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Subject: Medicinal Chemistry-III (BP 801T)

Unit: III

Topic: Calibration and validation of UV-VSI Spectrophotometer

Presentation outline

Introduction

Calibration

Validation

Analytical instruments

     UV Visible Spectrophotometer

     IR Spectrophotometer

     Spectrofluorimeter

     HPLC

     HPTLC

     GC

Introduction

Analytical instruments are used for a specific analysis of drugs and pharmaceuticals.

So, regular performance verification are made to ensure that the instruments used in the analytical purpose should be properly validated and calibrated "to demonstrate that it is suitable for its intended purpose".

Calibration

Calibration is a process by which ensure that an instrument readings are accurate with reference to established standards.

Calibration is performed using primary reference standards.

Instruments need to be calibrated before using. For example- weighing balance, p^H meter,........etc

Need for calibration

Calibration can be called for:

➢ with a new instrument.

➢ when a specified time period is elapsed.

➢ when a specified usage (operating hours) has elapsed.

➢ when an instrument has had a shock or vibration which potentially may have put it out of calibration.

➢ sudden changes in weather.

➢ whenever observations appear questionable.

Validation

Validation is a detailed process of confirming that the instrument is installed correctly, that it is operating effectively, and that it is performing without error.

The field of validation is divided into a number of subsections as follows-

➤ Cleaning validation
➤ Process validation
➤ Analytical method validation
➤ Computer system validation

Equipment validation

It demonstrate that equipment used in validation studies is suitable for use and is comparable to equipment used for routine analysis.

Qualification

Action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results.

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

Parts of qualification

The activity of qualifying system of equipment is divided into four subtypes-

- ➤ Design qualification (DQ)
- ➤ Installation qualification (IQ)
- ➤ Operational qualification (OQ)
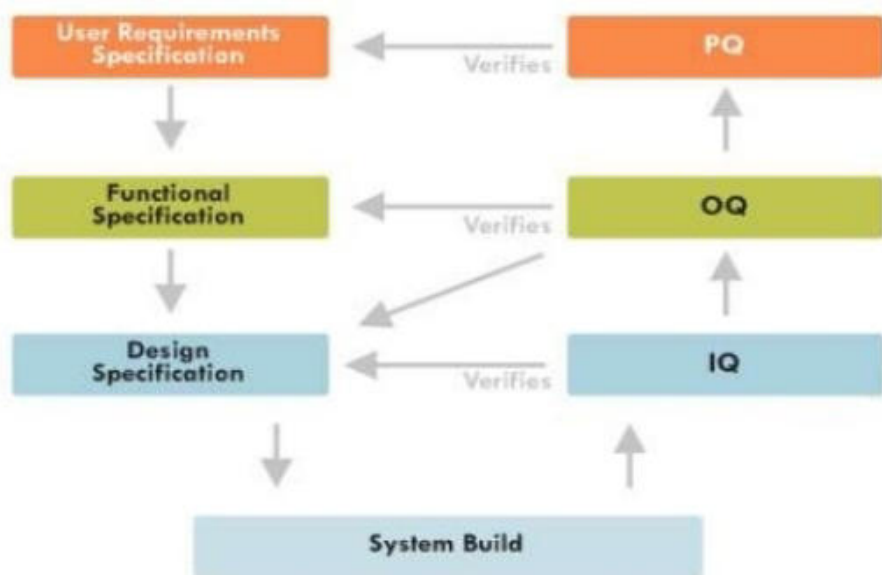- ➤ Performance qualification (PQ)

Design qualification (DQ)- Demonstrates that the proposed design will satisfy all the requirements that are defined and detailed in the User Requirements Specification.

Installation qualification (IQ)- Demonstrates that the process or equipment meets all specifications, is installed correctly, and all required components and documentation needed for continued operation are installed and in place.

Operational qualification (OQ)- Demonstrates that all facets of the process or equipment are operating correctly.

