



Reference materials for the calibration of UV/visible light spectrophotometers

Introduction

This Guide provides information about reference materials that can be used when calibrating or checking the performance of a UV/Visible light spectrophotometer (herein referred to as a spectrophotometer). The known absorbance of reference materials at particular wavelengths can be used to validate the scale of absorbance (photometric accuracy), the wavelength accuracy of a spectrometer and identify stray light problems.

Other parameters such as temperature, sample path length and solvent may influence the measurement result but are not discussed here.

Wavelength Calibration

The wavelength calibration of a spectrophotometer is carried out by using the instrument to identify the wavelengths at which features of a reference occur (such as absorbance or emission lines). The observed wavelengths are compared with the calibrated or known values for the reference. In some instruments, it is possible to refine the wavelength scale, or introduce a correction, based on the results of such an investigation. However, regardless of whether such a correction can be made, the results of a wavelength calibration can be used to assess the measurement uncertainty, when using the instrument, due to errors in the wavelength scale.

All wavelength calibration measurements should be made using the spectral bandwidth that is required for the measurements of interest. Similarly, reference materials with broad features such as absorbing solutions and glass filters should have known or calibrated values at that bandwidth.

When calibrating a scanning instrument, it is important to use the same scanning speed as is used for actual measurement.

Wavelength calibration should be carried out at wavelengths as close as possible to the wavelengths of interest during use of the instrument. The number of points to be checked will depend on the wavelength accuracy requirements of the measurement. Some modern instruments have built-in wavelength checks using deuterium or glass filters and these should be used between the annual full service/calibration.

Any of the following three reference materials may be acceptable, depending on the use of the spectrophotometer.

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 and can

vary strongly with bandwidth so glass filters must be calibrated before use; nominal values are listed in Table 1.

Table 1. Nominal wavelengths of selected absorption maxima for holmium and didymium glass filters.

Wavelength of maximum (nm)	
Holmium glass	Didymium Glass
241.5*	440.8
279.4	481.2
287.5	528.8
333.7	573.0
360.9	586.0
386.0	685.0
418.4	741.2
445.9	807.1
453.2	879.3
536.2	
637.5	

*not seen in all batches

Solution of holmium (III) in perchloric acid.

A solution of holmium oxide in perchloric acid provides sharper and less matrix-dependent lines than when the matrix is glass and makes available a spectral line at 241 nm that is not always measurable in glass. This solution is available as a reference material from some National Metrology Institutes (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 wavelengths of prominent lines of the holmium solution depend on the spectral bandwidth of the spectrophotometer; consensus values for spectral bandwidths of 0.1 nm, 1 nm and 3 nm are listed in Table 2. These wavelengths are considered intrinsic to holmium oxide and can be used without external calibration provided the solution is prepared carefully.

Note: Extreme care is needed in the handling and storage of perchloric acid – consult relevant health and safety documentation or guidance.

Table 2. Wavelengths and expanded uncertainties (of the absorption maxima of holmium (III) in perchloric acid for three values of spectral bandwidth [1]).

0.1 nm spectral bandwidth		1 nm spectral bandwidth		3 nm spectral bandwidth	
Absorption maximum (nm)	Expanded uncertainty* (nm)	Absorption maximum (nm)	Expanded uncertainty* (nm)	Absorption maximum (nm)	Expanded uncertainty* (nm)
240.98	0.04	241.12	0.05	241.03	0.06
249.80	0.05	249.87	0.06	250.06	0.09
259.98	0.08	260.22	0.07	260.12	0.09
278.16	0.04	278.13	0.05	278.04	0.06
287.02	0.04	287.19	0.06	287.61	0.07
293.34	0.04	293.39	0.05	293.33	0.07
333.49	0.04	333.47	0.04	333.48	0.07
345.47	0.04	345.39	0.04	345.52	0.07
361.29	0.05	361.25	0.03	361.09	0.06
385.38	0.04	385.61	0.04	385.99	0.09
416.05	0.04	416.26	0.05	416.86	0.08
450.63	0.04	-	-	-	-
452.02	0.04	451.40	0.05	451.28	0.05
467.78	0.03	467.82	0.03	468.11	0.06
473.75	0.05	473.52	0.03	473.53	0.06
485.21	0.04	485.23	0.04	485.21	0.06
536.43	0.04	536.56	0.04	537.19	0.07
640.43	0.03	640.50	0.03	641.11	0.06
652.68	0.05	652.69	0.04	653.12	0.18

* The expanded uncertainties are given at the 95% confidence level with a coverage factor of 2.1. See reference [2] for an explanation of terms.

Low pressure discharge lamps containing mercury, cadmium, zinc, neon, argon or krypton.

