

Performance of UV-Vis spectrophotometers

Introduction:

UV-Visible (UV-Vis) spectrophotometry is commonly used in analytical laboratories for qualitative and quantitative analyses. The popularity of the UV-Vis technique stems from its ease of use and the speed of the analysis. The absorbance data from the UV-Vis measurement can be related to the concentration of the sample by Beer's Law. UV-Vis methods are so simple that the fundamental performance of the spectrophotometers required to generate reliable data is sometimes neglected by the analysts. The various factors which affect the reliability of the UV-Vis measurement will be discussed in this article.

The performance verification tests required by major Pharmacopoeias for UV-Vis spectrophotometers are listed in Table 1. The required performance tests include wavelength accuracy, stray light, resolution and photometric accuracy. There are other factors such as noise, baseline flatness and stability that will affect the performance of the spectrophotometers as well.

Table 1: Performance Tests for UV-Vis Spectrophotometers

Performance Test	Standard	Pharmacopoeia			
		USP 24	BP 1998	EP 3 Ed.	JP (XII)
Wavelength accuracy	Deuterium lamp	X	X	X	X
	Mercury vapour lamp	X	X	X	X
	Holmium oxide glass filter	X	-	-	-
	Didymium glass filter	X	-	-	-
	Holmium oxide in HClO ₄	-	X	X	-
Stray Light	Potassium chloride solution	-	X	X	-
Resolution	Toluene in hexane solution	-	X	X	-
Photometric accuracy	Neutral density glass filters	X	-	-	-
	Metal on quartz filter	X	-	-	-
	Potassium dichromate solution	X	X	X	X
Noise	-----	-	-	-	-
Baseline flatness	-----	-	-	-	-
Stability	-----	-	-	-	-

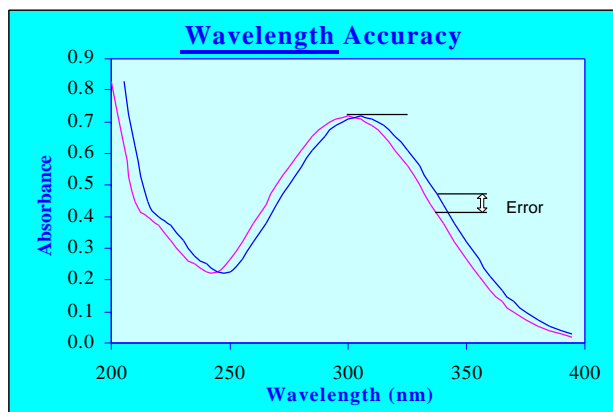
Performance Attributes:

1. Wavelength Accuracy

Wavelength accuracy is defined as the deviation of the wavelength reading at an absorption band or emission band from the known wavelength of the band. The wavelength deviation can cause errors in the qualitative and quantitative results of the UV-Vis measurement. It is quite obvious that if the spectrophotometer is not able to maintain an accurate wavelength scale, the UV profile of the sample measured by the instrument will be inaccurate. The true λ_{\max} of the analyte cannot be characterized accurately.

In addition to the qualitative problem, wavelength deviation affects the quantitation in two ways: the accuracy and sensitivity. Typically, most UV-Vis assays will specify that the measurements are to be taken at the λ_{\max} and the absorption profile of the analyte is fairly broad. A slight deviation in wavelength will have very little effect on the absorbance value (Figure 1). However, if the selected wavelength is not close to the λ_{\max} of the analyte of interest, then a small deviation in wavelength will cause a big change in the absorbance value of the measurement. Significant wavelength deviation will also steer the measurement away from the optimal wavelength at the λ_{\max} which effectively reduces the extinction coefficient and hence the sensitivity of the measurement.

Figure 1: Effect of wavelength accuracy on UV-Vis measurements



There are many standards that can be used to verify the wavelength accuracy of a spectrophotometer. The advantages and disadvantages of various wavelength verification standards are summarized in Table 2.