Performance qualification (PQ)- Demonstrates that the process or equipment performs as intended in a consistent manner over time.
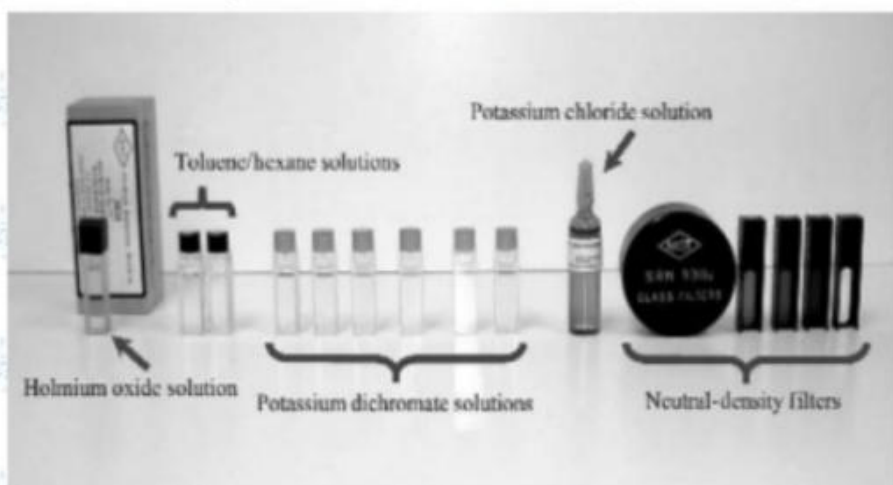
I.Q & O.Q & P.Q Report

User Requirements Specification	→ Verifies ←	PQ
Functional Specification	→ Verifies ←	OQ
Design Specification	→ Verifies ←	IQ
System Build		

Calibration of UV-VIS spectrophotometer

- ➢ Wavelength accuracy

- ➢ Stray light

- ➢ Resolution

- ➢ Photometric accuracy

- ➢ Noise

- ➢ Baseline flatness

- ➢ Stability

Reference standards for UV-Vis spectrophotometer

Wavelength accuracy:-

It is defined as the deviation of the wavelength reading at an absorption band or emission band from the wavelength of the band.
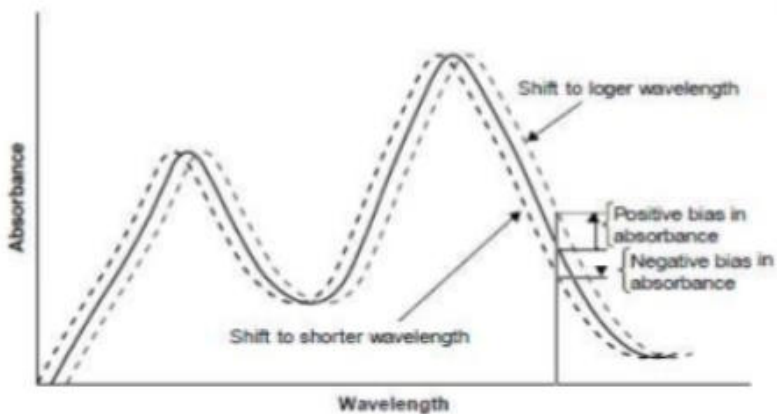

Figure 10.2. Effect of wavelength accuracy on UV–Vis measurements.

Test:-

Wavelength accuracy verification is checked by comparing the recorded wavelength of the peak against the value of reference standard.

