



A SYSTEMATIC APPROACH FOR QUALIFICATION OF UV-SPECTROPHOTOMETER

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ABSTRACT

Spectrophotometers must be qualified before the start to analysis the sample. Qualification is done by using the reference substance, and the absorbencies of all other substances are measured relative to the reference substance. The Qualification of uv spectrophotometer is required that the instrument is suitable for its intended use and establish the documentary evidence that the Spectrophotometer(s) are capable to perform and meet the acceptance criteria of the test like: Control of wavelength, control of absorbance and Limit of stray light etc.

KEYWORDS: Spectrophotometers must be qualified before the start to analysis the sample.

INTRODUCTION

UV/Visible spectrophotometers are widely used by many laboratories – including those found in academia and research as well as industrial quality assurance. The technique is mainly used quantitatively (although some qualitative analysis can also be performed). For any type of critical determination, whether it be clinical, pharmaceutical or industrial QC, environmental analysis or research, it is essential that the instrument is performing according to specification. In some of these applications, it is important that the instrument performance is monitored regularly and that there is documentary evidence that this is the case. This will apply to any measurements to do with human or animal health (clinical or pharmaceutical/nutraceutical), food or labs offering an accredited measurement service. Validation is also a key requirement of Good Laboratory Practice (GLP).

Different Types of Spectrophotometers

A. Single Beam: In this type, all the light passes through the sample. To measure the intensity of the incident light the sample must be removed so that all the light can pass through. This type of spectrometer is usually less expensive and less complicated.

B. Double Beam: In this type, before it reaches the sample, the light source is split into two separate beams. From these one passes through the sample and second one is used for reference. This gives an advantage because the reference reading and sample reading can take place at the same time..

C. Visible Light (400-700 nm): Visible spectrophotometers can use incandescent, halogen, LED, or a combination of these sources and these spectrophotometers vary in accuracy. Plastic and glass cuvettes can be used for visible light spectroscopy.

D. Ultraviolet Light: UV spectroscopy is used for fluids, and even solids. Cuvettes, only made of quartz, are used for placing the samples.

Details of Parameter to Be Checked During Qualification

1. Control of wavelength.
2. Atomic line spectra
3. Control of Absorbance.
4. Limit of stray light.
5. Resolution
6. Resolution Power
7. Absorption Cells

1: Control of Wavelength

Test can be performed by Holmium oxide filter or Rare Earth oxide solution

For non-diode array instruments, wavelength accuracy and precision are determined over the operational range using at least six replicate measurements. For wavelength accuracy, the difference of the mean measured value to the certified value of the CRM must be within ± 1 nm in the UV region (200–400 nm), and in the visible region (400–700 nm) must be within ± 2 nm. For wavelength precision, the standard deviation of the mean must not exceed 0.5 nm.

For diode array instruments, only one wavelength accuracy measurement is required, and determination of precision not needed. The difference between the certified and measured value of the CRM must not exceed ± 1 nm in the UV region (200–400 nm), and in the visible region (400–700 nm) must not exceed ± 2 nm.

Two different procedures were mentioned based on operational range of spectrophotometer. These are as follows

Acceptance Criteria for Accuracy.

Acceptance Criteria for UV region at 200-400 nm		Acceptance Criteria for VISIBLE region at 400-700nm	
Measured wavelength	Acceptance criteria ± 1 nm	Measured wavelength	Acceptance criteria ± 2 nm
241.1	240.1 – 242.1	416.3	414.3 - 418.3
249.9	248.9 – 250.9	451.4	449.4 - 453.4
278.1	277.1 – 279.1	467.8	465.8 - 469.8
287.2	286.2 – 288.2	485.2	483.2 - 487.2
333.5	332.5 – 334.5	536.6	534.6 - 538.6
345.4	344.4 – 346.4	640.5	638.5 – 642.5
361.3	360.3 – 362.3		
385.6	384.6 – 387.6		

By using Rare Earth Glasses (Alternate procedure): A Holmium glass to be used for verification of wavelength in UV-VIS region for 1.0 nm spectral bandwidth.

Acceptance Criteria for Accuracy.