Table 2: Wavelength standards comparison

Wavelength Standard	Advantage	Disadvantage
Emission lines from deuterium lamp	Sharp spectral lines Present in the UV light source	Limited to visible wavelengths
Emission lines from mercury vapour lamp	Sharp spectral lines Cover both UV and visible regions	Not commonly built into the instrument
Holmium oxide and Didymium filters	Easy to use NIST SRM available Independent of the resolution power of the instrument	Limited to visible wavelengths Broad spectral lines Re-certification required once every two years
4% Holmium oxide in 10% perchloric acid	Easy to use NIST SRM available Cover both UV and visible regions Re-certification required every eight years	Dependency on the resolution power of the instrument Corrosive solution

2. Stray Light

Stray light is defined as the detected light of any wavelength that is outside the bandwidth of the selected wavelength. The causes for stray light are scattering, higher order diffraction or poor instrument design. Stray light causes a decrease in absorbance and reduces the linearity range of the instrument. High absorbance measurements are more severely impacted by stray light. Consider the follow scenarios at 1% transmittance level (See Table 3). If there is no stray light, the absorbance value as governed by equation 1 should give a value of 2.

$$\text{Absorbance (A)} = -\log \text{Transmittance (T)} \quad (\text{Equation 1})$$

Where T is the ratio of intensity of the transmitted light (I) and incident light (I_o)

In the presence of stray light,

$$T = (I + I_s)/(I_o + I_s) \quad (\text{Equation 2})$$

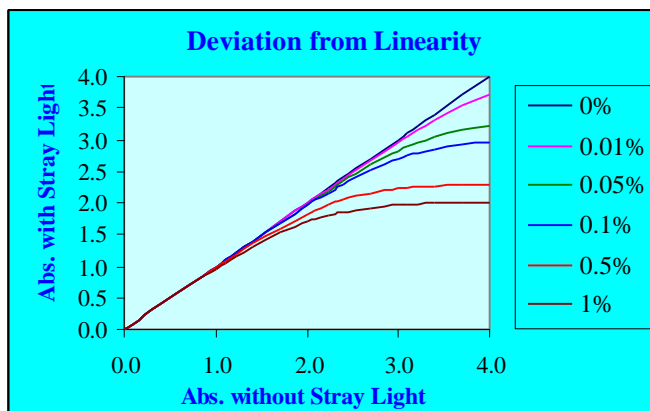
where I_s is intensity of the stray light.

Table 3: Stray light estimation

Is (Stray light)	Ii (Transmitted)	I = Is + Ii	Io	T= I/Io	A = -Log T
0%	1%	1%	100.0%	0.0100	2.000
0.1%	1%	1.01%	100.1%	0.0101	1.996
0.5%	1%	1.05%	100.5%	0.0149	1.826
1%	1%	2%	101.0%	0.0198	1.703

The changes in absorbance due to different levels of stray light at 1% transmittance are estimated in Table 3. As the results indicate, stray light can cause a significant reduction in absorbance value. At 1% transmittance, stray light at 1% of the incident light intensity can cause a 15% drop in absorbance. The effect of various levels of stray light on the absorbance value is show in Figure 2. The stray light problem causes a deviation from linearity at high absorbance. In general, the linearity of the absorbance response is limited by stray light at high absorbance and by noise at the low absorbance range. Absorbance ranging from 0.3 to 1 are less susceptible to stray light and noise problems and hence becomes the preferred absorbance range for UV-Vis analyses.

Figure 2: Effect of stray light on absorbance measurement



For the stray light test, various cut-off filters or solutions can be chosen depending on the wavelengths of interest. Table 4 outlines three typical solutions for stray light measurement at 200, 220 and 340nm. These solutions have the optical characteristics to allow light at slightly higher wavelengths than the measuring wavelengths to pass through and block off light at the measuring wavelength.

Table 4.

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

3. Resolution

According to the Raleigh criteria, two peaks are considered to be resolved if the minimum between the peaks of the detector output signal is lower than 80% of the maxima (Figure 3). The resolution of a UV-Vis spectrophotometer is related to its spectral band width (SBW). The smaller the spectral band width, the finer the resolution. The SBW depends on the slit width and the dispersive power of the monochromator. For diode array instruments, the resolution also depends on the number of diodes in the array.

The accuracy of the absorbance measurement depends on the ratio of the spectral bandwidth (SBW) to the natural band width (NBW) of the spectral band to be measured. The NBW is a physical characteristic of the analyte of interest. The accuracy is not likely to be affected if the ratio of NBW and SBW is greater than 20. If the ratio is less than 10 (SBW increasing), the measured spectrum becomes distorted as shown in figure 4.

Figure 3.

