Calibration of Analytical Instruments- UV-VIS Spectrophotometer and HPLC

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Calibration

- What is Calibration?

  Calibration is an operation which is performed to assure that the instrument readings are accurate with reference to the established standards.
- The aim of the calibration program is to ensure that all measuring and testing equipment included in the program are calibrated within the manufacturers accuracy specifications or the tolerance required for the application.

Calibration

Calibration may be called for:

- A new instrument.
- After an instrument has been repaired or modified.
- When a specified time period has elapsed.
- Before and/or after a critical measurement.
- After an event, for example
  - After an instrument has had a shock, vibration, or has been exposed to an adverse condition which potentially may have put it out of calibration or damage it.
  - Sudden changes in weather.
- Whenever observations appear questionable or instrument indications do not match the output of surrogate instruments.
- As specified by a requirement, e.g., customer specification, instrument manufacturer recommendation.

Calibration of UV-VIS Spectrophotometer

- Calibration of UV-VIS spectrophotometer involves following parameters:
  - ***Calibration for wavelength accuracy.***
  - ***Calibration for absorbance measurement.***
  - ***Gratings performance or stray light test.***
  - ***Resolution power.***

Calibration of UV-VIS Spectrophotometer

Calibration for wavelength accuracy:

- Take two empty cuvettes, into one cuvette add two drops of benzene and drain it.
- Place the cuvette in sample cell holder with benzene vapors occupied in it.
- Place the empty cuvette in reference cell holder.
- Scan it in between 240-270nm.
- The maximum absorbance should be at 253.9 ± 0.51 nm.
- Absorbance should be below 1 (one).

Calibration of UV-VIS Spectrophotometer

Calibration for absorbance measurement:

- Prepare 60ppm of potassium dichromate by using 0.01M sulfuric acid as solvent.
- Place the solution in sample cell holder.
- Place blank 0.01M sulfuric acid in reference cell holder.
- Measure the absorbance values at different λmax values.

λmax	**Absorbance**
257	0.864 ± 0.01
235	0.747 ± 0.01
313	0.292 ± 0.01
350	0.639 ± 0.01

Calibration of UV-VIS Spectrophotometer

- ***Gratings performance or stray light test:***
  - Prepare 1.2% potassium chloride solution in distilled water.
  - Distilled water is taken as reference.
  - Place the potassium chloride solution in sample cell holder.
  - Absorbance is measured at 195-220nm.
  - Absorbance is must be greater than 2 at 198nm.

Calibration of UV-VIS Spectrophotometer

- ***Resolution power:***
  - When prescribed in a monograph, record the spectrum of a 0.02% v/v solution of toluene in hexane .
  - The ratio of the maximum absorbance at about 269 nm to that at the minimum absorbance at about 266 nm should not be less than 1.5 unless otherwise specified in the monograph.

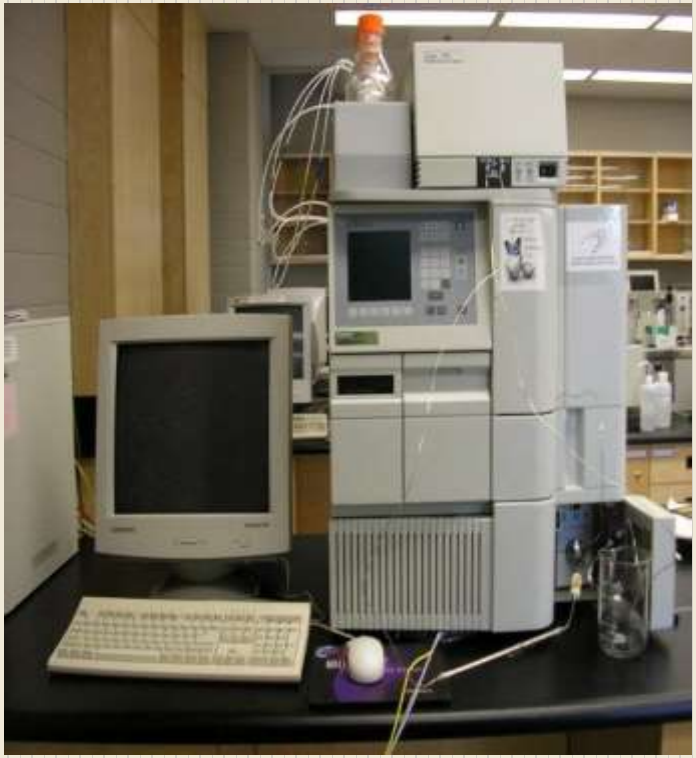
CALIBRATION REPORT

Name of the institution:			
Name of the Instrument: UV-Visible Spectrophotometer			
Make:	Serial No.:	Date of Calibration:	Calibration Due Date:
Signature:			
	Calibrated By	Person Responsible	Approved By

