White Paper

Agilent
Trusted Answers

UV-Vis Spectrophotometry Solution for the Pharmaceutical Industry

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

Introduction

UV-Vis spectroscopy is one of the most commonly used analytical techniques in pharmaceutical research, manufacturing, and quality control and 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 qualification 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 United States Pharmacopoeia (USP) and the European Pharmacopoeia (Eu.Ph.), all of which require that the performance of UV-Vis spectrophotometers is regularly qualified.

The USP chapter <857> ultraviolet-visible spectroscopy guides the instrument qualification protocols for UV-Vis spectrophotometers.[1] This white paper outlines how the Agilent Cary 60 UV-Vis spectrophotometer (see Figure 1) can be qualified according to USP chapter <857> to ensure that the instrument is suitable for its intended use (described in Table 1).

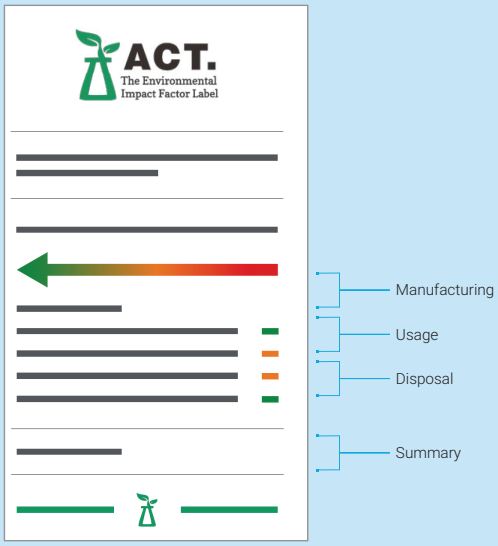
Figure 1. The Agilent Cary 60 UV-Vis spectrophotometer.

The Agilent Cary 60 UV-Vis spectrophotometer is a flexible, powerful, and reliable UV-Vis system, ideal for routine analysis in pharmaceutical laboratories. Built around the unique Xenon lamp, the Cary 60 UV-Vis combines high quality data collection with low cost of ownership; the 10-year lamp lifespan, reflected in a 10-year replacement warranty, reduces replacement and revalidation costs and minimizes instrument downtime.

Towards a more sustainable lab

The Cary 60 UV-Vis has been independently audited for its environmental impact and has received the ACT (Accountability, Consistency, Transparency) label, verified by My Green Lab. The label provides information about the environmental impact of the Cary 60 UV-Vis throughout its entire life cycle.

The Cary 60 UV-Vis improves the environmental impact of laboratories without impeding productivity or scientific progress.



The ACT label provides information about the environmental impact of manufacturing, using and disposing of a product, and its packaging.

Table 1. United States Pharmacopeia (USP) system qualification tests recommended by Agilent for the Agilent Cary 60 UV-Vis spectrophotometer.