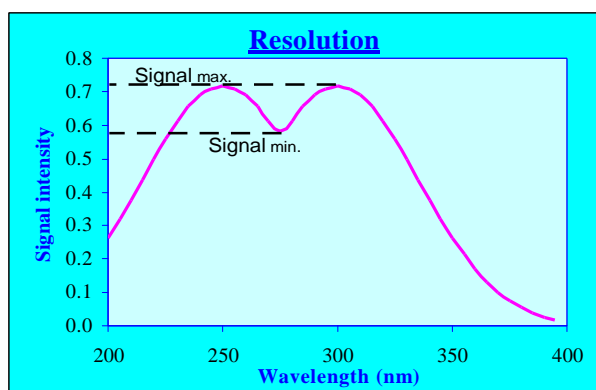
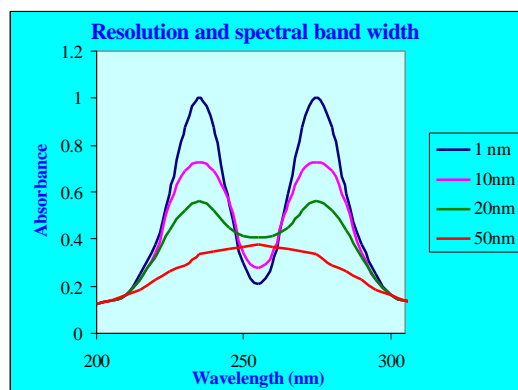


Figure 4.



In general, insufficient resolution leads to a decrease in extinction coefficient across the wavelength axis and therefore inaccurate quantitation. The sensitivity of the measurement is also compromised. From a qualitative point of view, the fine features in the spectrum may be lost. A solution mixture of 0.02 % v/v toluene in hexane (UV grade) is used to test the resolution power of the spectrophotometer. The ratio of the absorbance at λ_{\max} (269 nm) and absorbance at λ_{\min} (266 nm) should be greater than 1.5.

4. Noise

Noises in the UV-Vis measurement are mainly originating from the light source and electronic components. 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 at low absorbance. Electronic noise from electronic components affects the accuracy of the measurements at high absorbance. High noise level will reduce the limit of detection and render the instrument less sensitive.

5. Baseline Flatness

Most UV-Vis spectrophotometers have dual light sources. A deuterium lamp is used for the UV range and a tungsten lamp is used for the visible range. The intensity of the radiation coming from the light sources is not constant over the whole UV-Visible range. The response of the detector also varies over the spectral range. A flat baseline demonstrates the ability of the instrument to normalize the output of the lamp and detector responses.

6. Stability

Variations in lamp intensity and electronic outputs between the measurements of I_0 and I result in instrument drifts. These changes can lead to error in the value of the measurements especially over a long period of time. The resulting errors in the measurements may be positive or negative. The stability test checks the ability of the instrument to maintain a steady state over time so that the effect of the drift on the accuracy of the measurements is insignificant.

7. Photometric Accuracy

Photometric accuracy is determined by the difference between the measured absorbance and the established standard value. Most quantitation applications using UV-Vis would involve the measurement of the standards and samples of comparable concentrations in rapid succession on the same instrument. As long as the photometric measurements are reproducible and the response is linear over a defined range, the absolute photometric accuracy may not be critical. However, photometric accuracy is critical for measurement which comparing the extinction coefficients between different instruments. Photometric inaccuracy will lead to errors in quantitation.

There are many standards that can be used to verify the photometric accuracy of a spectrophotometer. The advantages and disadvantages of various commonly used photometric accuracy standards are summarized in Table 5.

Table 5:

Photometric Accuracy Standard	Advantages	Disadvantages
Neutral Density Glass Filters	Optically Neutral. Not restricted by the bandwidth Available for a range of transmittance/absorbance NIST SRM available	Limited to visible wavelengths
Metal on Quartz Filters	Optically Neutral. Not restricted by the bandwidth Available for a range of transmittance/absorbance Cover both UV and Visible range NIST SRM available	Possible inter-reflections between optical surface
Potassium Dichromate in acidic solution	Cover both UV and Visible range	Relatively unstable Careful preparation is required Corrosive and oxidative Results are sensitive to the analytical technique used

Since photometric accuracy of the instrument depends on almost all of the performance attributes discussed above, it is better to perform the photometric accuracy test at the final stage of the regular performance verification. If the factors that may affect the photometric accuracy have been successfully verified, there is a better chance of passing the photometric accuracy test. There is no point of doing the photometric accuracy test if there are known problems in the other performance attributes.

In conclusion, many performance verification tests have to be done on a UV-Vis spectrophotometer on a regular basis in order to guarantee the reliability of the results. A general understanding of the effects of each performance attributes on the outcome of the measurements will help to improve the UV-Vis experimentation.

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