Application Note: 51111

U.S. and European Pharmacopoeias and UV-Visible Spectrophotometers

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Key Words

- Certified Reference Materials
- European Pharmacopoeia
- Operational Qualification (OQ)
- Performance Verification
- Regulatory Compliance
- U.S. Pharmacopoeia

Introduction

UV-Visible spectrophotometers are versatile instruments that measure a variety of different samples in diverse laboratory settings. In some laboratories, instruments are regulated by agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). Thermo Scientific products provide a comprehensive suite of resources to help your laboratory achieve compliance with regulatory agencies. This includes software enabling 21 CFR Part 11 compliance to satisfy the FDA requirements for electronic records and electronic signatures, traceable certified reference materials produced in our own ISO/IEC 17025 accredited standards laboratory, and rigorous validation documentation to help your laboratory achieve and maintain compliance.

Installation qualification (IQ), operational qualification (OQ), and performance verification (PV) tests are performed using certified reference materials (CRMs). CRMs are traceable to primary standards recognized by the National Institutes of Science and Technology (NIST) or the National Physical Laboratory (NPL). Traceability ensures equivalent measurements are made regardless of the instrument or the region of the world where the measurement is made. ISO/IEC 17025 accreditation provides the assurance that our standards laboratory always uses the appropriate processes and procedures. We are the only UV-Visible spectrophotometer manufacturer to have a fully accredited laboratory that produces standards traceable to both NIST and NPL.

Documents like the U.S. Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia (BP), the Japanese Pharmacopeia and the Australian TGA provide specific testing protocols and, in some cases, specifications for instrument performance. These guidelines, in conjunction with the Thermo Scientific *UV Validator*, help your laboratory achieve and maintain regulatory compliance. The *UV Validator* guides you step-by-step through the process of IQ and OQ, writing standard operating procedures (SOPs), and storing records in one location for easy access during an audit.

This application note will focus on the requirements for UV-Visible spectrophotometers specified in the USP and EP. It is designed to help you understand what is and is not required in the individual Pharmacopoeias and specifically what reference material is needed to perform each test. Since both the USP and EP frequently allow alternative methods for testing, a list of CRMs and re-calibration services available is also supplied.

USP and EP Specifications for UV-Visible Spectrophotometers

Section 851 of the USP describes the requirements for UV-Visible spectrophotometers. Additional specifications are provided in some of the individual monographs that use UV-Visible spectrophotometers to perform the assay, such as the quantification of Lycopene [PF30(2)]. Likewise, the requirements for UV-Visible spectrophotometers are given in section 2.2.25 of the EP. The EP details some specific performance requirements in this section, such as the permitted tolerance for wavelength accuracy. However, like the USP, additional specifications are found in the individual monographs of the EP.

It can be inferred from both the USP and EP that your instrument must meet the general requirements specified in the instrument specific section and the performance expectation of the individual monographs. Therefore, if you are performing an assay given in a specific monograph, you are bound by the tolerance given in that monograph.

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General Test Descriptions

Photometric Accuracy, or absorbance accuracy, is the deviation between the absorption value measured on a spectrophotometer and the precisely known absorbance value of a traceable standard. Photometric accuracy is extremely important when the extinction coefficient of an analyte is being determined or when single point quantification measurements are performed.

Resolution is related to the physical slit width of the spectrophotometer, which in turn determines the spectral bandwidth (SBW) of the instrument. By definition, resolution is the ability to resolve two adjacent peaks. Therefore, resolution and spectral bandwidth are not identical measurements. Figure 1 shows the variation in the toluene in hexane test on a Thermo Scientific Evolution™ 600 instrument as a function of SBW. From Table 1 you can see the strong dependence of the SBW on the ratio obtained from the peak and the valley of the test.

