Calibration of Analytical Instruments as Per ICH & USFDA Guidelines

Abstract

Calibration of analytical instruments such as UV-Visible spectrophotometer, FTIR, HPLC,GC, Flourimeter and Flame photometer will be discussed. The focus will be on practical methods to ensure accurate and reliable measurements in analytical chemistry.The guidelines provide a framework for validating the performance of instruments, including specifications for calibration frequency, acceptable limits, and documentation.

Keywords: Calibration, spectrophotometer, analytical instruments, regulatory approval

INTRODUCTION

Calibration of analytical instruments is a critical process to ensure accuracy, reliability, and compliance in pharmaceutical analysis. According to the International Council for Harmonisation (ICH) and the U.S. Food and Drug Administration (USFDA) guidelines, regular calibration is mandated to maintain the integrity of data generated during drug development and quality control. These guidelines provide a framework for validating the performance of instruments, including specifications for calibration frequency, acceptable limits, and documentation. Adhering to these standards ensures that the instruments produce consistent, reproducible results, which are essential for regulatory approval and patient safety [1-3].

Comment [hd1]: Provide reference for recent guidelines.

Comment [hd2]: Mention about the frequency at which these calibaration should be done as perICH & USFDA guidelines

In the chapter, the calibration of analytical instruments such as UV-Visible spectrophotometer, FTIR, HPLC,GC, Flourimeter and Flame photometer will be discussed. The focus will be on practical methods to ensure accurate and reliable measurements in analytical chemistry.

CALIBRATION OF UV-VISIBLE SPECTROPHOTOMETER

There are four steps involved.

I) Control of Wavelength

II) Control of Absorbance

III) Limit of Stray Light.

IV) Resolution Power.

I. Control of Wavelength[4]

- Precisely weigh one gram of holmium oxide, dissolve it in a solution of 1.4 M HClO4 (perchloric acid), and use the same solvent to increase the volume to 25 ml.
- Choose the "CONTROL OF WAVELENGTH" Method file from the Instrument.
- To fix the base line, press the reference button after choosing the "control of wavelength" file.

Comment [hd3]: Enlist the machine names with brand names for which it will be useful.

- Next, add 1.4 M perchloric acid solution to the cuvette, place it in the sample cubicle, and set the reference to "zero."
- Place the holmium perchlorate solution in the sample cubicle after auto zero and hit the start button.
- Examine it and confirm the holmium perchlorate solution's absorption maxima.

Table 1: Accepted Tolerance

S.No	Maximum wavelength (nm)	Tolerance (nm)
1	241.15	± 1.0
2	287.15	± 1.0
3	361.5	± 1.0
4	536.3	± 3.0

II. Control of Absorbance[5]

- Absorbance parameter will check by using suitable filters or a solution of potassium dichromate($K_2Cr_2O_7$).
- Dry a portion of the potassium dichromate by heating it for two hours at 130 ^{0}C to constant weight.
- Accurately weigh out 60 mg of dried potassium dichromate, dissolve it in 0.005 M sulphuric acid solution, and use the same solvent to produce up to 1000 ml.
- Choose the "CONTROL OF ABSORBANCE" Method file from the Instrument.
- Press the Reference button for baseline adjustment after choosing the file.
- After that, press reference to zero and fill the cuvette with 0.005M sulphuric acid for the blank.
- Subsequently, determine the absorbance of the potassium dichromate sample at wavelengths of 235 nm, 257 nm, 313 nm, and 350 nm (UV region).and 430 nm (visible region).
- Calculate the specific absorbance of potassium dichromate at respective wavelength & check their maximum tolerance limit.

Table 2: Permitted tolerance

S.No	Maximum wavelength (nm)	Tolerance (nm)
1	235	± 1
2	257	± 1
3	313	± 1
4	350	± 1
5	430	± 3

III. Limit of stray light

- Any detected light that falls outside the given wavelength's band width is referred to as "stray light."
- The instruments' linearity range is reduced and absorbance is decreased due to scattered light.

