

# technical guide

## UV/Vis Spectrophotometer Calibration Procedures

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## 1 Introduction

Because of their inherent robustness there is a tendency to take the performance of UV/Vis spectrophotometers for granted. However, sound analytical practice requires that their performance be checked at regular intervals. This performance can be broken down into two parts – traceable calibration of the instrument and the checking of its ability to perform routine chemical analyses. The latter checks will be carried out by the laboratory using blanks and standards and are not described here. Periodic calibration of spectrophotometers requires measurement of wavelength accuracy, absorbance and stray light (Stray Radiant Energy). This guide describes procedures for making these measurements.

### 1.1 Traceability

NZS/ISO/IEC 17025 – *General requirements for the competence of testing and calibration laboratories* (section 5.6.1) requires that “All equipment used for tests and/or calibrations, including equipment for subsidiary measurements (e.g. for environmental conditions) having a significant effect on the accuracy or validity of the result of the test, calibration or sampling shall be calibrated before being put into service”.

Where calibrations cannot be strictly made in SI units, the laboratory: “shall provide confidence in measurements by establishing traceability to appropriate measurement standards such as:

- The use of certified materials (CRMs) provided by a competent supplier to give a reliable physical or chemical characterisation of a material;
- The use of specified methods and/or consensus standards that are clearly described and agreed by all parties concerned”.

Reference materials used to calibrate wavelength and absorbance scales of a spectrophotometer need to be certified by either:

- (a) A national metrology institute (NMI, e.g. the Measurement Standards Laboratory of New Zealand operated by Industrial Research Limited), or
- (b) An accredited calibration laboratory.

Alternatively, spectrophotometers may be calibrated directly by an IANZ accredited calibration laboratory.

## 2 Principal calibration requirements

The following measurements are considered to be critical:

- |                                 |       |
|---------------------------------|-------|
| Wavelength calibration          | (2.1) |
| Absorbance calibration          | (2.2) |
| Liquid sample holder absorption | (2.3) |
| Stray light control             | (2.4) |

### 2.1 Wavelength calibration

Any of the following three options may be acceptable, depending on the use of the spectrophotometers:

#### (i) Solution of holmium (III) in perchloric acid.

A solution of holmium oxide in perchloric acid provides sharper and less matrix-dependent lines than glass matrices and makes available a spectral line at 241 nm that is not easily measurable in holmium glass. This solution is available as a reference material from some NMIs and other suppliers. However, for those laboratories wishing to prepare their own solutions, holmium oxide can be obtained from chemical supply companies. The solution recommended for wavelength calibration is prepared by dissolving 4.0 g of holmium oxide in 96 g of 10% (volume fraction) aqueous perchloric acid. The solution can be warmed to aid dissolution but it is preferred that the solution be stirred overnight at room temperature to effect dissolution. The observed wavelengths of prominent lines of the holmium solution depend on the spectral slit width (SSW) of the spectrophotometer; values for SSWs of 1 nm and 3 nm are listed in Table 1. However few spectrophotometers used for routine analytical work are likely to have spectral slit widths (band widths) less than 3mm. (Check instrument specification if in doubt).

Note: Extreme care is needed in the handling and storage of perchloric acid.

**Table 1 Wavelengths of the absorption maxima of holmium (III) in perchloric acid for two values of spectral slit width (reference (g)).**

Absorption maximum (nm)	
1 nm	3 nm
241.1	241.0
249.9	250.1
278.1	278.1
287.2	287.6
333.5	333.5
345.4	345.5
361.3	361.1
385.6	386.0
416.3	416.9
451.4	451.3
467.8	468.1
485.2	485.2
536.6	537.2
640.5	641.1

**(ii) Glass filters containing holmium or didymium (a mixture of praseodymium and neodymium).**

The wavelengths of absorption maxima in holmium and didymium glasses can vary between batches; nominal values are listed in Table 2.

**Table 2 Nominal wavelengths of selected absorption maxima for holmium and didymium glass filters (reference (o)).**

Wavelength of maximum (nm)	
Holmium glass	Didymium glass
241.5 ± 0.2*	573.0 ± 3.0
279.4 ± 0.3	586.0 ± 3.0
287.5 ± 0.4	685.0 ± 4.5
333.7 ± 0.6	
360.9 ± 0.8	
418.4 ± 1.1	
453.2 ± 1.4	
536.2 ± 2.3	
637.5 ± 3.8	

\*not readily seen in glass

**(iii) Low pressure discharge lamps containing mercury, cadmium or zinc.**

Some spectrophotometers contain lamp housings for these lamps (Hg, Cd, Zn). Where this facility is provided, wavelength checks can be made very conveniently by operating the discharge lamp and rotating the monochromator to isolate the emitted spectral lines. For this and all wavelength calibrations the minimum slit width should be used. The mercury lamp has the advantage that its emission spectrum contains several lines over a reasonable range of wavelength. Wavelengths in air of prominent spectral lines of these elements are listed in Table 3.