commonly used wavelength standards such as

➢ Deuterium lamp,

➢ Mercury vapor lamp,

➢ Holmium oxide filter, and

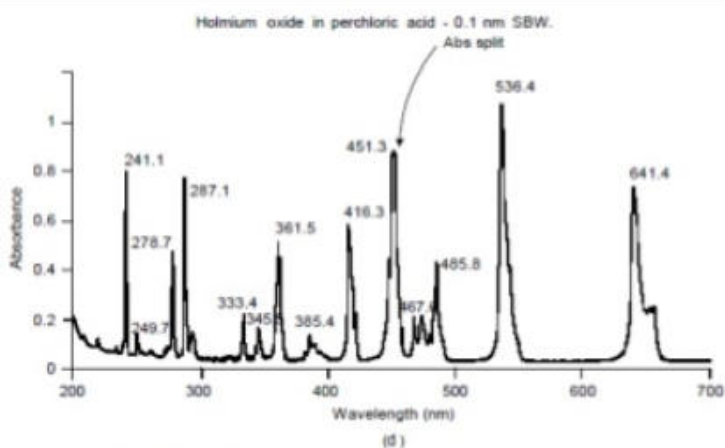➢ Holmium oxide solution (4% holmium oxide in 10% perchloric acid in a 1-cm cell)

Holmium oxide in perchloric acid - 0.1 nm SBW.

Figure 10.3. (d) Absorption spectrum of holmium oxide solution.

Table 10.3. Wavelength Assignment: 4% Holmium Oxide in 10% Perchloric Acid

SRM 2034 Band	Spectral Bandwidth		
	0.5 nm	1.0 nm	2.0 nm
1	241.01	241.13	241.08
2	249.76	249.87	249.98
3	278.13	278.10	278.03
4	287.01	287.18	287.47
5	333.43	333.44	333.40
6	345.52	345.47	345.49
7	361.33	361.31	361.16
8	385.50	385.66	385.86
9	416.09	416.28	416.62
10	–	451.30	451.30
11	467.80	467.83	467.94
12	485.27	485.29	485.33
13	536.54	536.64	536.97
14	640.49	640.52	640.84

Acceptance

±1 nm in the UV range (200 to 380 nm) and

±3 nm in the visible range (380 to 800 nm).

Three repeated scans of the same peak should be within ±0.5 nm.

Stray light:-

Stray light is defined as the detected light of any wavelength that is outside the bandwidth of the wavelength selected.

Test:-

Three test solutions prepared and measured the stray light at 200 nm, 220 nm and 340 nm

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.

At 1% transmittance, stray light at 1% of the incident light intensity can cause a 15% drop in absorbance

Table 10.5. Stray Light Measurement

Spectral Range (nm)	Solution	Measurement Wavelength (nm)
175–200	Aq. KCl (12 g/L)	200
210–260	Aq. NaI (10 g/L)	220
300–385	Aq. NaNO$_2$ (50 g/L)	340

Resolution power:-

The resolution of a UV-VIS spectrophotometer is related to its spectral bandwidth(SBW). The smaller the spectral width, the finer the resolution.

The SBW depends on the slit width and the dispersive power of the monochromator

Test:-

0.02%v/v toluene in hexane is used to test the resolution power of the spectrophotometer. The absorbance measured at 269 nm and 266 nm.

Acceptance:-

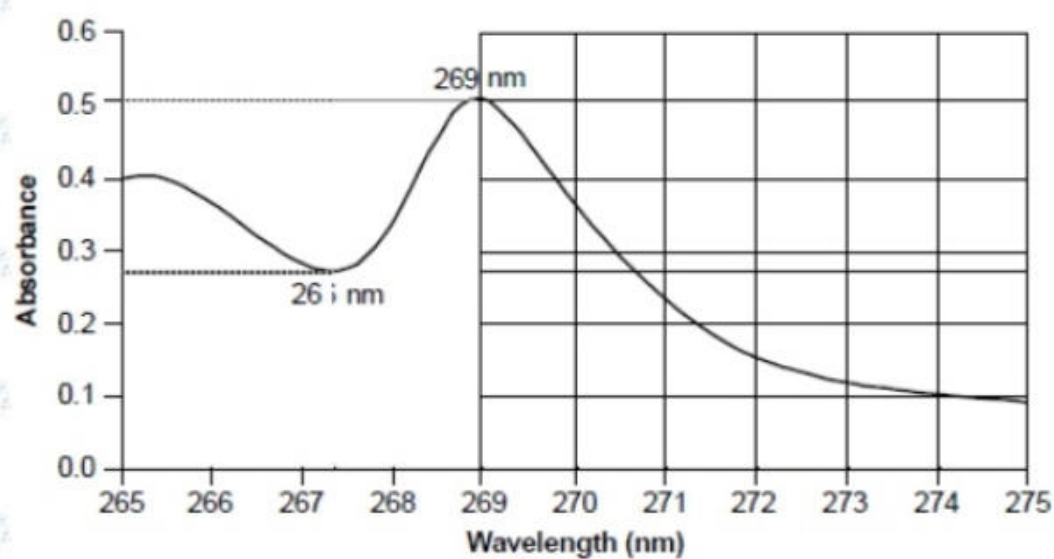The ratio of the absorbance at 269 nm and absorbance at 266 nm should be grater than 1.5