Procedure:

- Dry a certain amount of potassium chloride (KCl) by heating it to 130 °C until it reaches a constant weight.
- Carefully weigh up 1.20 gram of dried potassium chloride (KCl) and dissolve it in 100 millilitres of distilled water (1.2 % w/v).
- Select the Method File " LIMIT OF STRAY LIGHT"
- Press the Reference button for baseline correction after choosing the File.
- Using water as a blank at 200 nm, measure the absorbance of the above solution.
- Absorbance should be greater than 2.0.

IV. Resolution Power[6]

- Make a 0.02 % w/v toluene solution in hexane.
- Choose the "Resolution Power" Method File in the instrument.
- After choosing the file, adjust the baseline by pressing the Reference button.
- Utilising hexane as a blank solution, calculate the absorbance of the above solution at 266 and 269 nm.
- The absorbance maxima at 269 nm should not be greater than 1.5 times that of the minima at 266 nm.
- Record the report in both the instrument logbook and the internal calibration certificate.

CALIBRATIONOF FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER (FTIR)

Comment [hd4]: Machine names with brand names for which it will be useful-mention.

Procedure:

- Switch the sytem
- Establish the following instrument parameters:
  i) Resolution 2.0 ii) Apodization Strong iii) Range 4000 - 400 cm inverse iv) Mode Ratio
- Give the instrument half an hour to warm up.
- Use the instrument in accordance with SOP.
- Close the FTIR lid after inserting the polystyrene film into the sample holder.
- Press the "Scan Mode" button.
- Next, a polystyrene film spectrum appears on the screen.
- Go to Print Function to print the spectrum.
- Go to "DATA & then to PEAK" once the spectra printer is finished.
- The screen shows the peak data of the polystyrene film's spectrum.
- To print these data, select the Print Function.
- Examine the correctness of the wave function against the subsequent limit.

1. Wave Number Accuracy Limit[7]

Table 3: Accuracy limit for wavenumber

Wavenumber (cm^{-1})	**Limit (± cm^{-1})**
3060	1.5
2849.5	1.5
1601.2	1.5
1583.0	1.0
1154.5	1.0
1028.3	1.0

II. Resolution Performance of the Apparatus[8]

- Record the 0.05 mm thickness polystyrene film and observe the percentage transmittance (% T).
- The difference between the percentage transmittance at 2851 cm^{-1} and 2870 cm^{-1} should be NLT 18%**.**
- The difference between the percentage transmittance at 1583 cm^{-1} and 1589 cm^{-1} should be NLT 12%**.**

CALIBRATION OF HPLC

Comment [hd5]: Machine name and make for which it will be useful- mention

Various calibration parameters are

1. Flow Rate Accuracy

2. Injector Accuracy

4.Wavelength Accuracy

5. Detector Linearity

6. Injector Linearity

8. Column Oven Temperature Accuracy

I. Flow Rate Accuracy

- Apply Milli-Q water to all solvent lines to prime them.
- Choose a flow rate of 0.500 ml/min.
- To ensure that the pressure is stable and the system is stabilised, give it an adequate fifteen minutes.
- At the same time as you insert the outlet tube into a 10 ml volumetric flask, set the stop watch.
- When the bottom meniscus reaches the 10 ml point on the flask, stop the stopwatch.

- Keep track of the amount of time that has passed.
- Likewise, verify the flow rates at 1.0 and 2 ml per minute.

Table 4: Acceptance criteria for accurate flow rate[9]

Set flow (ml /min)	Actual time require to collect the upto the mark in minutes	Criteria for acceptance (minutes)
0.5	20	19. 6 -20.4
1	10	9.8 – 10.2
1.5	5	4.9 – 5.1

II. Injector Accuracy[10]

i) Attach the column to the detector inlet and pump.
ii) Make the mobile phase by mixing water and methanol in a 70:30% w/v ratio.
iii) Select a 1-minute run period and a 0.5 ml/min flow rate.
iv) Assign 25± 2 ^{0}C as the column temperature.
v) Add Milli-Q water to fill the standard HPLC vial to the third and securely fasten the wire with the cap.
vi) Determine the vial's weight and note it as W_1 grams.
Vii) Insert the vial into the chromatographic system and use it to make SIX injections totalling 50 µL.
ix) Weigh the vial once more and record the weight as w2 grammes following the injections.
x) Use the following formula to find the average volume administered each injection.