**Table 3 Emission lines of zinc, cadmium and mercury (reference(h)).**

Element	Wavelength (nm)	Element	Wavelength (nm)
Mercury*	185.0	Mercury	435.8
Zinc	213.9	Cadmium	467.8
Cadmium	228.8	Cadmium	480.0
Mercury	253.7	Mercury	546.1
Mercury	365.0	Mercury	579.1
Mercury	404.7	Cadmium	643.8

\* wavelength in vacuum; all others in air

For spectrophotometers with a deuterium lamp, the two deuterium emission lines at 486.0 nm and 656.1 nm (reference (o)) can also be used for wavelength calibration.

*Note: When calibrating a scanning instrument it is most important to use the slowest scanning speed available in order to minimise the effect of the response time of the instrument.*

Traditionally the minimum recommended number of points to be checked on the wavelength scale has been one in the UV range and three in the visible range. However modern instruments are very linear and for such instruments two points are likely to be sufficient. Some modern instruments have built-in wavelength checks using deuterium or glass filters and these should be used between the annual full service/calibration by the relevant accredited agency.

**2.2 Absorbance Calibration**

As the concentration of an absorbing species is usually directly proportional to the measured absorbance, the linearity and the stability of the photometric scale must be confirmed. The stability of the photometric reading must be adequate so variations during the course of the determination do not limit precision. It is recommended that absorbance calibration is carried out at absorbances and wavelengths routinely used by the laboratory. Typically this would include six to eight absorbance values over the range 0 to 3 at 546 nm for an instrument used in the visible and four to six absorbance values at one wavelength in the range 230—320 nm for instruments used at wavelengths shorter than 330 nm. For newer instruments it may be possible to verify the absorbance scale at only one wavelength. However, evidence validating that conclusion for a number of measurements would need to be provided to IANZ.

The following reference materials are preferred:

**2.2.1 Visible region****(a) Neutral density glass filters, specifically at 546 nm.**

Certified neutral density filters are available from some NMIs and chemical supply companies. These filters will need a first recertification after 5 years and thereafter every 10 years.

**(b) Analytical reagent grade copper sulphate in 1% sulphuric acid.**

The required solution is prepared by dissolving 20.0 g of copper sulphate pentahydrate (CuSO<sub>4</sub>·5H<sub>2</sub>O) in water to which is carefully added 10 ml sulphuric acid (d=1.83) and then diluting to 1 litre. The reference is a solution of sulphuric acid of the same concentration. Absorbances of this solution in 10 mm cells at different wavelengths are given in Table 4.

Copper sulphate solutions of different concentrations can be used to check the linearity of absorbance scales. The use of the absorbances at 600 nm and 650 nm is particularly useful for examining the low end of the absorbance scale.

**Table 4 Absorbance values for CuSO<sub>4</sub>·5H<sub>2</sub>O (20.0g.L<sup>-1</sup>) in 1% H<sub>2</sub>SO<sub>4</sub> (adapted from reference (o)).**

Wavelength (nm)	Absorbance
600	0.068
650	0.224
700	0.527
750	0.817

The absorbance data which form the basis for these operational checks were in all probability obtained with high quality instruments operating on narrow bandwidths (i.e. less than 10 nm) and the agreement between measured and reference absorbance should therefore be better than 2% of the absorbance. In testing instruments with relatively wide bandwidths it is likely that the absorbances obtained with test solutions will be lower than those in Tables 4 and 5 (reference (o)).

### 2.2.2 Ultraviolet

#### (a) Analytical reagent grade potassium dichromate in 5 x 10<sup>-3</sup> mol.L<sup>-1</sup> sulphuric acid.

Potassium dichromate solutions in sulphuric or perchloric acid can be used either in sealed cuvettes or by using prepared solutions in sealed ampoules.