Some spectrophotometers contain lamp housings for low pressure discharge lamps, or light from such lamps can be coupled into the spectrophotometer. Where this facility is provided, wavelength checks can be made very conveniently by operating the discharge lamp and isolating the emitted spectral lines. The mercury lamp has the advantage that its emission spectrum contains several lines over a reasonable range of wavelengths. Wavelengths in air of some prominent spectral lines of these elements are listed in Table 3.

For spectrophotometers with a deuterium lamp, the two deuterium emission lines at 486.0 nm and 656.1 nm [4] can also be used for wavelength calibration.

Table 3. Emission lines of zinc, cadmium, mercury [3].

Element	Wavelength (nm)	Element	Wavelength (nm)
Mercury*	184.950	Mercury	435.833
Zinc	213.856	Cadmium	467.815
Cadmium	228.802	Cadmium	479.991
Mercury	253.652	Mercury	546.074
Mercury	365.015	Mercury	579.066
Mercury	404.656	Cadmium	643.847

* wavelength in vacuum; all others in air

Absorbance Calibration

The absorbance calibration of an instrument is carried out by measuring the absorbance of reference materials on the spectrophotometer. The differences between measured values of absorbance and calibrated or known values can be used to assess the measurement uncertainty due to error in the absorbance scale. A correction factor for the absorbance scale error is not usually applied.

Reference materials should be chosen to cover both the range of absorbances and the range of wavelengths required for routine work in the laboratory. Typically, for an instrument used in the visible, this would include several absorbance values over the range 0 to 3 at significant visible wavelengths. In addition, for instruments used at wavelengths shorter than 330 nm, several absorbance values should be included for at least one wavelength in the range 230 nm - 320 nm. The following reference materials are preferred.

Absorbing neutral density glass filters, at wavelengths between 380 nm and 1000 nm

Certified neutral density filters are available from some NMIs and chemical supply companies.

Metallised quartz neutral density filters

Metallised quartz filters that reflect rather than absorb are also available for use as absorbance standards throughout the UV and visible wavelengths; in using these, care must be taken in testing for isochromatic stray light (see section 3.2) because of the likelihood that these filters may introduce stray light. 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.

Analytical reagent grade copper sulphate in 1 % sulphuric acid.

The required solution is prepared by dissolving 20.0 g of copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 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 relative to the solvent in 10 mm cells at different wavelengths are given in Table 4.

Table 4. Absorbance values for $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (20.0 g.L⁻¹) in 1% H_2SO_4 [5].

Wavelength (nm)	Relative Absorbance	Expanded Uncertainty*
600	0.068	0.002
650	0.224	0.002
700	0.527	0.002
750	0.817	0.002

* The expanded uncertainties are given at the 95% confidence level with a coverage factor of 2.0. See reference [2] for an explanation of terms.

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.

The absorbance data which form the basis for these operational checks were obtained with instruments operating on narrow bandwidths (i.e. less than 10 nm), after preparation of the solution and leaving it for longer than 5 days to allow for settling of any turbidity and at 25°C.

Analytical reagent grade potassium dichromate in 5×10^{-3} mol.L⁻¹ 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 solutions depending on the range of absorbance to be tested:

- Solution A: 50mg \pm 0.5mg in 1 litre of 5×10^{-3} mol.L⁻¹ sulphuric acid for the absorbance range 0.2—0.7.
- Solution B: 100mg \pm 1.0mg in 1 litre of 5×10^{-3} mol.L⁻¹ 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 5×10^{-3} mol.L⁻¹ sulphuric acid as reference. Table 5 contains nominal values for the relative absorbances of these solutions in 10 mm cells. (More precise values for five concentrations are given in reference [6] for SRM 935a in solutions made up with perchloric acid). It is important to note that the observed absorbances will be 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 relative absorbance values for $\text{K}_2\text{Cr}_2\text{O}_7$ in 5×10^{-3} mol.L⁻¹ H_2SO_4 [7].

Wavelength (nm)	Absorbance	
	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 5×10^{-3} mol.L⁻¹ 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.

Stray light control

Stray radiation, often referred to as stray light, falls into two categories (see figure 1):

Light from the monochromator at wavelengths different from that selected, which follows the path of the beam through the sample – often referred to as *heterochromatic* stray light.

Light of the selected wavelength which is scattered into the beam path without passing through the sample – often referred to as *isochromatic* stray light.

Tests should be made for the presence of both categories of stray light as part of the absorbance calibration of a spectrophotometer. The test is not a calibration itself; if a result is obtained outside the required tolerances, it may be necessary to service the instrument.