igure 10.7. Resolution check: spectrum of a 0.02% v/v toluene in hexane solution.

Noise:-

Noise in the measurement affects the accuracy at both ends of the absorbance scale.

Photon noise from the light source affects the accuracy of the measurements leads to low absorbance.

Test:-

Air is scanned in the absorbance mode for 10 min.peak to noise is recorded at 500nm. Root mean square noise is then calculated.

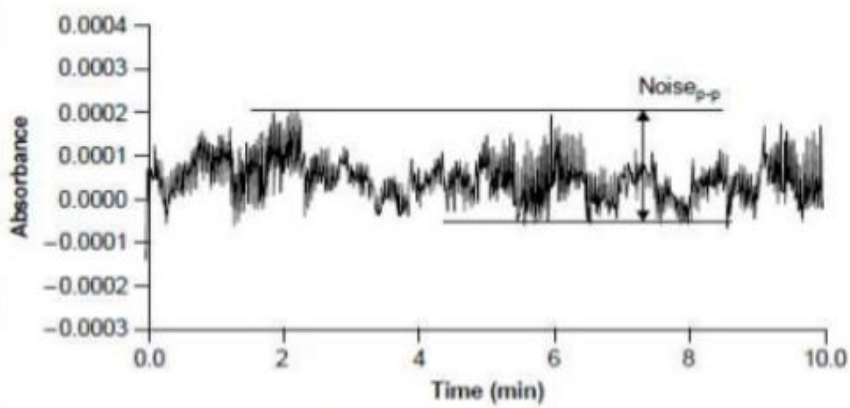## Acceptance:-

The RMS noise should be less than 0.001 AU

Figure 10.8. Noise measurement.

Baseline flatness:-

The flat baseline test demonstrates the ability of the instrument to normalize the light intensity measurement and the spectral output at different wavelengths through out the spectral range.

Test:-

Air is scanned in the absorbance mode. The highest and lowest deflections in the absorbance unit are recorded.

Acceptance:-
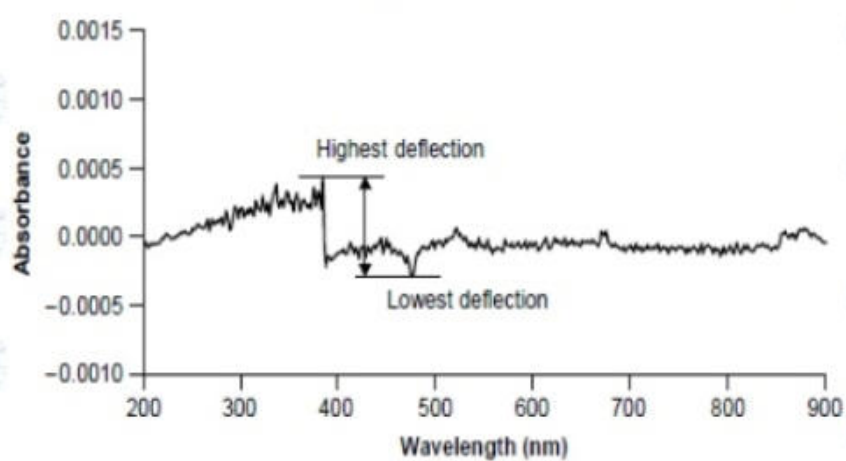
The deflection is typically less than 0.01AU

Figure 10.9. Baseline flatness.