$(W_1-W_2) \times 100/6$ = mean injected volume (µL).
Acceptance criteria: 50±1 should be the mean injected volume.

III. Wavelength Accuracy[11]

Procedure:

- Establish an instrument method with a wavelength in nanometres (nm), inject a blank, then proceed with standard preparation and record the absorbance height.

Acceptance requirements: Maximum absorbance of ±2 nm is required.

PDA Detector Accuracy:

- Choose 3D mode and configure the wavelength range to be 200–400 nm.
- Once the Chromatographic Conditions are met, inject 20 µl of the Standard preparation.
- Extract and save the chromatograms at wavelength of 202-208 nm and 269-275 nm, with a 1 nm interval between each.
- Make a note of the Wavelength Maximum (or Height of Absorbance)
- **Acceptance Criteria:** Either 205±2 nm or 272±2 nm should be the maximum absorbance.

IV. Linearity of Detector[12]

Standard solution preparation : Weigh accurately, transfer 60 mg of caffeine into a 100 ml volumetric flask, dissolve it, and then add mobile phase to bring the volume up to specification.

Detector linearity solution 1 (0.06 mg/ml): Fill the volume of the 100 ml volumetric flask with mobile phase after adding 10 ml of the Standard Solution.
Detector Linearity Solution 2 (0.048 mg/ml): Fill the 100 ml volumetric flask to the with mobile phase after adding 8 ml of the standard solution.
Detector Linearity Solution 3 (0.03 mg/ml): Fill the 100 ml volumetric flask to the with mobile phase after adding 5 ml of the standard solution.
Detector Linearity Solution 4 (0.24 mg/ml): Fill the 100 ml volumetric flask to the with mobile phase after adding 4 ml of the Standard Solution.
Detector Linearity Solution 5 (0.012mg/ml):): Fill the 100 ml volumetric flask to the with mobile phase after adding 2 ml of the standard solution

Procedure: I

- Inject a blank first, then the linearity solution for the detector.
- Plot the graph between Concentration and Peak Responses (Areas) after recording the peak responses of the caffeine standard solution.

Acceptance Standards: The regression coefficient (R^2) should be less than 0.99 and the plot should be linear.

V. Injector Linearity

Preparation of Standard solution:

- Weigh and transfer approximately 60mg of caffeine precisely into the 100 ml volumetric flask.
- Pour 10 millilitres of Standard Preparation into a 100 ml volumetric flask and use mobile phase to fill the remaining volume.

Procedure:

- Introduce 5 micro litre of the Mobile Phase as a Blank Injection.
- Then spike 5µl, 10 µl, 20 µl, 50 µl, 8 µl, of standard preparation & Record the Peak areas.
- Make a curve showing the volume injected versus the peak areas.

Criteria for acceptance: The regression coefficient (R^2) should be less than 0.99%, and the plot should be linear.

VI. Column Oven Temperature Accuracy

- A calibrated digital thermometer is used to test it at 30 ^{0}C and 60 ^{0}C.
- Set the temperature of the column oven to 30 0C and wait for the temperature to stabilise after inserting the thermometer probe.
- Take note of the temperature reading on the thermometer.
- Similarly, maintains the temperature precision of the column oven at and 60 ^{0}C.
  Acceptance Criteria: The oven temperature obtained from the thermometer display must be within a margin of error of ± 2.0°C from the predetermined temperature.

Comment [hd6]: Confirm is it 8 only?