Test Category	USP Test Description and Limits	
Control of Wavelength Method	At least six replicate measurements, reporting the mean and standard deviation (wavelength precision) for each analysis wavelength and the difference of the mean measured value to the certified value of the Certified Reference Material (CRM) (wavelength accuracy).	
Control of Wavelength Recommended Reference Material	Holmium in perchloric acid solution	200 to 400 nm ± 1 nm 400 to 780 nm ± 2 nm ≤ 0.5 nm standard deviation
	Didymium solution	400 to 900 nm ± 2 nm ≤ 0.5 nm standard deviation
	Holmium oxide glass filter	200 to 400 nm ± 1 nm 400 to 780 nm ± 2 nm ≤ 0.5 nm standard deviation
	Xenon emission lines	Recommended atomic line at 541.9 nm < ± 0.50 nm
Control of Absorbance Method	At least six replicate measurements, reporting mean and standard deviation of absorbance for each analysis wavelength.	
Control of Absorbance Recommended Reference Material	Certified Potassium dichromate ($K_2Cr_2O_7$) solutions	UV (200 to 400 nm) < 1 Abs, use 20 to 60 mg/L, accuracy: ± 0.010 Abs, precision: ≤ 0.005 Abs > 1 Abs, use 80 to 100 mg/L, accuracy: ± 1.0% Abs, precision: ≤ 0.5% Abs VIS (400 to 780 nm) < 1 Abs, 600 mg/L, accuracy: ± 0.010 Abs, precision: ≤ 0.005 Abs
	Certified NIST neutral density standards	VIS (400 to 780 nm) < 1 Abs, accuracy: ± 0.008 Abs, precision: ≤ 0.005 Abs > 1 Abs, accuracy: ± 0.80 % Abs, precision: ≤ 0.50% Abs
Stray Light Method	Procedure B: Measure the absorbance of the cut-off solution filters specified against a 10 mm cell filled with an appropriate reference and record the maximum absorbance value (A) or the minimum percentage Transmittance (%T) at the recommended wavelength.	
Stray Light Recommended Reference Material	%T at 198 nm reported 190 to 210 nm	Aqueous potassium chloride (12 g/L) Procedure B: A_{max} ≥ 2.0 A or $\%T_{min}$ < 1%T
	%T at 220 nm reported 210 to 270 nm	Aqueous sodium iodide (10 g/L) Procedure B: A_{max} ≥ 2.0 A or $\%T_{min}$ < 1%T
	%T at 320 nm reported 250 to 330 nm	Acetone Procedure B: A_{max} ≥ 2.0 A or $\%T_{min}$ < 1%T
	%T at 370 nm reported 300 to 400 nm	Aqueous sodium nitrite (50 g/L) Procedure B: A_{max} ≥ 2.0 A or $\%T_{min}$ < 1%T
Resolution Method and Limits	Ratio of absorbance at 269 and 266 nm	Toluene in hexane, 0.02% v/v Ratio ≥ 1.3

The Cary 60 UV-Vis is controlled by easy-to-use Agilent Cary WinUV software built around several application-focused software modules, each designed to reduce complexity and increase productivity by streamlining method setup, data collection, and data analysis (Figure 2). In this whitepaper, the "Validate" module will be used for fast and easy qualification for a Cary 60 UV-Vis to ensure the instrument being used is suitable for its intended use as part of operational qualification (OQ).

The optional Agilent Cary WinUV Pharma software is a comprehensive software package to help achieve compliance with 21 CFR part 11 and EU Annex 11.[2] This software supports 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 tests described in this white paper.