Stability:-

The lamp intensity is a function of the age of the lamp, 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.

Test:-

Air is scanned in the absorbance mode for 60 min at specific wavelength(340nm).The highest and lowest deflections in the absorbance unit are recorded.
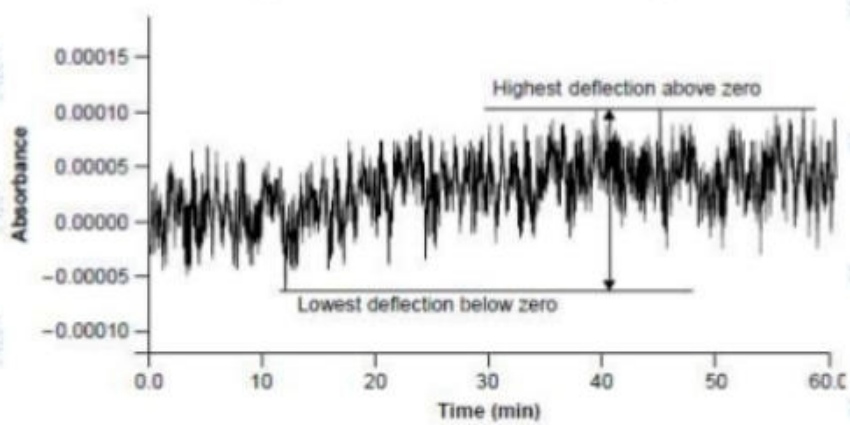
Acceptance:-

The deflection is less than 0.002 AU/h

Figure 10.10. System stability.

Photometric accuracy:-

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

Test:-

Either neutral density filters or potassium dichromate solutions are used.

Acceptance:-

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

Table 10.9. Standard Values for the BP/EP Potassium Dichromate Solution

Wavelength (nm)	Absorbance (AU)	A (1%, 1 cm)	Tolerance (A)
235	0.748	124.5	122.9–126.2
257	0.865	144	142.4–145.7
313	0.292	48.6	47.0–50.3
350	0.640	106.6	104.9–108.2
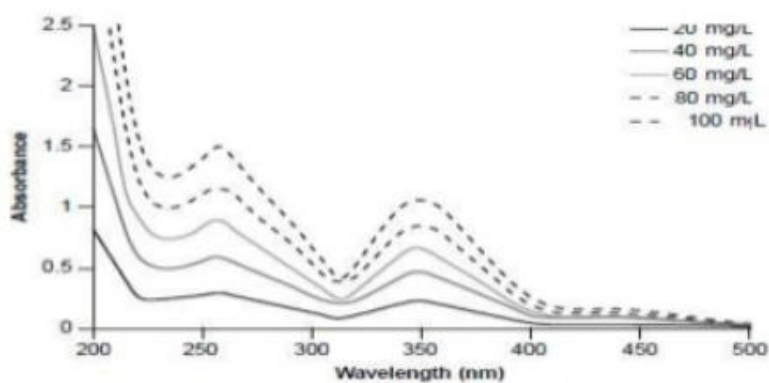
Figure 10.12. Photometric accuracy: potassium dichromate solutions.

Linearity:-

The linear dynamic range of the measurement is limited by stray light at high absorbance and by noise at low absorbance.

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

Test:-

A series of Potassium dichromate solution of concentration 20,40,60,80 and 100mg/L in 0.005M sulfuric acid. The absorption of various wavelength are plotted against the concentration of the solution and the correlation coefficients are calculated.

Acceptance:-

Correlation coefficient $r \geq 0.999$