibration, check the following parameter:

1. Accuracy
2. Reproducibiliy
3. Eccentricy

Accuracy:

Verify the balances for accuracy with the minimum weight (least count×100),5%, 20%, 50%,and 90% capacity of respective balances.

Record the displayed weight in respective monthly calibration formats.

Tolerance limit:

The variation, if any, should be ± least count of the balance or ± 0.2 % of the **certified value of standard weight** used, whichever is higher, and for *analytical Balance*, the variation should be ± least count of the Balance or ± 0.1 % of the **certificate value of standard weight** used whichever is higher.

Reproducibility:

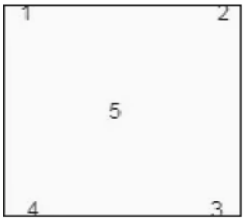
Check the Reproducibility by using the minimum *standard weight of balance capacity*. Place the weight in the middle of the weighing pen and observe the displayed value. Repeat the procedure **nine times** for the standard weight and record the reading.

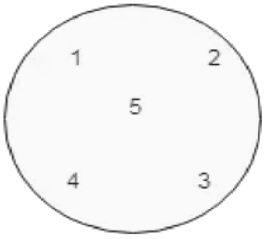
Calculate SD and % RSD for both the standard weight by the following formula.

% RSD= SD*100/ X

Acceptance criteria: %RSD should not be more than 2.0%.

Record the observations in monthly calibration record as per “monthly calibration record ofweighing balance.”





Eccentricity:

Take minimum weight and keep at on specified position as shown below calculate SD and %RSD by the following formula as specified above

Acceptance criteria:

% RSD not more than 2.0 %. Record the details of calibration in labels with Marker Pen. Write the identification number of the standard weight used during verification and calibration in the designated place of verification and calibration records. The calibration record shall be verified by a quality assurance by putting **a "reviewed by quality assurance"** stamp along with initials and date. Calibration of analytical Balance in the quality control department shallbe done as per their respective SOP.

Calibration of balances by external agency:

Internal calibration (software calibration) of weighing balances shall be done by external agencies. Calibration certificate received from an external agency shall be reviewed comprising the detail of balance and calibration summary.

Action to be taken if out of calibration:

If the Balance is out of calibration, refer to sop **"handling of out of calibration"** and rise deviation as per SOP "handling of deviation," and fix the label "out of calibration" as per SOP (status labeling) and inform to Department Head of Investigation. Raise maintenance memo and inform the engineering department for rectification action. If required, take a balance of configuration from another section and department for carrying out the weighing activity. In such situations, carry out Daily verification of transferred Balance and used for Weighing. If the parameter of Daily verification does not meet the specification, monthly calibration should be done. Once rectification of the Balance is done, calibrate the Balance and record the same in respective calibration records. the Balance shall be released for further use only on satisfactory closure of the deviation with a detailed result of Investigation

Accuracy:

Standard Mass To Test Accuracy	1st wt. value	2nd wt. value	3rd wt. value	4th wt. value	5th wt. value
Tolerance limit					
Observed wet value					
weight I.D.					

Reproducibility:

Minimum weight mass=						Weight I.D. No.=					
Sr.No.	1	2	3	4	5	6	7	8	9	10	Mean (x)
(xi)											
Sr.No.	1	2	3	4	5	6	7	8	9	10	Total=∑(xi-x)2
=∑(xi-x)2											
SD= N= Is the no. of values						%RSD= SD x 100/ Means(x) (Limit: NMT 2%)					

Eccentricity:

Minimum weight mass=			Weight I.D. No.=			
Position	Upper left (1)	Upper right (2)	Bottom left (3)	Bottom right (4)	Center (5)	Mean (x)
Observed wt. (xi)						
(xi-x)2						Total=∑(xi-x)2
SD= N= Is the no. of values			%RSD= SD x 100/ Means(x) (Limit: NMT 2%)			

CALIBRATION OF UV-VISIBLE SPECTROPHOTOMETER

In Pharmaceutical UV-Visible spectrophotometer is widely used for analytical purposes in the Quality control department. So to maintain its accuracy and working conditions, calibration ofUV-Visible spectrophotometer is performed timely.

