Spectroscopy Solutions for Pharmaceuticals:

Confidence in Compliance to USP <857> Using the Agilent Cary 60 UV-Vis Spectrophotometer

White Paper

Introduction

UV-Vis spectroscopy is one of the most commonly used analytical techniques in clinical chemistry, pharmaceutical research, and quality control/quality assurance (QA/QC). Regulatory requirements for UV-Vis instruments used in these environments ensure that they are subject to design qualification (DQ), manufacturing quality control, lifecycle management, and installation/operational qualification (IQ/OQ), all of which can be demonstrated through the manufacturing quality records and equipment validation certification of the instrument manufacturer. Laboratory managers and administrators must set up appropriate controls on laboratory access, and ensure that system suitability tests (SSTs) and standard operating procedures (SOPs) are documented and followed. Commonly, the guidelines used to generate these tests and procedures are defined by global pharmacopeias including the USP and EP, all of which require that the performance of UV-Vis spectrophotometers is regularly verified.

Agilent Technologies

The updated USP chapter <857> guides instrument validation protocols for UV-Vis spectroscopy. Table 1 outlines the main changes that have occurred, and this white paper outlines how to ensure that your instrument meets the performance criteria required using an Agilent Cary 60 UV-Vis spectrophotometer running on the updated, Windows 10-compatible Agilent Cary WinUV software.

Performance Test Requirements According to USP Chapter <857>

The automatic regulatory testing capabilities embedded within the Cary WinUV Validate application (provided with every Cary 60 UV-Vis spectrophotometer) deliver trusted reliability in the laboratory, and ensure that the instrument being used is suitable for its intended use as part of operational qualification (OQ). Agilent also offers a comprehensive portfolio of service plans and regulatory compliance services to help achieve validation of the Cary 60 UV-Vis spectrophotometer, software, and its components to ensure the highest levels of reliability and performance.

The update to USP <857> describes important revisions to four main performance capabilities:

- Control of wavelengths (wavelength accuracy and precision)
- Control of absorbance (photometric accuracy and precision)
- Stray light
- Resolution

The testing protocol for each of these, along with the details of the tests that are automatically run by the Cary WinUV Validate application, and examples of data from the Cary 60 UV-Vis spectrophotometer will be outlined in the following sections. To execute these tests, USP<857> recommends that the instrument performance be qualified over the intended operational range. This will vary according to the analytical protocol of different laboratories. In addition, USP<857> indicates that certified reference materials (CRMs) should be sourced from a credible supplier, such as Agilent, and should be recertified at regular intervals to maintain the validity of the certified values.

Table 1. Updates to USP<857> for OQ of UV-Vis Spectrophotometers

USP <857> Requirement	What has changed?	Agilent Cary WinUV software test protocol
Control of wavelengths	New accuracy tolerances: 200–400 nm ±1 nm 400–700 nm ±2 nm New precision tolerances: <0.5 nm standard deviation	At least six replicate measurements, reporting mean and standard deviation for each analysis wavelength.
Control of absorbances	UV accuracy (potassium dichromate) new tolerances: <1 Abs, <±0.01 Abs >1 Abs, <±1 % Abs New precision test for UV (potassium dichromate): <1 Abs, <±0.005 Abs >1 Abs, <±0.5 % Abs	At least six replicate measurements, reporting standard deviation for each analysis wavelength.
	Visible accuracy (NIST filters) new tolerances: <1 Abs, <±0.008 Abs >1 Abs, <±0.8 % Abs New precision test for visible (NIST filters): <1 Abs, <±0.005 Abs >1 Abs, <±0.5 % Abs	At least six replicate measurements, reporting standard deviation for each analysis wavelength.
Stray light	Aqueous potassium chloride (12 g/L), tolerance <1 %T	%T at 198 nm reported
	Aqueous sodium iodide (10 g/L), tolerance <0.05 %T*	%T at 220 nm reported
	Acetone, tolerance <1 %T	%T at 320 nm reported
	Aqueous sodium nitrite (50 g/L), tolerance <0.05 %T*	%T at 370 nm reported
Resolution	The USP now requires resolution to be measured (Toluene in Hexane, 0.02 % v/v)	Ratio of absorbance at 269 and 266 nm