Calibration of HPLC

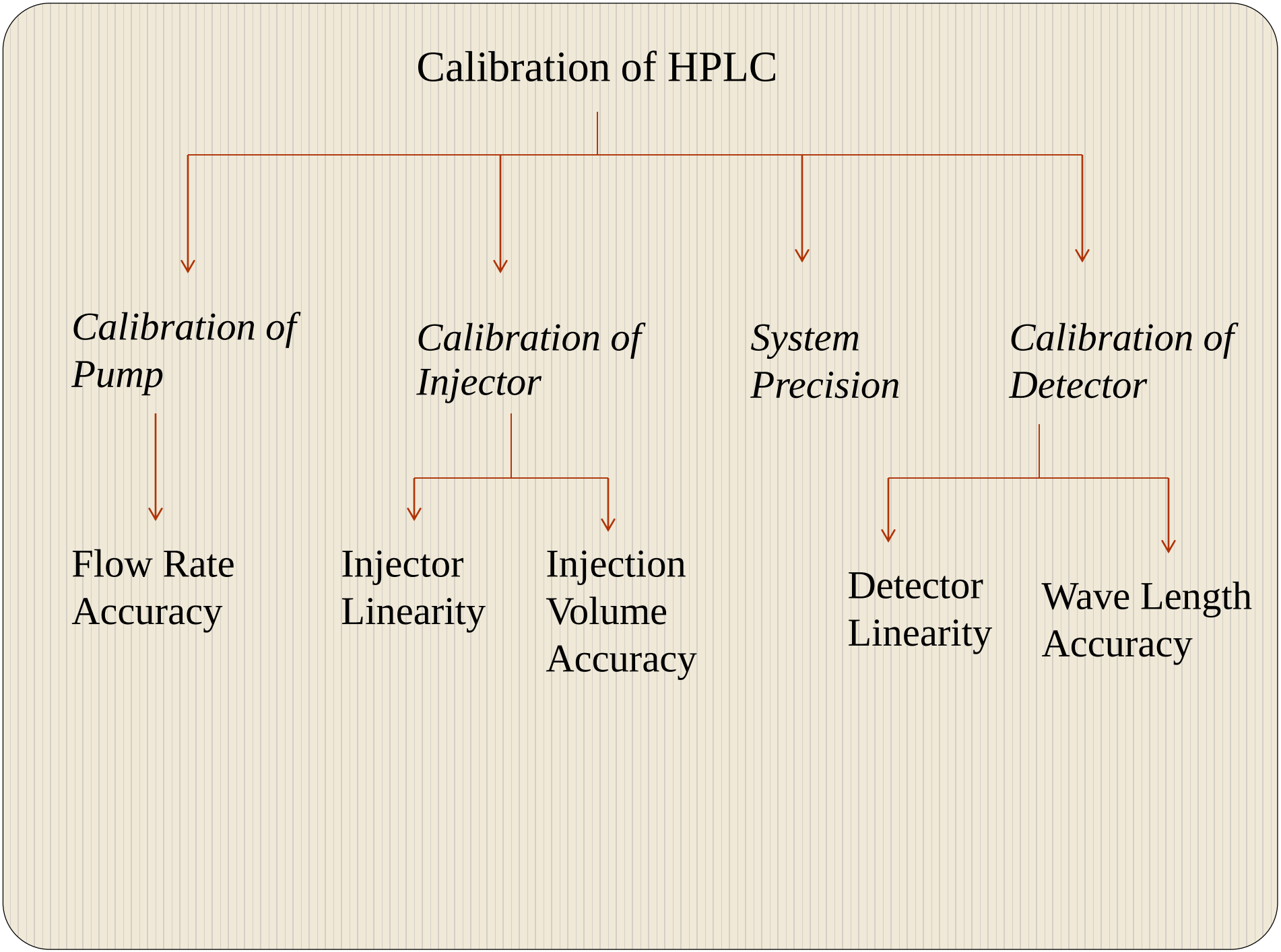
- When it is performed?
  - Most HPLC systems in pharmaceutical laboratories are calibrated every 6 to 12 months.
  - Periods longer than 12 months are not recommended, while periods shorter than 3 months are deemed un-necessary.
  - It also required after annual maintainance (or) major repairs though only effected modules, and not the entire system need to be calibrated.