Calibration of UV-Visible spectrophotometer by parts

Check the following During calibration of UV-Visible spectrophotometer

- Absorption cells
- Control of absorbance
- Photo metric linearity
- Limit of stray light
- Resolution power
- Control of Wavelengths

Absorption cells

Fill the cuvette with milli-Q water at 240 nm, and absorption is not greater than 0.093 of the

individual cuvette. Rotate both cuvettes (180°) one by one.

Acceptance criteria: On rotation of cuvette should give absorbance difference not more than0.005.

Control of absorbance:

For UV region:

Prepared 0.005M sulfuric acid:

Weigh accurately 60.0 mg of potassium dichromate, which previously dried to constant weight at 130°C in 1000.0ml and dissolved in 0.005 M sulphuric acid. Measure the absorbance at 235nm, 257 nm, 313nm, and 350 nm using 0.005 M H2SO4 asblank.

For visible region:

Weight about 60.0mg of potassium dichromate, which previously dried to constant weight at 130°C and transferred to 100.0 ml volumetric flask, dissolved in 0.005 M sulphuric acid. Measure the absorbance at 430 nm using 0.005M sulphuric acid as blank.

Photometric Linearity:

Acceptance criteria

Wavelengths(nm)	Specificabs.	Max. Tolerance
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2

Wavelengths(nm)	Specificabs.	Max.Tolerance
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0

Photometric Linearity:

Weight 50 mg Potassium dichromate and dissolved in 0.005 M Sulphuric acid in a 50 ml volumetric flask. Further, dilute the above solution in the following way

1 ml ≥ 100 ml

2 ml ≥ 100 ml

3 ml ≥ 100 ml

4 ml ≥ 100 ml

5 ml ≥ 100 ml

Take consecutive three readings of each dilution at wavelength 235,257, 313, and 350 nm.

Acceptance criteria:

At each wavelength mean value ≥of 0.999

Limit of stray light

Record absorbance of 1.2% solution of Potassium chloride in water at 200nm using water as blank

Acceptance criteria: Absorbance should be greater than 2.0

Resolution power

Record the spectrum of 0.02% v/v solution of toluene in hexane at 250nm to 300 nm.

Acceptance criteria

The absorbance maximum at 269 nm to the minimum at about 266 nm should not be less than 1.5.

Control of Wavelengths

Dissolved 1.0 g of holmium oxide in .1.4M perchloric acid to 25ml to prepare 4.0% w/v solution of holmium oxide. Record spectrum of holmium perchlorate solution from 200 nm to 600 nm using 1.4 Mperchloric acid as a reference solution.

Acceptance criteria

Wavelength	Maxi. Tolerance
241.14	240.15 to 242.15
287.15	286.15 to 288.15
361.50	360.50 to 362.50
536.30	533.30 to 539.30

Out of Calibration

Suppose the instrument or any parts of it are found out of calibration. Immediately Stopped any activity and Labeled the instruments "Out of Calibration." Follow the SOP for "Handling ofout of calibration."

CALIBRATION OF HPLC

Definition of Calibration: ICH

The demonstration that a particular instrument or device produces results within specified limits by comparison with those produced by a reference or traceable standard over an appropriate range of measurements.

Calibration of HPLC: Various Calibration parameters are:

- Flow rate accuracy

- Injector accuracy
- System Precision
- Wavelength accuracy
- Detector linearity
- Injector linearity
- Gradient Performance Check
- Column oven temperature accuracy

Flow Rate Accuracy:

1. Prime all the solvent lines with Milli Q water.
2. Set the flow rate to 0.500 ml/m.
3. Wait for about 15 m to stabilize the system and ensure that the pressure is stable.
4. Insert the outlet tubing into a 10 ml volumetric flask and start the stop watch simultaneously.
5. Stop the stopwatch when the lower meniscus reaches the 10 ml mark on the flask.
6. Record the elapsed time.
7. Similarly check the flow for 1.0 ml/mand 2.0 ml/m.