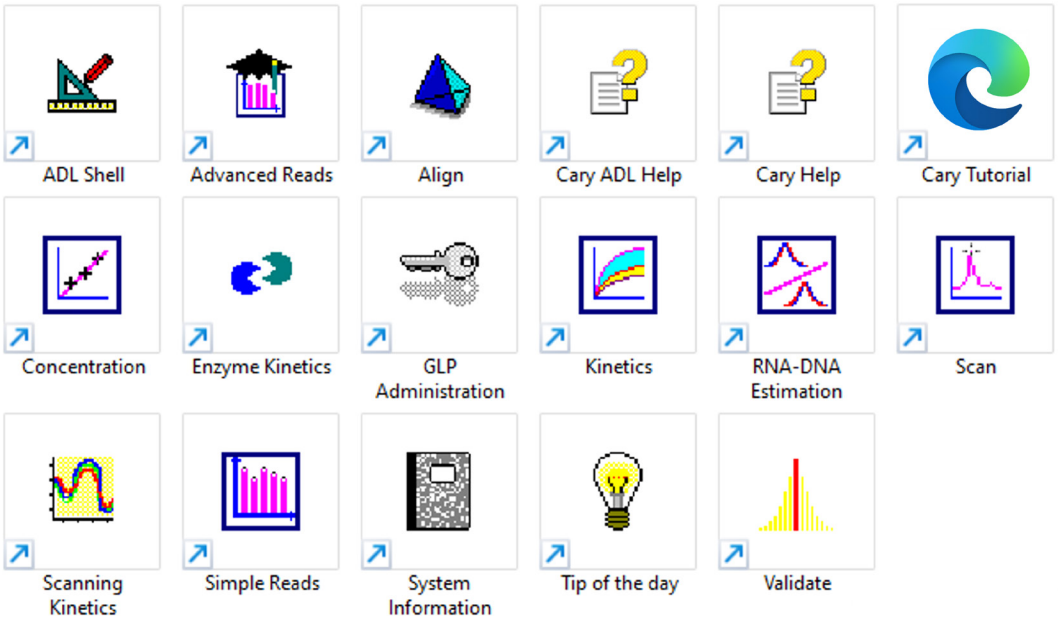


Figure 2. The Agilent Cary WinUV software includes application-focused software modules.

Instrument qualification of the Cary 60 UV-Vis

The USP <857> describes requirements for the four main operational qualifications:

- 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 chapter <857> recommends that the instrument be qualified over the intended operational range. This will vary according to the analytical protocol of different laboratories. In addition, USP chapter <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.

Control of wavelengths

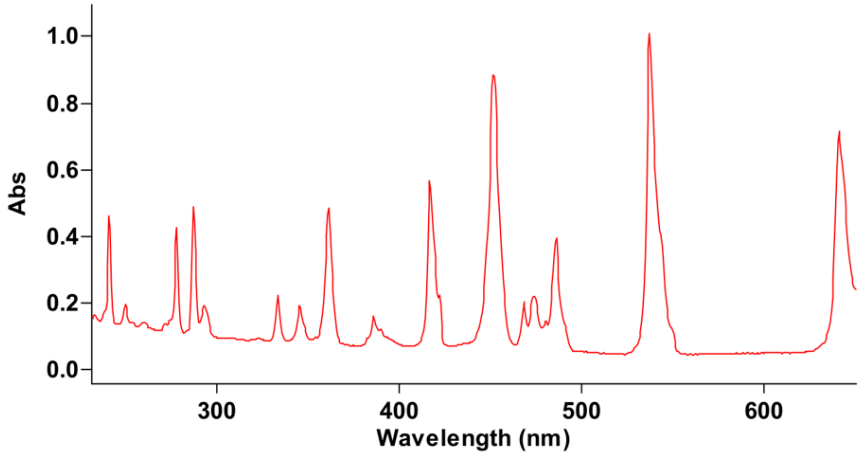
Wavelength accuracy and precision

The wavelength accuracy and precision tests are used to ensure that the wavelength axis of the UV-Vis spectrum is accurate and precise (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 and holmium-fused glass optical filters (from 240 to 640 nm), commonly available as CRMs, yield well-characterized peaks (Figure 3) across the usable range of the UV-Vis spectrum.

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 (Figure 3) are then identified. 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.

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 chapter <857> requires that the precision of UV-Vis instruments is better than 0.5 nm across the operational range of the instrument.

Wavelength (nm)	Accuracy (nm) ±	Pass/Fail	Precision	Pass/Fail
640.50	640.8708	PASSED	0.1103	PASSED
536.60	537.0117	PASSED	0.0824	PASSED
485.20	485.7658	PASSED	0.0000	PASSED
467.80	468.1545	PASSED	0.0837	PASSED
451.40	451.4664	PASSED	0.1791	PASSED
416.30	416.4723	PASSED	0.0000	PASSED
385.60	385.7199	PASSED	0.0863	PASSED
361.30	361.0958	PASSED	0.0803	PASSED
345.40	345.3062	PASSED	0.0854	PASSED
333.50	333.3471	PASSED	0.0637	PASSED
287.20	287.0298	PASSED	0.0000	PASSED
278.10	277.7230	PASSED	0.0000	PASSED
249.90	249.6488	PASSED	0.0483	PASSED
241.10	240.8386	PASSED	0.0000	PASSED



Wavelength Accuracy and Precision Test - Holmium Perchlorate Method PASSED

Figure 3. Wavelength scan of holmium perchlorate using an Agilent Cary 60 UV-Vis spectrophotometer and Agilent Cary WinUV software, with tabulated averaged wavelength data from six scans for each peak position. Accuracy is displayed as the averaged wavenumber of the found peaks from six replicate measurements. The software shows a pass/fail result based on the difference between it and the standard wavelength. Precision is shown as the standard deviation of the six replicate measurements.