*Cary 60 UV-Vis guaranteed specifications

Control of Wavelengths

Wavelength accuracy

The wavelength accuracy test is used to ensure that the wavelength axis of the UV-Vis spectrum is accurate (correct and within acceptable limits) across the intended operational range. It recommends use of atomic line spectra or rare earth oxides that yield well-characterized absorption bands, and enables the comparison of the UV-Vis spectrophotometer wavelength readings to published values. The analysis of rare earth oxides holmium perchlorate (from 200–600 nm) and didymium (from 700–860 nm), commonly available as CRMs, yields well-characterized peaks (Table 2) across the useable range of the UV-Vis spectrum.

Table 2. Prescribed Wavelengths for the Determination of Wavelength Accuracy and Precision for Holmium Perchlorate and Didymium

Holmium perchlorate (nm)	Didymium (nm)
241.1	731.6
278.1	740.0
287.2	794.1
333.5	799.0
345.4	864.4
361.3	
385.6	
416.3	
451.4	
467.8	
485.2	
536.6	
640.5	

To conduct a wavelength accuracy test according to USP<857>, the Cary 60 UV-Vis spectrophotometer performs a scan, and the peaks that can be resolved on the resulting spectrum (Figures 1 and 2) are then identified, and the precise location of each is cross-checked with the certified data for that standard. USP Chapter <857> requires that wavelength accuracy in the UV and visible regions of the spectrum must be ±1 nm and ±2 nm, respectively.

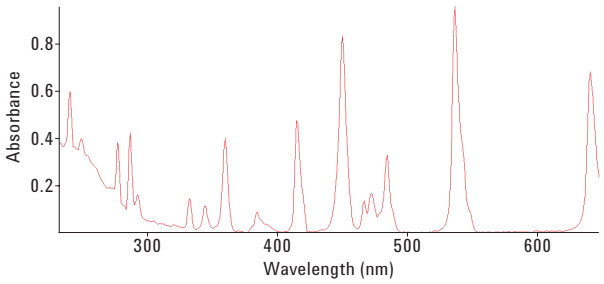

Figure 1. Wavelength scan of holmium perchlorate using an Agilent Cary 60 UV-Vis spectrophotometer and Agilent Cary WinUV software.

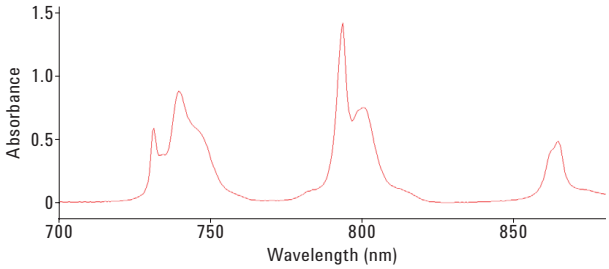

Figure 2. Wavelength scan of didymium using an Agilent Cary 60 UV-Vis spectrophotometer and Agilent Cary WinUV software.

Wavelength precision

The wavelength precision test assesses how reproducibly a scanning UV-Vis spectrophotometer can measure at each specific wavelength in the wavelength range. Wavelength precision is tested by calculating the standard deviation of at least six replicate measurements of the absorbance peaks (Figure 3). USP<857> requires that the precision of UV-Vis instruments is better than 0.5 nm across the operational range of the instrument.

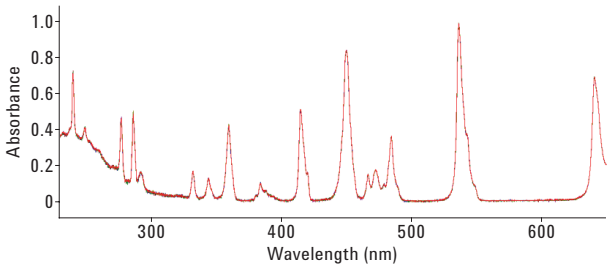

Figure 3. Six overlaid wavelength scans of holmium perchlorate using an Agilent Cary 60 UV-Vis spectrophotometer and Agilent Cary WinUV software.