CALIBRATION OF GAS CHROMATOGRAPHY

Various calibration parameters are

1. Flow Rate Accuracy

2. Column Oven Temperature Accuracy

3. System Precision

4. System Precision for Head Space Auto Sampler

5. Detector Linearity

6. Detector Noise & Drift Test.

1. Flow Rate Accuracy [13, 14].

A digital flow meter should be connected to the detector outlet.
The carrier gas flow (helium) should be set and the observed flow should be noted in a replicate.
The process should be repeated for the other carrier gases, such as hydrogen and air, and the result should be recorded in GC calibration
The acceptance criteria state that the carrier gas flow rate should be within 10% of the set flow.

II. Column Oven Temperature:

- Attach the column to the detector port.
- After inserting the thermometer probe into the column oven and adjusting the temperature to 40 ^{0}C, observe until the temperature stabilises.
- Over a ten-minute period, record the temperature as it was measured using three separate probe readings.
- Repeat the process for temperatures of 100 ^{0}C, 150 ^{0}C, and 190 ^{0}C.

Acceptance Criteria: The oven temperature obtained from the thermometer display has to be within ± 2.0°C of the predefined temperature..

III. System Precision

Standard Solution preparation:

Place 20 millilitres of methanol, ethanol, and acetone into a 100 millilitre volumetric flask and finish it off with ethyl acetate.

Procedure:

- After injecting a blank, six replicates of the standard solution are carried out..
- Make a note of peak areas &RT (retention time).

Acceptance Criteria: Percentage RSDof RT NMT 1.0 % & Peak area Should NMT 5 %.

IV. System Precision for Head Space Auto Sampler:

Preparation of Standard Solution:

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Comment [hd7]: Write in proper words what does it mean.

It is prepared by taking methylene chloride (0.6 gr), chloroform (0.06 gr), trichloro ethane (0.08 gr), 1,4 diixane (0.38 gr) in 50 millilitre volumetric flask containing 40 ml of DMF (dimethyl formamide)& make upto the volume with DMF.

Procedure:

- Transfer 0.5 ml of the standard solution into six distinct vials, cover with a septum, and secure with magnetic caps and crimps.
- Arrange a blank vial and set these vials on top of the head space sample.
- Fill the Head Space Sample Tray with the vials.
- Standard Vials are placed after Blank Vials.

Acceptable limits: Retention time RSD should be 1.0% and peak area should be not more than15%.

V. Detector Linearity:

Preparation of Standard Solution*:*

i) Detector Linearity Solution A: Pour 10 millilitres of ethanol, methanol and acetone into a 100 millilitre volumetric flask and use ethyl acetate to dilute to the desired level.

ii) Detector Linearity Solution B: Pour 15 millilitres of ethanol, methanol and acetone into a 100 millilitre volumetric flask and use ethyl acetate to dilute to the desired level.

iii) Detector Linearity Solution C*:*Pour 20 millilitres of ethanol, methanol and acetone into a 100 millilitre volumetric flask and use ethyl acetate to dilute to the desired level.

iv) Detector Linearity Solution D: Pour 25 millilitres of ethanol, methanol and acetone into a 100 millilitre volumetric flask and use ethyl acetate to dilute to the desired level.

V. Detector Linearity Solution E: Pour 30 millilitres of ethanol, methanol and acetone into a 100 millilitre volumetric flask and use ethyl acetate to dilute to the desired level.

Procedure:

- Inject blank, followed by detector linearity solution & record the peak responses.
- Draw a Standard Plot b/w the Concentration vs The Peak Responses.

Acceptance Criteria:

The Plot Should be Linear & Regression Co-efficient (R2) Should NLT 0.99 %.

Vi. Detector Noise & Drift Test

- Run the system for a single run for up to 15 minutes after the GC is ready.
- Using software, compute noise and drift after the run is over.

Acceptance Criteria:

Noise levels should not exceed 100 microvolts and drift should not exceed 2500 microvolts / hour.

CALIBRATION OF FLOURIMETER

1. Sensitivity of Flourimeter

2. Wavelength Accuracy

3. Accuracy of Spectral Slit Width Calibration
4. System for Detecting Linearity
5. Quenching of Flourescence Intensity

1. Sensitivity of Flourimeter

- Sensitivity of flourimeter is done with the help of primary standard quinine sulphate solution.
- Using 0.1M sulphuric acid,make a 1 ppm solution of quinine sulphate.