Acceptance criteria: The time taken to collect the water should be with in ± 2.0% of the actual value.

Table 2: Flow Rate Accuracy

Set Flow	Actual time required to collect up to the mark in m	Acceptance criteria (in m)
0.5 ml/m	20.0	19.6 – 20.4
1.0 ml/m	10.0	9.8 – 10.2
2.0 ml/m	5.0	4.9 – 5.1

Injector Accuracy:

1. Connect the pump and detector inletwith union.
2. Prepare mobile phase consisting of amixture of water and Methanol (70:30 v/v)
3. Set a flow rate of 0.5 ml/m and a run time of 1 m.
4. Set the column temperature at 25± 2°C.
5. Fill a standard HPLC vial to 2/3rd with Milli-Q water. Seal the vial properly with a cap.

6. Weigh the vial and record the weightas W_1 grams.

7. Place the vial in the chromatographic system and perform 6 injections of 50µl volume from this vial.

8. Weigh the vial again and note theweigh after the injections as W_2 grams. Calculate the mean volume injected perinjection as follows:

Mean injected volume (µl) = $(W_1 - W_2)$ ×100/6

Acceptance criteria: The mean injectedvolume should be 50.0±1.0 µl.

System Precision:

Standard Preparation: Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase. Transfer 10ml of this solution into a 100ml volumetric flask and dilute to the volume with mobile phase.

Procedure: Inject blank, followed by standard preparation in 6 replicates. Note down the areas and retention times.

Now calculate the %RSD of retention time and peak areas for 6 replicates injections.

Acceptance criteria: The %RSD of retention time & peak area should be <1.0%.

Wavelength Accuracy:

Procedure: Create and instrument method with a wavelength in nm and inject blank, followed by Standard preparation and note down the height or absorbance.

Acceptance criteria: The maximum absorbance should be ±2nm.

PDA Detector Accuracy:

Select 3D mode and set the wavelength range as 200-400nm.Inject 20 µl of standard preparation once into the chromatographic system. Extract and record the chromatograms at wavelengths of 202 to 208nm with an interval of 1nm and at 269 to 275 nm with an interval of 1nm.Note down the height or absorbance.

Acceptance criteria: The maximum absorbance should be at 205±2nm and 272±2nm.

Detector Linearity:

Standard Preparation: Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase.

- **Detector linearity solution 1(0.06 mg/ml):** Transfer 10ml of Standard Preparation into a 100ml volumetric flask and dilute to the volume with mobile phase
- **Detector linearity solution 2(0.048 mg/ml):** Transfer 8ml of Standard Preparation into a 100ml volumetric flask and dilute to the volume with mobile phase.

❖ **Detector linearity solution 3(0.03 mg/ml):** Transfer 5ml of Standard Preparation into a 100ml volumetric flask and dilute to the volume with mobile phase.

Acceptance criteria in percentage composition (Height (%) should be with in±1.0% of the set composition.

Column Oven Temperature Accuracy:

It is evaluated with a calibrated digital thermometer at 30°Cand 60°C .Place the thermometer probe in the column oven and set the column oven temperature at 30°C.Wait till the temperature stabilizes.

Record the temperature displayed on the thermometer. Similarly performs the column oven temperature accuracy test at 60°C.

Acceptance criteria: The resulting oven temperature from the thermometer display shouldbe within ±2°C of the settemperature.