The WinUV advantage

The Validate application of the Cary WinUV software makes ensuring that your instrument meets the Control of Wavelengths (Wavelength Accuracy and Precision) tests efficient and easy by automatically running the testing protocols for holmium and didymium. In addition, the wavelength accuracy can be confirmed using lines that are intrinsic to the unique Agilent Xenon flash lamp source. The Cary 60 UV-Vis meets the wavelength accuracy and precision performance requirements specified by USP<857>. The Cary WinUV software generates a report that allows the user to review the tests performed and the values obtained for each replicate measurement.

Control of Absorbances

Photometric accuracy

Photometric accuracy and precision tests are used to establish the linearity of a UV-Vis spectrophotometer across the operational absorbance range of the instrument. In essence, these tests are used to ensure that a UV-Vis spectrophotometer yields reliable quantitative measurements. All tests for these parameters rely on the Beer-Lambert Law, which dictates that a linear relationship exists between absorbance and sample concentration. Tests for USP<857> are conducted on solutions of potassium dichromate, which has absorbance peaks in the UV region of the spectrum; NIST-traceable filters (930E) can be similarly used for the visible region of the spectrum.

To test photometric accuracy in the UV region of the spectrum according to USP<857>, a solution of potassium dichromate in dilute perchloric acid is measured, and the absorbance intensity at 235 nm, 257 nm, 313 nm, and 350 nm is determined. The photometric accuracy is determined automatically by the Validate application of the Cary WinUV software, with the capability to analyze up to three absorbance levels in the range of 0–200 mg/L potassium dichromate. When below 1 Abs, the absorbance accuracy must be ±0.01 Abs, and when above 1 Abs, ±1 % of the absorbance measured. Following USP<857>, photometric accuracy in the visible range of the spectrum uses NIST-traceable filters that absorb between 440–635 nm. The Cary WinUV Validate application allows for up to three NIST-traceable filters to be measured automatically, with the user able to enter the expected absorbance, accuracy, and precision. The application generates a report showing the mean result (accuracy) and a Pass or Fail response as determined by the entered tolerances (Figure 4).

Wavelength (nm)	Accuracy (nm) ±	Pass/Fail	Precision	Pass/Fail
235.00	0.7270	PASSED	0.0000	PASSED
257.00	0.8450	PASSED	0.0000	PASSED
313.00	0.2849	PASSED	0.0000	PASSED
350.00	0.6270	PASSED	0.0000	PASSED

Photometric Accuracy Test - K2Cr2O7 Method PASSED

Figure 4. Six replicates of 60 mg/L potassium dichromate were measured, and the Agilent Cary WinUV Validate application generated a report for photometric accuracy and precision (standard deviation).

Photometric precision

USP <857> requires that photometric precision be determined for the UV-Vis spectrophotometer in the intended operational range. Absorbance precision is determined as the standard deviation of six replicate measurements with the tolerances described in Table 1. Figure 4 shows a typical report provided by the Cary WinUV Validate application using a Cary 60 UV-Vis spectrophotometer.

The WinUV advantage

The Cary WinUV Validate application allows up to three test solutions of potassium dichromate, or three absorbance levels for NIST filters, to be measured automatically. The user can easily enter the expected absorbance, accuracy, and precision allowing the intended operational range of the Cary 60 UV-Vis to be qualified. All measurements are displayed in the report with Pass/Fail recorded for each test.

Stray Light

The test for stray light quantifies light that is detected by the UV-Vis spectrophotometer, but is actually from a wavelength other than that selected reaching the detector. Because the detector in the instrument cannot differentiate between the types of light that it measures, all light is measured. This means that any stray light that is detected can yield inaccuracy and problems with quantitative analyses because it can decrease photometric selectivity and create a nonlinear photometric response (degrade the Beer-Lambert Law relationship). The stray light tests use solutions that have no transmission within a specified wavelength range, so that any light reaching the detector indicates the presence of stray light.