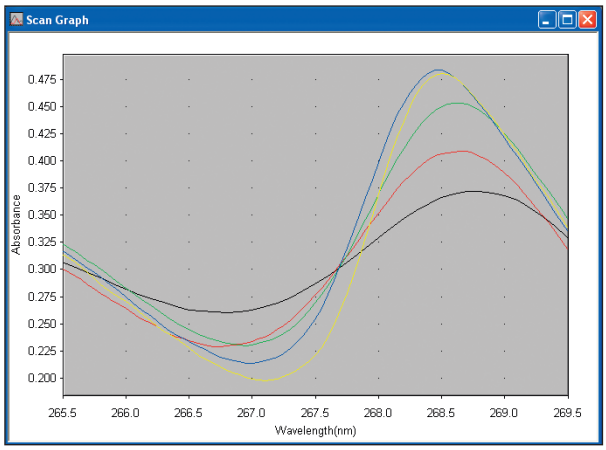

Figure 1: The toluene in hexane performance verification test at various spectral bandwidths. Scans at 0.2 nm (blue), 0.5 nm (yellow), 1.0 (green), 1.5 (red), and 2.0 (black) are shown. Typical ratios are shown in Table 1.

SBW (nm)	0.5	1.0	1.5	2.0	3.0
Ratio	2.5	2.1	1.6	1.4	1.0

Table 1: Nominal minimum/maximum absorbance ratios of the toluene in hexane test as a function of spectral bandwidth (SBW)

It is important to remember that as the physical and spectral bandwidth decrease, so does the energy throughput, thus the signal-to-noise ratio of the measurement is lower. It is always recommended that the SBW of the instrument not exceed 10% of the natural bandwidth of the absorption band.

Stray Light is stray radiant energy present in the spectrophotometer. Instrumental stray light evaluates the efficiency of the instrument, which is influenced by scattering, higher order diffraction, and the performance of optical components and the detector(s). Stray light limits instrument performance at high absorbance or low transmittance values. For more information, request Thermo Scientific Technical Note 51170, *Stray Light – Measurement and Effect on Performance in UV-Visible Spectroscopy.*

Wavelength Accuracy can be defined as the discrepancy between the wavelength value reported by the instrument for a peak and that of a precisely known peak. This test effectively evaluates the performance of the moving mechanical components responsible for positioning the monochromator. While these components are very stable, they are susceptible to the effects of thermal changes and vibration. For more information, request Thermo Scientific Technical Note 51171, *Wavelength Accuracy – Measurement and Effect on Performance in UV-Visible Spectroscopy.*

The sections that follow describe the general requirements of the EP given in section 2.2.25 and the USP in section 851.

European Pharmacopeia Specifications

Section 2.2.25 entitled *Absorption Spectrophotometry, Ultraviolet and Visible* of the EP details how to perform UV-Visible absorption measurements. This section also describes the individual performance verification tests and provides some specific instrument performance specifications. Detailed specifications can also be found in the individual monographs.

Apparatus. This first section of the EP defines the instruments that are appropriate for absorption measurements. It states that the instruments must be "***capable of producing monochromatic radiation in the range of 200 nm to 800 nm***" and have a ***"device suitable for measuring the absorbance"***, i.e. a detector. This is a general requirement and is satisfied by all Thermo Scientific UV-Visible spectrophotometers.

Control of Wavelengths or Wavelength Accuracy. This section describes the verification of the wavelength scale. The EP specifies four methods of determining wavelength accuracy: (1) the absorption maxima of a holmium perchlorate solution, (2) the emission line of a hydrogen discharge lamp, (3) the emission line of a deuterium discharge lamp, and (4) the emission lines of a mercury vapor lamp. A table of absorption maxima for each of these methods is given in Table 2. The EP states, ***"the permitted tolerance is ± 1 nm for the ultraviolet range and ± 3 nm for the visible."*** This section also states that, "[s]uitable certified reference materials may also be used." This requirement is satisfied or exceeded by all Thermo Scientific UV-Visible spectrophotometers.