Calibration of HPLC



- Calibration of HPLC involves following parameters:

  - ***Calibration of Pump***
  - ***Calibration of Injector***
  - ***System Precision***
  - ***Calibration of Detector***


Calibration of HPLC
Calibration of Pump
Calibration of Injector
System Precision
Calibration of Detector
Flow Rate Accuracy
Injector Linearity
Injection Volume Accuracy
Detector Linearity
Wave Length Accuracy

Calibration of HPLC

Calibration of Pump

Flow Rate Accuracy:

- Prime all the solvent lines with HPLC grade water.
- Set the flow rate to 0.5 ml/min.
- Wait for about 15min to stabilize the system and ensure that the pressure is stable.
- Insert the outlet tubing into a 10 ml volumetric flask and start the stop watch simultaneously.
- Stop the stopwatch when the lower meniscus reaches the 10 ml mark on the flask.
- Record the elapsed time.
- Similarly check the flow for 1.0 ml/m and 2.0 ml/m.
- The time taken to collect the water should be with in ± 2.0% of the actual value.

Calibration of HPLC

Calibration of Injector

Injector linearity

- Duplicate injections of 10µg/ml caffeine solution are injected by setting 5µl, 10µl, 15µl and 20µl respectively one after one as injection volumes.
- The mean peak areas for the above injected volumes are noted.
- Calculate the correlation coefficient by using linearity curve and the value should be with in the acceptance criteria i.e., NLT 0.99.

Calibration of HPLC

Calibration of Injector

Injection volume Accuracy

- HPLC vial is filled with water (HPLC grade) and weighed, it's weight is recorded as W_1
- The vial is placed in the injection tray, then inject six times.
- After completion of six injections remove the vial and weigh, it's weight is recorded as W_2
- The injection volume is calculated using the formula,

$$Injection\ volume(\mu l) = \frac{W_1 - W_2}{0.99602 \times 6}$$

- **Acceptance criteria:** The mean injected should be 50.0±1.0 µl.

Calibration of HPLC

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%.

Calibration of HPLC

Calibration of Detector

Detector Linearity

- Prepare a standard stock solution of caffeine (1000µg/ml).
- From the stock solution prepare solutions of concentration 1, 5, 10, 50, 100µg/ml respectively.
- Duplicates of each concentration are injected.
- The mean peak areas of all the concentrations are recorded.
- Calculate the correlation coefficient value by using linearity curve and it should be within the acceptance criteria .i.e., 0.99.

Calibration of HPLC

Calibration of Detector

Wavelength Accuracy

- The wavelength of detector is adjusted to 266nm.
- Then inject duplicates of 10µg/ml solution of caffeine and then the peak area response is recorded.
- The wavelength was increased successively at an increment of +1nm (267, 268, 269……, 276nm) and the peak area is recorded at all the wavelengths.
- The maximum peak area response has to be obtained at 273±2nm (acceptance criteria).

CALIBRATION REPORT

Name of the institution:			
Name of the Instrument: HPLC			
Make:	Serial No.:	Date of Calibration:	Calibration Due Date:
Signature:			
	Calibrated By	Person Responsible	Approved By

References:

- Validation standard operating procedures, 2nd edition, by Syed Imtiaz Haider, pg no.:117 – 125.
- http://www.pharmaguideline.com/2010/05/calibration-of-uv-visible.html
- http://qualityassurancepharma.blogspot.in/2010/12/calibration-of-ultraviolet.html
- http://qualityassurancepharma.blogspot.in/2010/12/operation-calibration-of-hplc-lc.html
- http://www.gmp7.com/Standard-Operation-Procedures/hplc-calibration_p8985.html
- http://www.pharmaguideline.com/2011/01/hplc-caibration.html


Thank You