Procedure and performance limits

The USP<857> prescribes a new method for measuring stray light that uses differing pathlength cells, and comparing the measurement from the sample and reference positions in the instrument. For the Cary 60, the alternative method described in USP<857> is used in which each analytical solution is measured at a single wavelength, and the % transmission (%T) is reported. This method is valid, and can be used to demonstrate that any Cary 60 UV-Vis spectrophotometer is USP<857> compliant for stray light.

Cary WinUV advantage

The stray light tests are performed automatically by the Validate application of Cary WinUV, and the Cary 60 UV-Vis spectrophotometer passes the stray light test if the %T is less than the prescribed tolerance (Figure 5).

KCl at 198 nm reading 0.190034 %T PASSED
KCl at 198 nm Tolerance <= 1.000000%T

NaI at 220 nm reading 0.015290 %T PASSED
NaI at 220 nm Tolerance <= 0.050000%T

K2Cr2O7/NaNO2 at 370 nm reading 0.008922 %T PASSED
K2Cr2O7/NaNO2 at 370 nm Tolerance < 0.050000%T

Acetone at 320 nm reading 0.017077 %T PASSED
Acetone at 320 nm Tolerance < 0.050000%T

Stray Light Test PASSED

Figure 5. The Agilent Cary WinUV Validate application reports the %T measured for each stray light analtyical sample, and reports a Pass or Fail against the tolerance for the Agilent Cary 60 UV-Vis.

Resolution

The resolution of a UV-Vis spectrophotometer is the narrowest spectral bandwidth that the instrument can achieve, and is important when measuring samples that have complex spectra or spectra that have multiple, near-overlapping absorbance peaks.

Procedure and performance limits

The resolution test involves measuring the spectrum of a 0.020 % v/v solution of certified samples of toluene in hexane (UV grade) between 275 to 265 nm, and calculating the ratio of the absorbance maxima and minima that are found at approximately 269 and 266 nm, respectively. The calculated absorbance ratio is dependent upon the spectral bandwidth of the instrument used.

Validate Report: What Happens When You Pass All Tests?

Agilent recommends that the full suite of tests be regularly undertaken to verify performance, and help build a database of instrument operating parameters that will aid troubleshooting and streamline maintenance procedures. This is easy to do with the Validate application of Cary WinUV software, which provides a fully automated method for verifying that your instrument is performing to the specifications prescribed in Chapter <857>. The user must simply enter the values and tolerances from their CRMs, and place each sample into the instrument when prompted. All collected results are displayed in the Validate report with Pass/Fail recorded for each test, with the Cary 60 UV-Vis spectrophotometer meeting the performance requirements specified by the USP<857>.

Selecting an instrument

The reputation of all quality-driven pharmaceutical laboratories is dependent upon maintaining the quality of the results they produce. This can only be obtained with the utmost confidence in the instrumentation used. Agilent Technologies is a trusted manufacturer with a strong presence in the pharmaceutical industry. The Agilent Cary 60 UV-Vis spectrophotometer is an integral instrument in the Agilent suite of analytical tools, and is manufactured according to a quality management system certified to ISO 9001. It meets or exceeds all the performance requirements prescribed by global pharmacopeia.

The Cary 60 UV-Vis spectrophotometer is built around the unique Agilent Xenon flash lamp source that has been proven to collect high quality data over the complete UV-Vis wavelength range. The patented Xenon flash lamp has the lowest cost of ownership, as lamps routinely last more than 10 years, thus minimizing lamp replacement and expensive instrument revalidation costs. It also provides usability benefits for room light immunity, efficiency savings (the Cary 60 has no warm up time), and performance benefits (great linear absorbance range and fast scanning for improved productivity).

The Cary 60 UV-Vis spectrophotometer also offers a comprehensive software package to help achieve compliance with 21 CFR part 11 and EU Annex 11 [1]. This software product helps achieve data integrity and traceability for all electronic records associated with the operation of the Cary 60 UV-Vis spectrophotometer, including the Validate application used to perform the instrument performance tests described in this white paper.

Reference

1. Support for Title 21 CFR Part 11 and Annex 11 Compliance: Agilent Cary WinUV Pharma, *Agilent Technologies White Paper*, publication number 5991-7268EN (**2016**).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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