Holmium Perchlorate Solution (nm)	Mercury Vapor Lamp (nm)*		Hydrogen Lamp (nm)	Deuterium Lamp (nm)
241.15	**253.7**	**404.66**	486.1	486.0
287.15	302.25	**435.83**		
361.5	313.16	**546.07**		
536.3	334.15	576.96		
	365.48	579.07		

** The Evolution 300/600 Mercury Lamp Accessory allows automated testing of the lines shown in bold print. Other lines are easily tested with manual scans.*

Table 2: Absorption maxima for EP standards (taken from Table 2.2.25.-1)

Control of Absorbance. This section describes the verification of the absorbance scale, also referred to as **photometric accuracy** or **absorbance accuracy**. The recommended method of verifying photometric accuracy is a solution of potassium dichromate. The EP gives recommendations on the appropriate solvent and concentrations for the preparation of dichromate solutions. The EP states, ***"the tolerance for the absorption is ± 0.01."*** This section also states that, "suitable certified reference materials may also be used." Solutions of potassium dichromate in far-UV sealed cuvettes are appropriate for performing this test and are available from Thermo Scientific. More information is provided in the CRMs sections on the following pages. This requirement is satisfied or exceeded by all Thermo Scientific UV-Visible spectrophotometers.

Limit of Stray Light. This section describes the verification of stray light. The recommended method of verifying stray light is a 12 g/L solution of potassium chloride. The absorbance of this solution in a 1 cm cell should be ***"greater than 2.0 at 198 nm when compared with water as compensation liquid."*** This section also states that, "[s]uitable certified reference materials may also be used." Solutions of potassium chloride in far-UV sealed cuvettes are available from Thermo Scientific. More information is provided in the CRMs sections on the following pages. This requirement is satisfied or exceeded by all Thermo Scientific UV-Visible spectrophotometers.

Resolution. This section defines the procedure for testing the resolution of the spectrophotometer, when prescribed in a specific monograph. The recommended method of testing is a 0.02% (v/v) percent solution of toluene in hexane. The minimum ratio of the absorbance at 266 nm to 269 nm is stated in the monograph. This section also states that, "suitable certified reference materials may also be used." Since the individual monographs define the appropriate limits of this test, please refer to the individual instrument specifications to determine the best instrument for your analysis. Currently, there are no monographs in the EP that specify a resolution requirement for a Visible or UV-Visible spectrophotometer. Typically a spectral bandwidth less than 2.0 nm is required to meet the British Pharmacopoeia (BP) specification. The Evolution 600, Evolution 300, Evolution 60 and the GENESYS™ 6 spectrophotometers meet or exceed this requirement.

Variable Slit Widths. A recent addition to the EP, this section defines the use of an instrument with variable slit-widths or spectral bandwidth (SBW). If using an instrument with a variable slit width, such as the Evolution 300 or Evolution 600 spectrophotometer, the "slit-width must be small compared with the half-width of the absorption band…" Typically, the half-width of the peak being analyzed should be greater than 10 times the SBW. This means an SBW of 1.0 nm should be used to analyze peaks greater than 10 nm wide at half height. The standard continues "…it [slit-width] must be as large as possible to obtain a high value of I_0." With this statement, the EP suggests that the largest possible SBW be used for quantitative analysis.

U. S. Pharmacopeia Specifications

Section 851 entitled *Spectrophotometry and Light Scattering* of the USP details how to perform UV-Visible absorption measurements. This section also describes the individual performance verification tests and the recommended standards for performing these tests; however, it provides no specific instrument performance specifications. These instrument specifications are found in the individual monographs for the assays.

Accuracy of Calibration - wavelength scale. This section describes the verification of the wavelength scale, also referred to as wavelength accuracy. The USP states, ***"the best single source of UV and visible calibration is the quartz-mercury arc."*** The section then continues by stating that, "a hydrogen discharge lamp may also be used." A table of calibration wavelengths for both lamps is given in Table 3.

Quartz Mercury Arc (nm)*		Hydrogen Lamp (nm)
253.7	365.48	486.13
302.25	**404.66**	656.28
313.16	**435.83**	
334.15		

** The Evolution 300/600 Mercury Lamp Accessory allows automated testing of the lines shown in bold print. Other lines are easily tested with manual scans.*

Table 3: Absorption maxima for USP wavelength accuracy standards

A mercury lamp accessory is available for the Evolution 300 and Evolution 600 spectrophotometers. Because the emission lines from the mercury lamp are fundamental properties of the element, there is no inherent calibration or certification necessary with this standard. Additionally, this accessory provides the best method for verifying wavelength accuracy as stated in the USP. Moreover, the Mercury Lamp accessory also allows you to calibrate the wavelength scale of the instrument exactly as it was calibrated at the factory. This saves the cost of a service call should re-calibration be necessary for any reason.