Acceptance Criteria for UV region at 200-400 nm		Acceptance Criteria for VISIBLE region at 400-700nm	
Measured wavelength	Acceptance criteria ± 1 nm	Measured wavelength	Acceptance criteria ± 2 nm
241.5	240.5 – 242.5	418.8	416.8 – 420.8
279.2	278.2 – 280.2	445.8	443.8 – 447.8
287.5	286.5 – 288.5	467.8	465.8 - 469.8
338.8	337.8 – 339.8	453.7	451.7 – 455.7
360.9	359.9 – 361.9	460.2	458.2 – 462.2
		536.5	534.5 – 538.8
		637.7	635.7 – 639.9

If the operational range of the spectrophotometer lies outside the range 240–650 nm: A 4% m/v solution of Didymium (a mixture of neodymium and praseodymium) in perchloric acid is used for wavelength verification in the 730–870nm region.

Acceptance Criteria for Accuracy.

Acceptance Criteria for VISIBLE region at 730 - 870 nm	
Measured wavelength	Acceptance criteria ± 2 nm
731.6	729.6 – 733.6
740.0	738.0 – 742.0
794.1	792.1 – 796.1
799.0	797.0 – 801.0
864.4	862.4 – 866.4

If operational range of the spectrophotometer lies within range 240–650 nm.

A 4% m/v solution of holmium oxide in perchloric acid to be used at 1.0-nm spectral band width and a path length of 1 cm are shown below.

Inference: Need to perform based on operational range of instrument.

2. Atomic Line Spectra

A commonly employed low-pressure mercury lamp has a number of intense lines that cover a large part of the UV and visible spectra. Two deuterium lines from the source at 486.0 and 656.1 nm often are used by manufacturers as an internal Qualification check and can be used for diagnostic purposes.

Element	nm	Acceptance criteria	Element	nm	Acceptance criteria for accuracy
Hg	253.7	NA	D2	486.0	± 0.5 nm
Hg	296.7	NA	Hg	546.1	NA
Hg	365.0	NA	Hg	577.0	NA
Hg	404.7	NA	Hg	579.1	NA
Hg	435.8	NA	D2	656.1	± 0.5 nm

Inference: Acceptance criteria for accuracy is ± 0.5 nm and Acceptance criteria for uncertainty is ± 0.01 nm for these wavelength measured in triplicate during Instrument self-check.

3. Control of Absorbance

To establish the transmittance accuracy, precision, and linearity of a given system, it is necessary to verify the absorbance accuracy of a system over its intended operational range by using the following procedures as appropriate for the wavelength and absorbance ranges required.

Test can be performed by: Acidic potassium dichromate solution in 0.001M perchloric acid and Neutral density glass filter.

The control of absorbance must include at least one assessment at each wavelength range in the 0–1 A region. If absorbance >1 A is to be used for quantitation, then control of absorbance must also be evaluated in the 1–3 A range. If verification of linearity is required, it should be evaluated using at least four different absorbance standards, at varying levels spanning the required range. The options and acceptance criteria are summarized.

Absorbance Range	Standard	UV (200–400 nm)	Vis (400–800 nm)
0–1	K ₂ Cr ₂ O ₇ solution	60 mg/L Evaluate at 235, 257, 313, and 350 nm. Accuracy ± 0.010 A λ Precision <0.005 A λ	600 mg/L Evaluate at 430 nm. Accuracy ± 0.012 A λ Precision <0.005 A λ
0–1	Neutral density glass filters	NA	Evaluate at 440, 465, 546.1, 590, 635nm. Accuracy ± 0.008 A λ Precision <0.005 A λ
1–3	K ₂ Cr ₂ O ₇ solution	200 mg/mL Evaluate at 235, 257, 313, 350 nm. Accuracy $\pm 1\%$ Precision $<0.5\%$	NA
1–3	Neutral density glass filters	NA	Evaluate at 440, 465, 546.1, 590, 635 nm. Accuracy $\pm 0.8\%$ Precision $<0.5\%$
Linearity	K ₂ Cr ₂ O ₇ solutions	20, 40, 60, 80, 100 mg/L Evaluate at 345 nm. $r^2 > 0.99$ Intercept = 0 at 95% CL	NA
Linearity	Neutral-density glass filters	NA	At least 4 certified filters $r^2 > 0.99$ Intercept = 0 at 95% CL

Test with Acidic potassium dichromate solutions in 0.001 M Perchloric acid.