NOTE: *For oven Temperature Accuracy, Chromatographic conditions and mobile phase refer to system precision test.*

Chromatographic Conditions for System Precision

Column Flow rateDetection	C_{18},150mm×4.6 mm,5µm
Column oven temperature	25°C ± 2°C
Mobile phase	Water: Methanol (70:30v/v)
Column Flow rate	1.0 ml/m
Detection	UV at 272 nm
injection volume	20 µl
Run time	15 m

Injector Accuracy:

1. Connect the pump and detector inletwith union.
2. Prepare mobile phase consisting of amixture of water and Methanol (70:30 v/v)
3. Set a flow rate of 0.5 ml/m and a run time of 1 m.
4. Set the column temperature at 25± 2°C.
5. Fill a standard HPLC vial to 2/3rd with Milli-Q water. Seal the vial properly with a cap.
6. Weigh the vial and record the weightas W_1 grams.
7. Place the vial in the chromatographic system and perform 6 injections of 50µl volume from this vial.

8. Weigh the vial again and note theweigh after the injections as W_2 grams. Calculate the mean volume injected perinjection as follows:

Mean injected volume (µl) = $(W_1 - W_2) \times 100/6$

Acceptance criteria: The mean injectedvolume should be 50.0±1.0 µl.

System Precision:

Standard Preparation: Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase. Transfer 10ml of this solution into a 100ml volumetric flask and dilute to the volume with mobile phase.

Procedure: Inject blank, followed by standard preparation in 6 replicates. Note down the areas and retention times.

Now calculate the %RSD of retention time and peak areas for 6 replicates injections.

Acceptance criteria: The %RSD of retention time & peak area should be <1.0%.

Wavelength Accuracy:

Procedure: Create and instrument method with a wavelength in nm and inject blank, followed by Standard preparation and note down the height or absorbance.

Acceptance criteria: The maximum absorbance should be ±2nm.

PDA Detector Accuracy:

Select 3D mode and set the wavelength range as 200-400nm.Inject 20 µl of standard preparation once into the chromatographic system. Extract and record the chromatograms at wavelengths of 202 to 208nm with an interval of 1nm and at 269 to 275 nm with an interval of 1nm.Note down the height or absorbance.

Acceptance criteria: The maximum absorbance should be at 205±2nm and 272±2nm.

CALIBRATION OF GAS CHROMATOGRAPH

Various Calibration parameters are:

- Flow rate accuracy
- Column oven temperature accuracy
- System precision
- System precision for head space autosampler
- Detector linearity

- Detector noise and drift test

Flow rate accuracy:

1. Connect the digital flow meter to thedetector outlet port.
2. Set the carrier gas (Helium) flow andwait till it reaches the set flow.
3. Note the observed flow in replicate.
4. Repeat the procedure for other carriergases such as Hydrogen and Air.
5. Record the result in GC calibrationprotocol.

Acceptance criteria: The flow rate ofcarrier gas should be ±10% of set flow.

Flow Rate Accuracy

Carrier gas	ml/min.
Helium	125
Hydrogen	40
Air	400

Column Oven Temperature Accuracy:

It is evaluated with a calibrated digital thermometer at 30°Cand 60°C .Place the thermometer probe in the column oven and set the column oven temperature at 30°C.Wait till the temperature stabilizes. Record the temperature displayed on the thermometer. Similarly performs the column oven temperature accuracy test at 60°C. **Acceptance criteria:** The resulting oventemperature from the thermometer displayshould be within ±2°C of the set temperature.

NOTE: *For oven Temperature Accuracy, Chromatographic conditions and mobile phase refer to system precision test.*

Column Oven Temperature Accuracy:

1. Connect the column to the detector port.
2. Place the thermometer probe in the column oven and set the column oven temperature at 40°C.Wait till the temperature stabilizes.
3. Note the observed temperature as read by the probe in triplicate over a period of 10 m.
4. Repeat the procedure for 100°C, 150°C and 190°C.

Acceptance criteria: The resulting oven temperature from the thermometer display should be within ±2°C of the set temperature System Precision:

Preparation of Standard solution: Transfer 20 ml of Methanol, Ethanol and Acetone into 100ml volumetric flask and make up with Ethyl acetate.