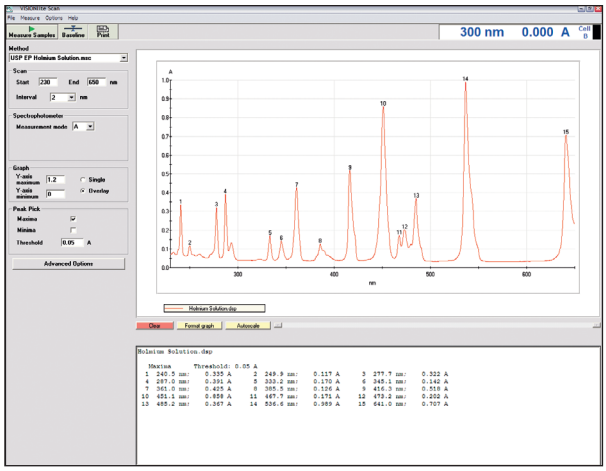

Figure 2: The peaks of a solution of holmium oxide in perchloric acid solution. The scan was acquired on a GENESYS 6 spectrophotometer using VISION*lite*™ software.

If continuous sources are not used, the USP specifies a specific NIST reference material, SRM 2034, a certified holmium oxide solution. Figure 2 shows the peaks of a solution of holmium oxide in perchorlic acid, a test used to verify wavelength accuracy.

The only rigid specification given in section 851 of the USP regarding wavelength accuracy is, **"…comparisons [should] be made at the wavelength at which peak absorption occurs. Should this differ by more than ± 1 nm from the wavelengths specified in the individual monograph, re-calibration of the instrument may be indicated."** This specification is located in a paragraph discussing the good practices for spectrophotometric measurements and does not appear in the section discussing wavelength accuracy tests. Some have inferred that this statement indicates a resolution of 1 nm is a requirement of the USP, however, it is merely a best practice suggestion for performing wavelength calibrations.

Accuracy of Calibration – Photometric Scale. This section describes the verification of the absorbance scale, also referred to as **photometric accuracy**. Here the USP simply states, **"a number of standard inorganic glass filters as well as standards solutions of known transmittances such as… potassium dichromate are available."** Here the USP references two specific NIST publications for SRM 930e and SRM 931e, these documents detail the use of liquid and glass filters for making photometric accuracy measurements.

Stray Light. No specification for stray light is given in Section 851 of the USP.

Resolution. No specification for resolution is given in Section 851 of the USP.

Thermo Scientific Certified Reference Materials

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 by decreasing the lead time required to receive traceable standards. Additionally, this laboratory provides recalibration services for many standards, regardless of the original manufacturer.

The Evolution 300 and Evolution 600 instruments feature an optional mercury lamp accessory. This accessory allows not only for verification of wavelength accuracy, but also allows the user to perform a complete re-calibration of the instrument if necessary. The Mercury Lamp tests performance and offers the ability to perform a wavelength calibration exactly as it is performed in the factory, all without a service call.

Information on our complete line of CRMs is provided in the chart on the following page. Please contact us if you have any additional questions regarding your particular application.