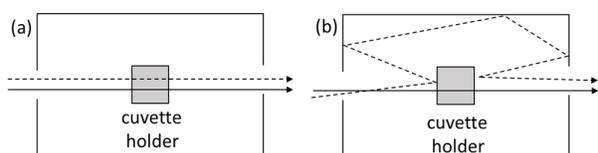


Figure 1. (a) Heterochromatic and (b) isochromatic stray light

Heterochromatic stray light

Heterochromatic stray light is often due to contamination of the monochromator grating, and therefore has a tendency to increase with time. For heterochromatic stray light, the test is to set the monochromator to select a wavelength λ_0 , insert a cut-off or a band-stop filter that should block light at that wavelength, and measure the transmittance or absorbance of the filter.

When using a cut-off filter, if the transmittance of a cut-off filter is higher than expected (or the absorbance lower than expected) the value obtained is a measure of stray light of wavelengths longer than λ_0 when the instrument is set to λ_0 .

Or, when using a band stop filter, if the transmittance is higher than expected (or the absorbance lower than expected) the value obtained is a measure of stray light of wavelengths above and below λ_0 when the instrument is set to λ_0 .

Clearly, more information can be obtained by using band-stop filters, but only methylene blue ($\lambda_0 = 600\text{--}660\text{ nm}$) is suitable and readily available. Consequently, cut-off filters, as illustrated in figure 2, are used more often.

The test for heterochromatic stray light does not identify “nearby” stray light or stray light of wavelengths shorter than λ_0 . Filters that may be used to test for this (at the corresponding wavelengths λ_0) include:

Various sharp-cut filter glasses

Liquid filters

- KCl 12 g/L aqueous at 175 nm –200 nm
- NaI 10 g/L aqueous at 210 nm –259 nm
- Acetone at 250 nm –320 nm
- NaNO_2 50 g/L aqueous at 300 nm –385 nm.

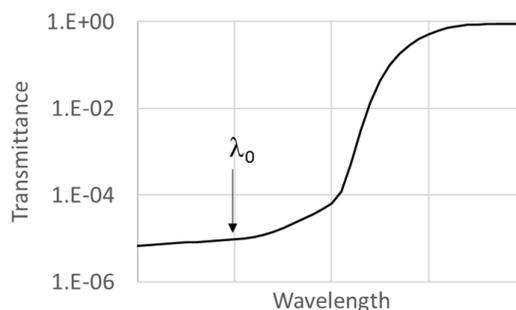


Figure 2. A test for heterochromatic stray light: measure the transmittance in the absorbing region of a cut-off filter covering the set wavelength (indicated by the arrow)

Isochromatic stray light

The test for isochromatic stray light compares the signal obtained when a completely absorbing stop is inserted in the cuvette holder to the signal 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 a cuvette. However, the results should be treated with caution, because this exaggerates any introduction of stray light by scattering in the sample compartment.

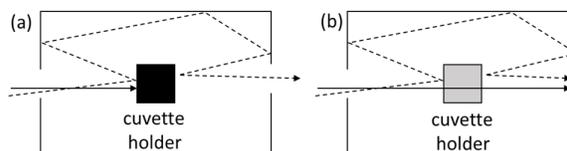


Figure 3. Test for isochromatic stray light: compare the signal in (a), with the cuvette stopped, to the signal in (b), obtained when all light exiting the sample compartment is stopped.

Availability of reference materials

Calibrated glass holmium, absorbing neutral density, reflectorized neutral density and a selection of glass cut-off filters are available from MSL. Alternatively, filters or solutions can be purchased from commercial suppliers and calibrated at MSL.

Several liquid filters or certified reference materials for making up solutions, for both absorbance and wavelength calibration, are available from the National Institute of Standards and Technology in Washington, USA (NIST). Alternatively, they can be made using AR grade chemicals. Reference [7] contains information on other suitable absorbance standards.

Further Information

If you want to know more about reference materials and calibration of spectrophotometers, contact MSL and book in for a Spectrophotometry Workshop. See the MSL website <http://measurement.govt.nz/>.

Prepared by A Koo. Version 1, July 2017.

This guide replaces IANZ TG4, version 3.

References

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- [2] *Guide to the expression of uncertainty in measurement*, JCGM 100:2008, (BIPM, 1st edition, 2008)
- [3] *Line Spectra of the Elements*, J Reader and C H Corliss, CRC Handbook of Chemistry and Physics, 79th Ed, Section 10 pp 1-87 (1998)
- [4] These and other spectral lines can be found at http://physics.nist.gov/PhysRefData/ASD/lines_form.html
- [5] *Filters for the reproduction of sunlight and daylight and the determination of color temperature*, Davies, R. and Gibson, K.S., NBS Miscellaneous Publication M114, National Bureau of Standards (1931).
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- [7] *Standards in Absorption Spectrometry*. UV Spectrometry Group, C Burgess and A Knowles (Eds). Chapman and Hall (1981)