Control of absorbances

Photometric accuracy

The control of absorbance tests is used to ensure that the photometric axis of the instrument is accurate and precise (correct and within acceptable limits) across the intended operational range. As such, it also establishes 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 chapter <857> are conducted on certified solutions of potassium dichromate, which has absorbance peaks in the UV region of the spectrum; certified 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 chapter <857>, a solution of certified potassium dichromate in dilute perchloric acid (0.001 M) is measured, and the absorbance intensities at 235 nm, 257 nm, 313 nm, and 350 nm are 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 to 100 mg/L potassium dichromate. When below 1 Abs, the absorbance accuracy must be ± 0.010 Abs, and when above 1 Abs, ± 1.0% of the absorbance measured (Figure 4). Following USP chapter <857>, photometric accuracy in the visible range of the spectrum uses certified NIST-traceable filters that absorb between 440 to 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 according to the calibrated CRM values. The application generates a report showing the mean result (accuracy) and a Pass or Fail response as determined by the entered tolerances (Figure 5).

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.

First K2Cr2O7 solution

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6
235.00	0.7374	0.7373	0.7369	0.7373	0.7370	0.7372
257.00	0.8572	0.8573	0.8573	0.8572	0.8573	0.8571
313.00	0.2875	0.2876	0.2876	0.2875	0.2876	0.2875
350.00	0.6371	0.6374	0.6372	0.6371	0.6369	0.6376

Wavelength (nm)	Average (Abs)	Pass/Fail	Precision	Pass/Fail
235.00	0.7372	PASSED	0.0002	PASSED
257.00	0.8572	PASSED	0.0001	PASSED
313.00	0.2875	PASSED	0.0001	PASSED
350.00	0.6372	PASSED	0.0003	PASSED

Photometric Accuracy Test - K2Cr2O7 Method PASSED

Figure 4. Tabulated average and precision for six replicate measurements of 60 mg/L potassium dichromate.

First Filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6
440.00	1.0438	1.0443	1.0440	1.0434	1.0441	1.0438
465.00	0.9615	0.9616	0.9612	0.9614	0.9615	0.9616
546.10	0.9725	0.9725	0.9727	0.9727	0.9724	0.9724
590.00	1.0118	1.0112	1.0120	1.0113	1.0112	1.0117
635.00	0.9650	0.9653	0.9656	0.9658	0.9666	0.9661

Wavelength (nm)	Average (Abs)	Pass/Fail	Precision	Pass/Fail
440.00	1.0439	PASSED	0.0003	PASSED
465.00	0.9615	PASSED	0.0001	PASSED
546.10	0.9725	PASSED	0.0001	PASSED
590.00	1.0115	PASSED	0.0004	PASSED
635.00	0.9657	PASSED	0.0006	PASSED

Second Filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6
440.00	0.7259	0.7260	0.7259	0.7259	0.7258	0.7258
465.00	0.6698	0.6698	0.6702	0.6699	0.6700	0.6699
546.10	0.6788	0.6787	0.6788	0.6787	0.6789	0.6787
590.00	0.7081	0.7084	0.7085	0.7084	0.7083	0.7081
635.00	0.6780	0.6783	0.6783	0.6784	0.6781	0.6777

Wavelength (nm)	Average (Abs)	Pass/Fail	Precision	Pass/Fail
440.00	0.7259	PASSED	0.0001	PASSED
465.00	0.6699	PASSED	0.0001	PASSED
546.10	0.6788	PASSED	0.0001	PASSED
590.00	0.7083	PASSED	0.0002	PASSED
635.00	0.6781	PASSED	0.0003	PASSED

