

Wavelength Accuracy – Measurement and Effect on Performance in UV-Visible Spectrophotometry

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Key Words

- Calibration
- Certified Reference Material
- Deuterium Lamp
- Holmium Oxide
- Mercury Lamp
- NIST
- NPL
- NTRM
- UV-Visible
- Wavelength Calibration
- Wavelength Accuracy

Introduction

Good laboratory practice requires that the performance of a UV-Visible spectrophotometer is verified on a routine basis. A performance verification of the wavelength accuracy should be included in the routine evaluation of any spectrophotometer. This application note will discuss environmental factors that influence wavelength accuracy and the algorithm used to calibrate the wavelength scale of Thermo Scientific Evolution™ spectrophotometers. We will also discuss how a factory recalibration can be performed, if necessary, and which certified reference materials are available to verify the wavelength accuracy of a Thermo Scientific UV-Visible spectrophotometer.

Factors Affecting Wavelength Accuracy

Physical Shock

The wavelength accuracy of an instrument may be affected if the spectrophotometer is jolted violently, dropped, or subjected to severe or prolonged vibration. It is recommended that a wavelength accuracy performance verification test be performed each time the instrument is moved.

Temperature

The wavelength calibration can also vary somewhat if the current operating temperature is significantly different from the temperature at which the spectrophotometer was last calibrated. Generally, all instruments that are calibrated at room temperature show wavelength differences around 0.2 nm for every 10 °C temperature change from the calibration temperature. Some Thermo Scientific spectrophotometers are equipped with tungsten and deuterium lamps that generate more heat than a Xenon lamp. It is essential that all instruments are warmed up to a normal operating temperature (usually over a period of 1 hour) before performing instrument verification tests.

Replacement of Key Optical Components

All spectrophotometers will require re-calibration after the replacement of any optical component. The wavelength accuracy of the instrument is greatly affected by the alignment of the optical path through the instrument. Therefore, any changes in the optical alignment of the instrument, through the replacement of optical components, will require a verification of the wavelength calibration and possibly a re-calibration of the instrument.

Mechanical Wear

Our UV-Visible spectrophotometers are designed to minimize the effects of wear. Therefore, mechanical wear should not effect the wavelength accuracy of the instrument. However, good laboratory practice dictates that the performance of spectrophotometers should be evaluated at regular intervals to ensure satisfactory operation.

Calibration Algorithms for the Evolution 300 and Evolution 600 Spectrophotometers

The Evolution 300 and Evolution 600 spectrophotometers guarantee exceptional wavelength accuracy over a wide wavelength range, not simply at a single wavelength. This ensures your instrument will perform the same at 260 nm as it does at 656.1 nm (the typical single line specification for wavelength accuracy). The calibration routines for the Evolution 300 and 600 are also unique and are discussed below.

The Evolution 300 and 600 spectrophotometers use a cubic through zero equation for wavelength accuracy calibration. The cubic fit eliminates the oscillatory features found in many of our competitors' instruments. The difference between a cubic through zero and a oscillatory (sinusoidal) fit is illustrated in Figure 1.

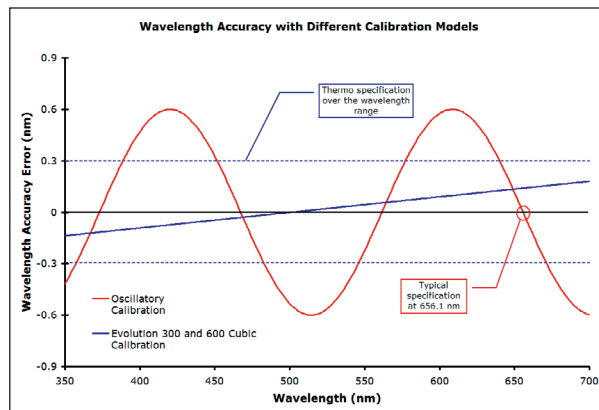


Figure 1: The error in wavelength accuracy due to different calibration methods. The calibration for the Evolution 300 and 600 is shown in blue, a typical oscillatory calibration is shown in red. The maximum error in wavelength accuracy for the Evolution 300 and 600 is shown by the horizontal blue dashed lines. The typical oscillatory calibration point is circled in red.