The Accuracy of Absorbance of 0–200 mg/L range, potassium dichromate solutions in 0.001M perchloric acid must be $\pm 1\%A$ (for values above 1.0A) or $\pm 0.010A$ (for values below 1.0A). , whichever is larger at 235, 257, 313 or 350 nm. And linearity shall be verified by measuring the absorbance of the solutions at 345 nm.

And the absorbance precision for Standard deviation with six replicate measurement for 235 and 313 or 350 nm, is must not exceed $\pm 0.5\%A$ (for values above 1.0A) or $\pm 0.005A$ (for values below 1.0A), whichever is larger.

(These solutions are available as CRMs or can be prepared according to NIST from SRM 935a.).

Inference: Need to perform equivalency between Potassium dichromate solutions in Sulfuric acid and

Potassium dichromate solutions in perchloric acid for control of absorbance parameter.

Absorptivity of Acidified Potassium Dichromate.

Absorptivity (L g ⁻¹ cm ⁻¹) at 23.5°					
K ₂ Cr ₂ O ₇ (mg/L)	235 nm	257 nm	313 nm	350 nm	430 nm
20	12.291	14.298	4.817	10.699	
40	12.335	14.354	4.823	10.709	
60	12.378	14.410	4.828	10.719	
80	12.421	14.466	4.833	10.728	
100	12.465	14.522	4.839	10.738	
120	12.512	14.578	4.517	10.750	
140	12.556	14.634	4.523	10.760	
160	12.600	14.690	4.529	10.770	
180	12.644	14.746	4.535	10.780	
200	12.688	14.802	4.541	10.790	
600					15.9

Solutions, Adapted from NIST SP 260-544. Neutral-Density Glass Filters.

The Accuracy of Absorbance of neutral-density glass filters must be $\pm 0.8\%A$ (for values above 1.0 A) or $\pm 0.0080 A$ (for values below 1.0A), whichever is larger at 440, 465, 546.1, 590, and 635 nm.

And the absorbance precision for Standard deviation with six replicate measurement for 440 and 635 nm, must not exceed $\pm 0.5\%A$ (for values above 1.0A) or $\pm 0.005A$ (for values below 1.0A), whichever is larger.

These gray glass filters are manufactured from doped glass and have a nominally flat spectrum in the region of the Qualification wavelengths. These filters are available as CRMs that are traceable to NIST SRM 930e, 1930, and 2930.

4. Limit of Stray Light

Although the measurement of absorbance or transmittance is a ratio measurement of intensities and therefore theoretically is independent of monochromatic source intensity, practical measurements are affected by the presence of unwanted radiation called “stray radiant energy” or “stray light”.

Stray light can be detected at a given wavelength with a suitable liquid filter. These solutions are available as CRMs or can be prepared at the concentrations shown in Table by using reagent-grade materials.

Spectral Ranges of Selected Materials for Monitoring Stray Light

Spectral Range (nm)	Recommended Wavelength (nm)	Liquid or Solution
190–205	198	Aqueous potassium chloride (12 g/L)
210–259	220	Aqueous sodium iodide or potassium iodide (10 g/L)
250–320	300	Acetone
300–385	340	Aqueous sodium nitrite (50 g/L)

When using a 5-mm path length cell (filled with the same filter) as the reference cell, and then measuring the 10-mm cell over the required spectral range, analysts can calculate the stray light value from the observed maximum absorbance using the formula:

$$S\lambda = 0.25 \times 10^{-2A\lambda}$$

$A\lambda$ = observed maximum absorbance

Acceptance criteria: $S\lambda$ is ≤ 0.01 . $A\lambda \geq 0.7A$.

This procedure simply requires the 10-mm cell measurement to be referenced against the 5-mm cell (filled with the same filter) and therefore can be achieved by either chronological or spatial referencing in any type of spectrophotometer. Alternatively, analysts can measure the absorbance of the filters specified in Table 3

against the appropriate reference, and record the maximum absorbance value. (An $S\lambda$ value of 0.01 is produced by an $A\lambda$ value of 0.7A, which equates to a maximum absorbance value of 2A measured by this alternate procedure.)

[Note—For some instruments where absorbance values greater than 3A cannot be reported directly, this procedure may require a two-step process whereby the sample beam initially is attenuated by a 1- to 2-A filter, the value of which is measured and recorded. After zeroing the instrument with this filter in place, measure the stray-light filter, and again record the absorbance value. The estimated stray light value is now the sum of these two absorbance readings.]