Thermo Scientific Certified Reference Materials

Parameter	Standard	Comments	Detail	Instruments Supported	Part Number
Photometric Accuracy	**Potassium Dichromate in Perchloric Acid**	Recommended by EP Recommended alternative standard for USP	Perchloric acid blank, and 60 mg/l potassium dichromate in 0.001 M perchloric acid Absorbance ≈ 1.0A Calibrated at 235, 257, 313, 350 nm	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95100E Recalibration: 9423UV95120E
	6 Potassium Dichromate Solutions in Perchloric Acid (UV-Visible)	Recommended by EP Recommended alternative standard for USP Set is useful for verifying linearity over a quantitative analysis range	Perchloric acid blank, 20, 40, 60, 80, and 100 mg/l potassium dichromate in 0.001 M perchloric acid Absorbance from 0.1A to 1.5A Calibrated at 235, 257, 313, 350 nm	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95200E Recalibration: 9423UV95220E
	NTRM 930 (Visible only)	Recommended by USP Recommended alternative in EP	1 empty filter holder (blank) 3 neutral density glass filters with nominal absorbance values of 0.5A, 0.7A, 1.0A Neutral Density filters Calibrated at 440.0, 465.0, 546.1, 590.0, 635.0 nm Holmium Oxide glass filter calibrated at wavelengths specified by NIST and NPL	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423CRM9300E Recalibration: 9423UV91760E
	NRTM 1930 (Visible only)	Recommended by USP Recommended alternative in EP	1 empty filter holder (blank) 3 neutral density glass filters with nominal absorbance values of 0.3A, 1.5A, 2.0A Neutral Density filters Calibrated at 440.0, 465.0, 546.1, 590.0, 635.0 nm Holmium Oxide glass filter calibrated at wavelengths specified by NIST and NPL	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423CRM1930E Recalibration: 9423UV91860E
	Nicotinic Acid Solutions in Hydrochloric Acid (UV-Visible only)	Useful for validating photometric accuracy in the deep UV at 213 and 261 nm	HCl blank and 6, 12, 18, and 24 mg/l nicotinic acid in 0.1N HCl Nominal absorbance values of 0.0A, 0.25, 0.50, 0.75, and 1.0A	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95530E
	Set of 9 Traceable Absorbance Filters (Visible only)	Useful for validating photometric accuracy up to 3.0A Wide range of this standard set provides excellent tool for linearity verification	1 clear glass filter (blank) 8 neutral density glass filters with nominal absorbance values of 0.1A, 0.2A, 0.4A, 1.0A, 1.5A, 2.0A, 2.5A, 3.0A	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	942317938091 Recalibration: 401322989811
Resolution	**Toluene in Hexane**	Recommended by EP for verification of instrument resolution	0.020% v/v solution of toluene in hexane and hexane blank Maximum absorbance closest to 268.7 nm Minimum absorbance closest to 267.0 nm Ratio = Abs(max)/Abs(min)	Evolution: 600,300,100,60 GENESYS 6	222-226600
Stray Light	**Potassium Chloride in Aqueous Solution**	Recommended by the EP	Measures stray light between 190 to 200 nm 12 g/l aqueous solution of KCl Water blank	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95520E
	Sodium Iodide in Aqueous Solution		Measures stray light at 220 nm 10 g/l aqueous solution of NaI Water blank	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95500E
	Sodium Nitrite in Aqueous Solution		Measures stray light at 340 nm 5% aqueous solution of $NaNO_2$ Water blank	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	222-226500
Wavelength Accuracy	**Mercury Lamp Accessory**	Recommended by the USP	Fundamental standard producing calibration lines from the UV to the near-IR	Evolution: 600, 300	Evolution 300 10020201 Evolution 600 10022101
	Holmium Oxide in Perchloric Acid Solution (UV-Visible)	Recommended by the EP Recommended alternative in USP	One sealed cell with 4% v/v solution of holmium oxide in 10% perchloric acid	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	222-226400
	2 calibrated / traceable wavelength filters (UV-Visible)	Recommended alternative in USP and EP	One Holmium GLASS filter calibrated against NPL & NIST Primary Standards One Didymium GLASS filter calibrated against NPL Primary Standard	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	942318503112 Recalibration: 401322989841
	Rare Earth in Sulfuric Acid (UV-Visible only)		One sealed cell with 4% v/v solution of a rare earth in sulfuric acid Calibrated at nominal wavelength values of: 254, 240, 223, 212, 201 nm	Evolution: 600,300,100,60 Helios: α, β, γ, δ GENESYS 6	9423UV95540E

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