- Check the instrument's sensitivity after setting the wavelength to 366 nm.

2. Wavelength Accuracy (Calibration of Light Source)

In which emission wavelength or excitation wavelength accuracy of Light Source will be measured.

Table 5: Permitted tolerance

Light Source	**Wavelength Region**	**Uncertainty (A.C)**
Xenon Source Lamp	Select excitation wavelength range b/w 400 - 500 nm	± 0.2 nm
Pen lamp or low pressure atomic lamp	Select excitation wavelength range b/w 200 - 450 nm	± 0.1 nm

3. Spectral Slit Width Accuracy

By measuring the spectral band width, the emission or excitation wavelength selector's spectral slit width accuracy can be determined[8].

.***Acceptance Criteria***: The uncertainty for emission or excitation wavelength should be ± 0.5 nm.

4. Linearity of Detection System

- The linear intensity range of a detection system can be evaluated using a variety of methods.
- Depending on the instruments used to adjust the light's intensity that reaches the detector, they can be divided into three categories.
  1. Two apertures
  2. Polarised or optical filters
  3. Fluorophoric Concentration
- The third approach is the most widely used and easiest to use.
- It makes use of a collection of solutions made by serially diluting a stock solution that is fluorescent.
- The observations at very low concentrations may be affected by fluorophore adsorption to cuvette walls, hence in this instance, a low concentration solution should be employed..

5. Quenching of Flourescence Intensity (Inner - Filter Effect)[15]

- 25 mg of quinine sulphate should be dissolved in enough 0.05M sulphuric acid resulting in 500 ml of solution..

- Prepare two series of solutions of Quinine sulphate, so that Concentration are

1. 0.1 to 0.5µg/ml (with differentiation of 0.1µg/ml).

2. 10 to 50µg/ml (with differentiation of 10µg/ml).

- Measure the fluorescence intensity of both of 2 series, it can be done by plot the flourescence intensity vs. concentration of quinine sulphate solution.
- The flourescence of weak solutions shows linear relationship with concentration.
- The stronger solutions show marked quenching effect due to inner filter effect (self-quenching).

CALIBRATION OF FLAME PHOTOMETER

There are 3 Calibration Parameters

I. Calibration Curve method (Linearity Test)

II. Direct Comparison Method

III. Selectivity or Specificity

I. Calibration Curve method (Linearity Test)[16]

In this method a series of standard solutions of the element to be prepared.

Procedure:

- Prepare a stock solution of NaCl i.e µg/ml.
- From the stock solution prepare a series of standard solution of sodium those are 10, 20, 30, 50, 50 µg/ml.
- Select the sodium filter in the instrument
- Maintain an air pressure ranging from 0.4 to 0.5 kg/sq cm and light the burner.
- Use distilled or deionised water& set the percentage flame intensity.
- Set 100 percentage flame intensity (or full scale in the instrument) by using the maximum concentration of standard solution in the calibration region (50 µg/ml).
- Then draw a calibration curve b/w concentration of solution & percentage (%) flame intensity.

Acceptance Criteria: Regression coefficients should not be less than (NLT) 0.98 and the plot must be linear.

II. Direct Comparison or Direct Curve Method

- In this method the percentage flame intensity of standard solution & sample solution is compared.
- Concentration of ion in sample solution is calculated by following formula

$$\frac{\%\ Flame\ intensity\ of\ the\ sample \times concentration\ of\ ion\ in\ standard}{\%\ Flame\ intensity\ of\ standard}.$$

III. Selectivity or Specificity

- This is accomplished by a method known as peak matching, in which, when sample and standard spectra are collected, at least three of the emission spectrum's peaks should match..
- For example calcium emits radiation at 422 nm, 554 nm, and 626 nm.
- If the spectrum of the sample shows emission maximum at these wavelengths then sample can be identified by using standard.

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