Potassium dichromate is dried for 30 to 60 minutes at 140-150°C and then used to prepare either of the following two solutions depending on the range of absorbance to be tested:

Solution A: 50mg ± 0.5mg in 1 litre of 5 x 10<sup>-3</sup> mol.L<sup>-1</sup> sulphuric acid for the absorbance range 0.2—0.7.

Solution B: 100mg ± 1.0mg in 1 litre of 5x10<sup>-3</sup> mol.L<sup>-1</sup> sulphuric acid for the absorbance range 0.4—1.4.

Absorbance measurements should be made in 10 mm cells at a temperature in the range 15-25°C using 5 x 10<sup>-3</sup> mol.L<sup>-1</sup> sulphuric acid as reference. Table 5 contains nominal values for the absorbances of these solutions in 10 mm cells. (More precise values for five concentrations are given in reference (f) for SRM 935a in solutions made up with perchloric acid). It is important to note that the observed absorbances are affected by the length of the cell and a significant deviation from the specific values may originate from non-standard cells. Cell lengths should be checked at purchase.

**Table 5 Nominal absorbance values for K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in 5 x 10<sup>-3</sup> mol.L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> (reference (o))**

Wavelength (nm)	Solution A	Solution B
235 (min)	0.626	1.251
257 (max)	0.727	1.454
313 (min)	0.244	0.488
350 (max)	0.536	1.071

Over small concentration ranges, potassium dichromate solutions in 5 x 10<sup>-3</sup> mol.L<sup>-1</sup> sulphuric acid obey the Beer-Lambert law and hence can be used to check the linearity of the absorbance scale on spectrophotometers. Otherwise, small deviations (up to 1.6%) can be expected from the the Beer-Lambert law.

#### b) Metallised quartz filters

Metallised quartz filters that reflect rather than absorb are also available for use as absorbance standards in the UV; in using these particular care must be taken in testing for isochromatic stray light because of the likelihood that these filters may introduce stray light, particularly in the grade of laboratory instruments used for routine analysis. These filters may also introduce multiple reflections with other components and must be handled with care because as surface devices they are vulnerable to scratching. Notwithstanding these comments, filters of this type have demonstrated stability and have application in the UV spectral range.

CRMs for options 2.2.1(b) and 2.2.2(a) are available from the National Institute of Standards and Technology in Washington, USA (NIST) and include but are not limited to those listed in references (e) and (f). Alternatively, they can be made using AR grade chemicals or CRMs. Reference (o) contains information on other suitable absorbance standards. It should be noted that the NIST crystalline potassium dichromate reference material is calibrated in solution with perchloric acid whereas the NIST CRM in sealed ampoules uses sulphuric acid as does reference (c).

### 2.3 Liquid sample holder absorption

For options 2.2.1(b) and 2.2.2(a) the absorbance of blank cuvettes/ampoules containing water must also be measured and allowed for in the calibration. Glass cells should be checked at 356 nm and 650 nm and quartz cells at 220 nm and 240 nm.

## 2.4 Stray light control

Stray radiant energy (SRE), often referred to as stray light, falls into two categories:

- Light from the monochromator at wavelengths different from, and mostly very different from, the selected passband, often referred to as heterochromatic stray light.
- Light in the selected wavelength passband which is scattered into the beam exiting the sample chamber without passing through the sample, often referred to as isochromatic stray light.

These categories are illustrated in Fig. 1. Tests should be made for the presence of both of these as part of the operational qualification of a spectrophotometer.

FIG. 1(a) Heterochromatic stray light

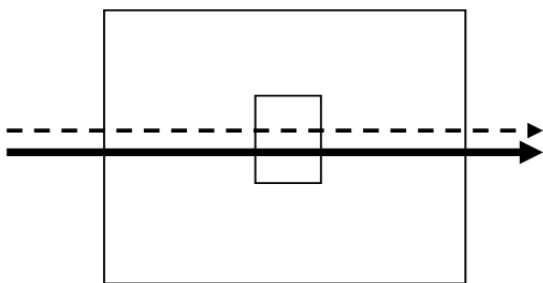
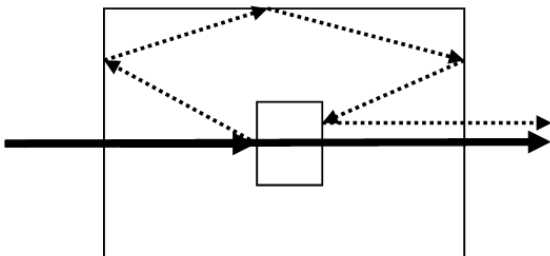