Third Filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6
440.00	0.5427	0.5426	0.5426	0.5427	0.5427	0.5425
465.00	0.4932	0.4933	0.4933	0.4933	0.4933	0.4931
546.10	0.5073	0.5072	0.5071	0.5071	0.5072	0.5072
590.00	0.5356	0.5358	0.5357	0.5355	0.5357	0.5357
635.00	0.5221	0.5220	0.5217	0.5220	0.5218	0.5220

Wavelength (nm)	Average (Abs)	Pass/Fail	Precision	Pass/Fail
440.00	0.5426	PASSED	0.0001	PASSED
465.00	0.4932	PASSED	0.0001	PASSED
546.10	0.5072	PASSED	0.0001	PASSED
590.00	0.5357	PASSED	0.0001	PASSED
635.00	0.5219	PASSED	0.0002	PASSED

Photometric Accuracy Test - NIST Filter Method PASSED

Figure 5. Tabulated average and precision for six replicate measurements of NIST standard filters.

Stray light

The test for stray light quantifies light that is detected by the UV-Vis spectrophotometer which is not of the selected wavenumber. 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 test limits

The USP chapter <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 chapter <857> is used, in which each analytical solution is measured at a single wavelength, and the percent transmission (%T) is reported (Figure 6). The test passes if the %T is less than the prescribed tolerance. This method is valid and can be used to demonstrate that any Cary 60 UV-Vis spectrophotometer is USP<857> compliant for stray light.

KCl at 198 nm reading 0.315876 %T PASSED
KCl at 198 nm Tolerance <= 1.000000%T

NaI at 220 nm reading 0.084088 %T PASSED
NaI at 220 nm Tolerance <= 1.000000%T

K2Cr2O7/NaNO2 at 340 nm reading 0.005689 %T PASSED
K2Cr2O7/NaNO2 at 340 nm Tolerance < 1.000000%T

Acetone at 300 nm reading 0.014744 %T PASSED
Acetone at 300 nm Tolerance < 1.000000%T

Stray Light Test PASSED

Figure 6. The Agilent Cary WinUV Validate application reports the %T measured for each stray light analytical 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 test 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 and 265 nm and calculating the ratio of the absorbance maxima and minima that are found at approximately 269 and 266 nm, respectively (Figure 7).

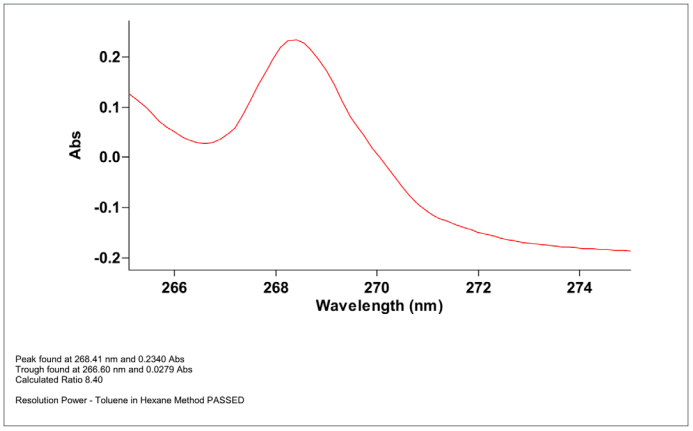


Figure 7. The Agilent Cary WinUV Validate application measures a spectrum of toluene in hexane, calculates the maxima:minima peak ratio, and reports a Pass or Fail result against the tolerance for the Agilent Cary 60 UV-Vis.

Instrument qualification workflow

The Cary WinUV Validate application allows the user to define an automated method to qualify the Cary 60 against the criteria outlined in USP chapter <857>.

The Validate application of the Cary WinUV software makes ensuring that the instrument meets the required tolerances efficient and easy by automatically running the testing protocols and providing step-by-step instructions for inserting reference materials and blanks.

The user may select from a range of test suites, which include a premade list of the recommended USP tests for the Cary 60 (Figure 8). The user can then enter relevant CRM serial numbers and values from the CRM certificate for each test type (Figure 9). When the user begins the method, they will be prompted to place each reference material in the instrument by the on-screen loading guide. This will continue until all tests are run and a report is generated.