In Figure 1, the error in wavelength accuracy for the Evolution 300 and 600 instruments is represented by the solid blue line. The horizontal, dashed blue lines represent the wavelength accuracy specification of the Evolution 300 and 600 instruments. Note that the typical oscillatory calibration has wavelength calibration errors that fall outside our Evolution spectrophotometer specifications. The oscillatory calibration is designed to have a minimum error at a single calibration point, usually the 656.1 nm deuterium line. This translates into essentially low error at this single wavelength, but much larger error values elsewhere, for example 510 or 600 nm, as shown in Figure 1.

Performing a Factory Re-calibration Using a Mercury Lamp Accessory

The Mercury Lamp accessory gives you ability to perform the exact same calibration that was performed on your Evolution 300 or 600 at the factory in your own laboratory.

The re-calibration can be performed from Local Control or VISION™ software simply by selecting the appropriate option from the menu. The Mercury Lamp re-calibration option is available on the Evolution 300 and 600 instruments when the Mercury Lamp is installed. The superior wavelength accuracy in the Evolution 600 results from a 10-point calibration. The calibration uses 7 Mercury Lamp accessory lines, 2 lines from the deuterium lamp, and the zero order line of the instrument to calibrate the instrument at each spectral bandwidth (SBW). This means the calibration for a SBW of 0.2 nm is performed and stored independently of the calibration at a SBW of 1.0 nm. The Evolution 300 is calibrated in a similar manner using Mercury Lamp lines, for more information refer to Table 1.

Wavelength Standards

There are several standards available for the verification of wavelength accuracy and a few are listed in Table 2. Some methods use primary standard such as the emission from a mercury lamp. The other methods rely on secondary traceable standards such as solutions of rare earth oxides. Methods that use primary standards are the most reliable and should be used whenever available.

Evolution 300 Wavelength Accuracy (nm)	Evolution 600 Wavelength Accuracy (nm)
253.65	253.65
296.73	296.73
404.66	404.66
435.84	435.84
546.07	546.07
760.95	763.51
871.68	871.68
1092.14	

Table 1: Mercury Lamp lines used for the Wavelength Accuracy Performance Verification tests in the Evolution 300 and 600 spectrophotometers

Standard 1: Emission Lines from a Mercury Source

The emission spectrum of a low-pressure mercury lamp has a number of intense lines that cover a large part of the UV and visible range. Figure 2 shows the emission spectrum of a Mercury Lamp accessory in an Evolution 600 spectrophotometer. The frequencies of these lines are fundamental physical properties of mercury, and are therefore invariant. The mercury emission spectrum is a primary wavelength standard.

The Mercury Lamp is a powerful accessory that allows the wavelength accuracy of the instrument to be evaluated using a primary standard. The mercury lamp is the most preferred method of wavelength accuracy calibration and is recommended by both the U.S. and the European Pharmacopoeias.¹

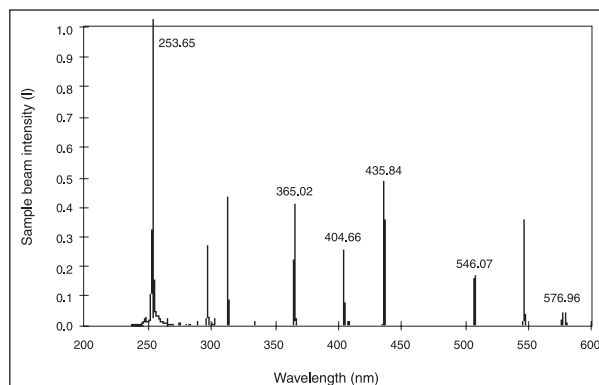


Figure 2: The Emission from a Mercury Lamp accessory measured on an Evolution 600 spectrophotometer

The Mercury Lamp accessory for the Evolution 300 and 600 spectrophotometers is inserted into a lamp position within the optical system. The lamp is easy to install, requires no alignment, and is automatically detected by both Local Control and VISION software. No additional power supply is necessary as the accessory is run completely from the spectrophotometer. Once installed it can be left in place and used only when required.

An automated wavelength accuracy performance verification (PV) test is available in the Local Control and VISION^{pro}™ or VISION^{security}™ software when the Mercury Lamp accessory is installed. This performance verification test evaluates the wavelength accuracy of the spectrophotometer at several wavelengths across the UV-Visible spectrum; these wavelengths are given in Table 1.