FIG. 1(b) Isochromatic stray light

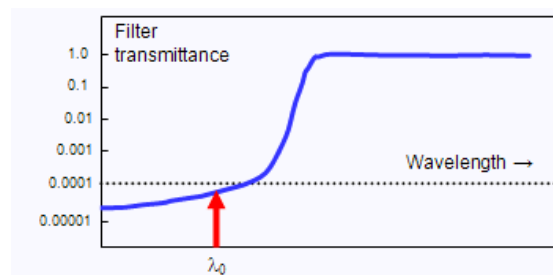


For heterochromatic stray light the test is to set the monochromator to select a wavelength  $\lambda_0$ , insert a cut-off or a band-stop filter that blocks, and measure the transmittance; the observed transmittance at  $\lambda_0$  is a measure of the SRE content of the monochromator output at longer wavelengths (cut-off filter) or wavelengths above and below  $\lambda_0$  (bandpass filter). In preference this should be done with a band pass filter but the only suitable and readily available one is methylene blue ( $\lambda_0 = 600\text{--}660\text{ nm}$ ). Consequently cut-off filters are used as illustrated in Fig. 2. Heterochromatic stray light and the resulting errors are dangerous because they are frequently unsuspected and, being often due to contamination of the monochromator grating, they have a tendency to increase with time. The test described here does not catch “nearby” SRE or SRE on the UV side of

UV-vis beams. Filters that may be used for this test and the corresponding wavelengths  $\lambda_0$  include;

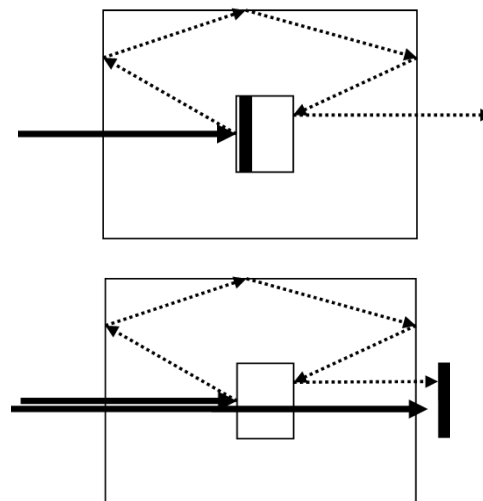
- Various sharp-cut filter glasses
- Liquid filters
  - ♦ KCl 12 g/L aqueous at 175–200 nm
  - ♦ NaI 10 g/L aqueous at 210–259 nm
  - ♦ Acetone at 250–320 nm
  - ♦  $\text{NaNO}_2$  50 g/L aqueous at 300–385 nm.

FIG. 2 Test for the heterochromatic stray light: measure the transmittance in the pass band of a cut-off filter that stops the monochromator set wavelength (indicated by the arrow)



For isochromatic stray light the test is to insert a completely absorbing stop in a cuvette and to compare the signal obtained with that which is obtained when a stop is placed at the exit of the sample compartment (see Fig. 3). The sensitivity of the test can be enhanced by inserting a mirror at the front surface of the cuvette; this exaggerates any introduction of stray light by scattering in the sample compartment and the results of such a test should be treated with caution.

FIG. 3 Test for isochromatic stray light: compare the signal with the cuvette stopped to that obtained when all light exiting the sample compartment is stopped.



### 3 Uncertainty of measurement

With the introduction of ISO/IEC 17025 there is now a requirement for all laboratories, testing laboratories as well as calibration laboratories, to determine the uncertainty of each of their measurements. Obviously for equipment being used to make measurements in testing the starting point for this determination will be an awareness of the uncertainty contribution provided from each measuring instrument. Accredited metrology laboratories are required to report the uncertainty for their calibrations and all IANZ endorsed calibration certificates will provide this information. As appropriate, this data can be directly used in the testing laboratory's own determination of uncertainty of measurement in testing.

When a testing laboratory chooses to carry out its own in-house calibration, the uncertainty of that calibration will need to be determined using appropriate methods. Depending on the degree of rigour required, there are a number of different approaches to this. Various guidance documents are available and testing laboratories should adopt methods appropriate for their purposes.

### 4 Acknowledgements

Dr John Clare – Measurement Standards Laboratory of New Zealand, Industrial Research Limited.

Mr John Small – Global Scientific (previously known as Medic Scientific).

Mr Graeme Thompson – previously of Biolab Scientific Limited.

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