Chromatographic Conditions for System Precision

Column	30m × 0.32mm,1.8µ,DB-624
Injector temperature	180°C
Detector temperature	250°C
Flow mode	Pressure
Carrier Gas flow rate Helium	25 kpa
Oven program	50°C(hold 5 m) raise to 10°0C
Split ratio	1:10
Injection volume	0.2 µl
Hydrogen flow	40 ml/m
Air flow	400 ml/m
Detector temperature	250°C

Procedure: Inject blank, followed by Standard preparation in 6 replicates. Note down the areas and Retention times.

Chromatographic Conditions for Head Space Auto Sampler

Column	30m × 0.32mm,1.8µ,DB-624
Detector	Flame ionization detector
Injector temperature	220°C
Detector temperature	260°C
Flow mode	Pressure
Carrier Gas flow rate Helium	25 kpa
Oven program	40°C(hold 5 m) raise to 200°C(hold 5 m)
Split ratio	1:10
Injection volume	0.2 µl
Hydrogen flow	40 ml/m
Air flow	400 ml/m

Head Space Conditions

Vial equilibrium	22 m
Vial pressure	0.5 m
Loop fill	0.5 m
Loop equilibrium	0.05 m
Inject	1.00 m
GC cycle time	38 m
Oven temperature	80°C
Loop temperature	100°C
Vial pressure	10.8 psi

Acceptance criteria: The %RSD of retention time should be not more than 1.0%& peak area should be not more than 5.0%.

System precision for head space autosampler:

Preparation of standard solution: Prepare a standard mixture solution by taking Methylene dichloride (0.6g), Chloroform (0.06g),Trichloroethane (0.08g),1,4-Dioxane (0.38g) in 50ml volumetric flask containing about 40ml of Dimethyl formamide.Finally makeup to volume with DMF(Solution-A).

Procedure: Take 0.5 ml of standard solution-A in 6 different vials and seal with septum, then magnetic caps and crimp. Place these vials on head space sampler; prepare a blank vial also. Load the vials in head space sampler tray. Blank vials followed by the standard vials.

Acceptance criteria: The %RSD of retention time should be NMT 1.0%& peak area should be NMT 15.0%.

Detector linearity:

Preparation of standard solutions:

- **Detector linearity solution A:** Transfer 10ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
- **Detector linearity solution B:** Transfer 15ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
- **Detector linearity solution C:** Transfer 20ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
- **Detector linearity solution D:** Transfer 25ml of each Methanol, Ethanol and Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate.
- **Detector linearity solution E:** Transfer30ml of each Methanol, Ethanoland Acetone into a 100ml volumetric flask and dilute to the volume with Ethyl acetate. Procedure: Inject blank, followed byDetector linearity solutions and record thepeak responses .Draw a standard plot between the concentrations Vs the peakresponses.

Acceptance criteria: The plot should be linear and regression coefficient (R2) should not be less than 0.99.

Detector Noise and Drift Test:

After GC is ready run the system up to 15 m through single run. After completion of run calculate noise and drift through software.

Acceptance criteria:

Noise NMT: 100 µV Drift NMT: 2500 µV/h.

Long answer type Questions (10 Marks)

1. Explain in detail the calibration of Gas Chromatography.
2. Explain in detail the calibration UV Visible spectrophotometer
3. Explain in detail the calibration of HPLC.
4. Define process validation and explain elements of Process validation.
5. Which departments are involved in process validation.

Short answer type questions (5Marks)

1. Enlist the parameters of analytical method validation.
2. Give purpose of validation of analytical methods.
3. Give the procedure for calibration of absorbance of UV Visible spectrophotometer.
4. Which are the parameters involved in calibration of UV Visible spectrophotometer?
5. Enlist the parameters for calibration of HPLC

Very Short answer type questions (2 Marks)

1. Define Calibration.
2. Define accuracy and Precision
3. Name the standards for calibration as per ICH guidelines
4. Enlist elements of process validation.
5. Write the limitations of process validation.
6. Name the parameters for calibration of GC.
7. Name the parameters for calibration of HPLC.
8. Name the parameters for calibration of UV Visible spectrophotometer.