Wavelength Standard	Standard Type
Emission of a Mercury Lamp	Primary
Emission of a Deuterium Lamp	Primary
Calibrated traceable Rare Earth Oxides in Solution	Secondary
Calibrated traceable Rare Earth Glasses	Secondary

Table 2: Primary and secondary wavelength standards

This automated PV wavelength accuracy test evaluates the wavelength accuracy of the instrument at the lowest available SBW (0.2 nm for the Evolution 600 or 0.5 nm for the Evolution 300). However, some researchers may wish to verify the wavelength accuracy of the spectrophotometer manually at a different SBW. This can be accomplished by scanning the Mercury Lamp emission spectrum. This test is also an excellent illustration for teaching aspects of wavelength accuracy and spectral bandwidth.

To perform the wavelength accuracy test manually using the Mercury Lamp accessory, select the desired wavelength scan range and configure the instrument as follows:

Local Control Software		VISION Software	
Scan Type	Standard Scan	Data Mode	Intensity
Mode	I (Intensity Mode)	PMT gain	100
PMT Gain	100	Bandwidth	<i>select desired</i>
Bandwidth	<i>select desired</i>	Scan Speed	30 nm/min
Speed	30 nm/min	Data Interval	0.1 nm
Data Interval	0.1 nm	Lamp Change	Mercury
Lamp Change	Hg		

Most of the energy in the mercury spectrum is emitted in the 253.65 nm line, the intensity of which is much greater than the other lines. When the spectrum is first scanned only the 253.65 nm line will be visible, however, the vertical scale can be expanded to show the entire emission spectrum. Exact values for the Mercury emission wavelengths are given in Table 3.

253.65	546.07
296.73	760.95
404.66	871.68
435.84	

Table 3: Fundamental emission lines from the Mercury Lamp accessory in nanometers (nm)

The permitted tolerances for wavelength accuracy are quoted in the specifications of individual spectrophotometers. The mercury lamp accessory enables the user to conveniently demonstrate that the wavelength accuracy of the instrument is within specification, whenever necessary.

If the required tolerances are not met, a wavelength re-calibration is necessary. Under normal circumstances, this would only be expected following replacement of an optical component. The mercury lamp re-calibration re-measures all relevant emission lines, then recalculates and replaces the software compensation equation. More details about the calibration procedure are given in the Calibration Algorithms for the Evolution 300 and Evolution 600 Spectrophotometers section, on page one.

Standard 2: Emission Lines from a Deuterium Source

The emission lines produced from the deuterium lamp are again characteristic of the source element; and as a fundamental physical standard, these wavelengths are invariant. The deuterium emission spectrum is therefore a primary wavelength standard. However, unlike the mercury source, only two characteristic lines are usable. These lines are located at 486.0 and 656.1 nm as shown in Figure 3.

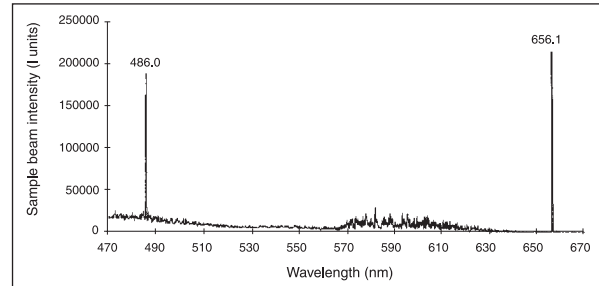


Figure 3: The emission of a deuterium lamp measured on an Evolution 600 spectrophotometer

The emission spectrum (over the desired range) can be easily obtained by selecting scan method parameters as above listed above for the Mercury Lamp, but with lamp change set to Deuterium (D2).

The permitted tolerances for wavelength accuracy are quoted in the specifications of Thermo Scientific UV-Visible spectrophotometers. If the required tolerances are not met, a wavelength calibration is necessary. Under normal circumstances this would only be expected following replacement of a major optical component. More details about the calibration procedure are given in the Calibration Algorithms for the Evolution 300 and Evolution 600 Spectrophotometers section, on page one. The deuterium lamp recalibration uses the deuterium emission line at 656.1 nm to adjust the software compensation equation and is only intended to remove the effects of small movements of optical components and shifts due to temperature, shock, or vibration. If after performing a wavelength accuracy PV test, the errors found is linear and no more than 0.5 nm in magnitude, then a deuterium calibration can be used. The process will take about five minutes and is fully described in the spectrophotometer or software manual. This option can only be used when an existing calibration is present.