Procedure A

The aim of this procedure is to produce the differential spectrum resulting from the subtraction of a spectrum produced by a 5-mm path length cell from that of a 10-mm cell, both filled with the same filter solution. This spectrum will contain a peak maximum absorbance value, and analysts can calculate the stray light value from the observed maximum absorbance using the formula.

$$s\lambda = 0.25 \times 10^{-2A\lambda}$$

$S\lambda$ = stray light value, in transmittance (T) (i.e., 0.01 T = 1.0%, T = 2.0 A).

$A\lambda$ = observed maximum absorbance.

This procedure requires the 10-mm cell measurement to be subtracted from the 5-mm cell, both filled with the same filter. This measurement can be achieved by either using the physical capabilities of the spectrophotometer, i.e., using the double beam capability, with the 5-mm cell as the reference, or by mathematically subtracting the spectra from the 10-mm cell from the 5-mm cell, chronologically produced by sequential measurement of both the 5- and 10-mm cells, using the single sample beam, with the spectrophotometer referenced to air (blank holder).

Acceptance criteria: $s\lambda \leq 0.01$ and $A\lambda \geq 0.7 A$

Procedure B

Analysts can measure the absorbance of the filters against a 10-mm cell filled with water as reference, and record the maximum absorbance value (A_{max})

Acceptance criteria: $A_{max} \geq 2.0 A$

Inference

Three additional solutions perform for limit of stray light such as aqueous sodium iodide or potassium iodide (10 g/L), Acetone and Aqueous sodium nitrite (50 g/L) are used in the Spectral Range (nm) 210–259 nm, 250–320 nm and 300–385 nm respectively.

5. Resolution

Determine the resolution of the spectrophotometer by using the following procedure. Measure the ratio of the absorbance of a 0.020% (v/v) solution of toluene in hexane (UV grade) at the maximum and minimum at

about 269 and 266 nm, respectively, using hexane as the reference. The absorbance ratio obtained depends on the spectral bandwidth of the instrument. For most pharmacopeial quantitative purposes, a spectral bandwidth of 2 nm is sufficient, and the acceptance criteria for the ratio is NLT 1.3.

The effect of spectral bandwidth and measurement temperature on the ratio is shown in Table Spectral Bandwidth and Measurement Temperature.

Temperature of Measurement	Spectral Bandwidth				
	0.5 ± 0.1 nm	1.0 ± 0.1 nm	1.5 ± 0.1 nm	2.0 ± 0.2 nm	3.0 ± 0.2 nm
20 ± 1°	2.4–2.5	2.0–2.1	1.6–1.7	1.3–1.4	1.0–1.1
25 ± 1°	2.3–2.4	1.9–2.0	1.6–1.7	1.3–1.4	1.0–1.1
30 ± 1°	2.1–2.2	1.8–1.9	1.5–1.6	1.3–1.4	1.0–1.1

Suitable CRMs may also be used for this measurement. Alternatively, a suitable atomic line can be scanned in single-beam mode, and the peak width at half peak height can be determined. This peak width at half peak height equates to the bandwidth of the spectrophotometer.

6. Resolution Power

Prepare a solution of 0.02 % v/v of toluene in methanol. Dilute 2 ml of Toluene to 200 ml with Methanol. Further dilute 2 ml of above solution to 100 ml with Methanol.. Replace Wavelength range; enter 275 nm in the start box and 255 in the end box Record the second derivative spectrum in the range 255 to 275 nm of 0.020 % v/v toluene in methanol using methanol in the reference cell.

Acceptance criteria: A small negative extreme located between two large negative Extreme at about 261 and 268 nm should be clearly visible. Ratio A/B is not less than 0.2.

7. Absorption Cells

Fill the cell with distilled water and measure its apparent absorbance against air at 240 nm for Quartz cells and at 650 nm for glass cells. With recording instruments it is desirable to scan Over the optical region of interest. The apparent absorbance should be not greater than 0.093 For 1 cm quartz cells (UV region) and 0.035 for 1 cm glass cells (Visible region). Rotate the Cell in its holder (180°) and measure the apparent absorbance again.

Acceptance criteria: Rotating the cells should give an absorbance difference not greater than 0.005.

REFERENCES

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