Use of Rare Earth Oxide Solutions

Rare earth oxides in solution are preferred when primary standards for performing wavelength accuracy PV tests are not available. These standards are prepared by dissolving rare earth oxides in dilute acidic media. The most frequently encountered standard of this type is holmium oxide in perchloric acid. Solutions of rare earth oxides are preferred over solutions as they are nearly independent of temperature² and have much sharper peaks compared to their glass counterparts.

These solutions are advantageous in their ability to quickly determine the wavelength accuracy of the instrument. The spectrum of these solutions, over the desired spectral range, can be obtained using a tradition wavelength scan. Using secondary standards is quicker and simpler than primary standards, thus making secondary standards ideals for routine checking of the wavelength calibration.

The holmium oxide solution standard (Part No. 222-226400) consists of holmium oxide dissolved in dilute perchloric acid and sealed by heat fusion in a far-UV quartz cuvette. The peak wavelengths are supplied with the calibration certificate and have nominal values of 241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 537, and 640 nm.³ The holmium oxide solution standard is calibrated at SBWs of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 nm. The holmium solution has peaks that are much sharper than those produced by a holmium glass filter, thus, much more sensitive to spectral bandwidth. For best results on a variable bandwidth instrument, the lowest available SBW should be used for the PV test. The peaks of a holmium oxide solution are shown in Figure 4.

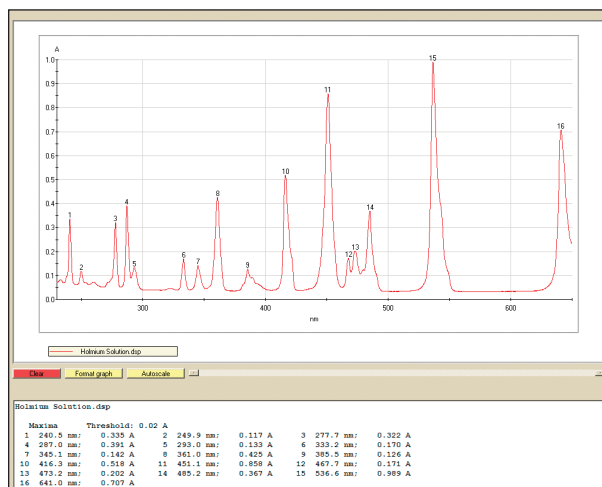


Figure 4: The peaks of a solution of holmium oxide in perchloric acid solution. The scan was acquired on an Evolution 100 spectrophotometer using VISIONlite™ software

The Far UV Wavelength Standard (Part No. 9423UV95540E) consists of rare earth oxide dissolved in dilute sulfuric acid, supplied in a heat-sealed Far UV quartz cuvette. The absorbance spectrum of this rare earth has a series of characteristic peaks in the far UV region at 201, 211, 222, 240, and 253 nm. The Far-UV standard is calibrated at SBWs of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 nm.

Use of Rare Earth Glasses

This technique uses glasses manufactured by fusing the appropriate rare earth oxide in silica. The two most frequently used are holmium and didymium (a mixture of neodymium and praseodymium). Manufacturing can cause batch-to-batch variation in these standards, however, this is overcome by calibrating each standard glass against certified reference glasses from the NPL and NIST via an ISO/IEC standard 17025 procedure. We maintain an ISO/IEC 17025 accredited standards laboratory in Cambridge, UK. This laboratory allows us to provide NIST and NPL traceable CRMs. Having a NIST and NPL traceable laboratory saves valuable time, decreasing the lead-time required to receive traceable standards. Additionally, this laboratory provides re-calibration services for all Thermo Scientific standards.

Rare earth glass standards allow the wavelength accuracy of an instrument to be determined quickly by performing a wavelength scan over the spectral region of interest. We offer a set of two calibrated-traceable wavelength standard filters (Part No. 942318503112). This set contains one holmium glass and one didymium glass filter calibrated against NPL and NIST primary standards.

References

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3. NIST Special Publication 260–120

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