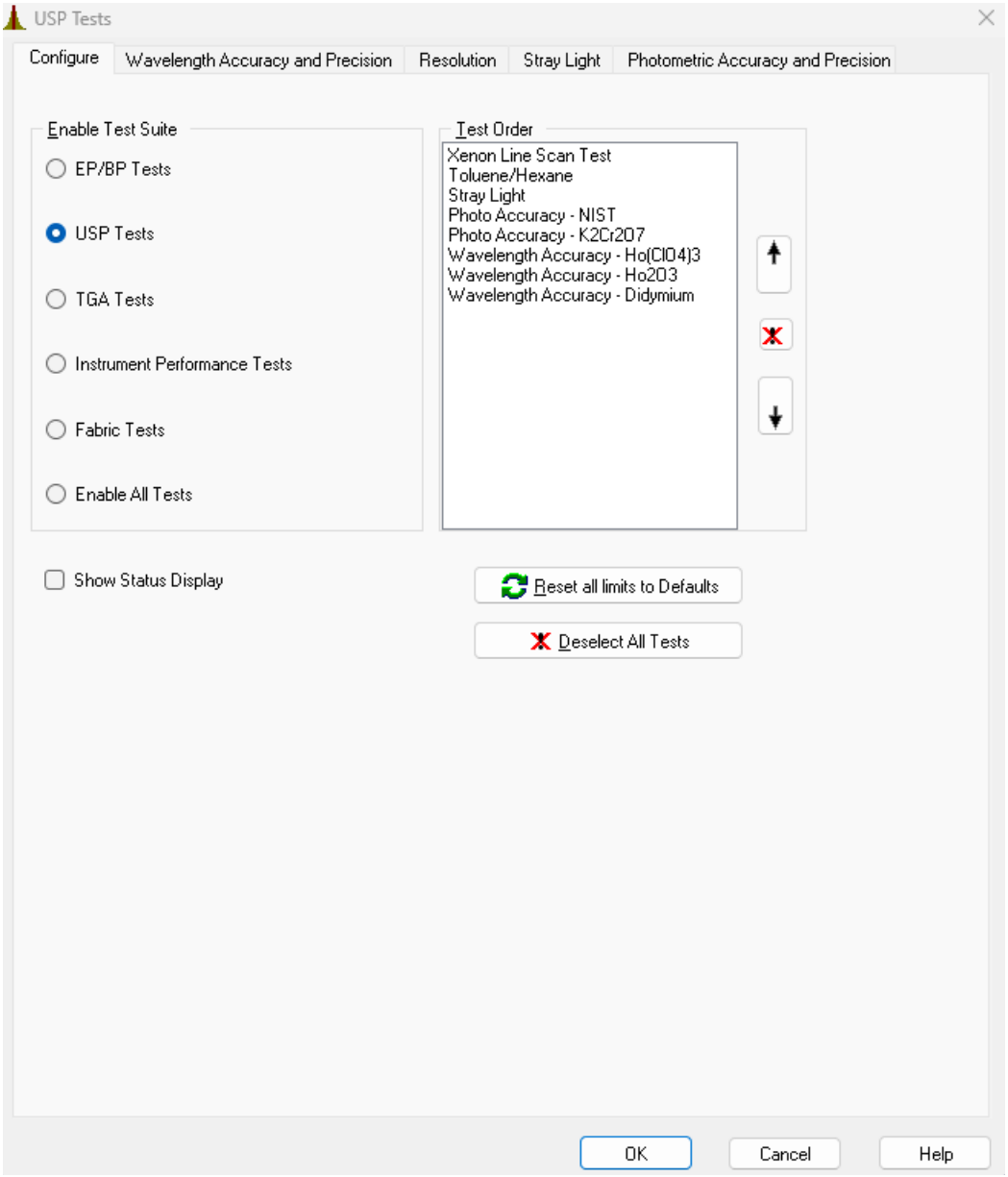


Figure 8. The Agilent Cary WinUV Validate application contains a premade USP test suite, which includes the recommended USP tests for the Cary 60.

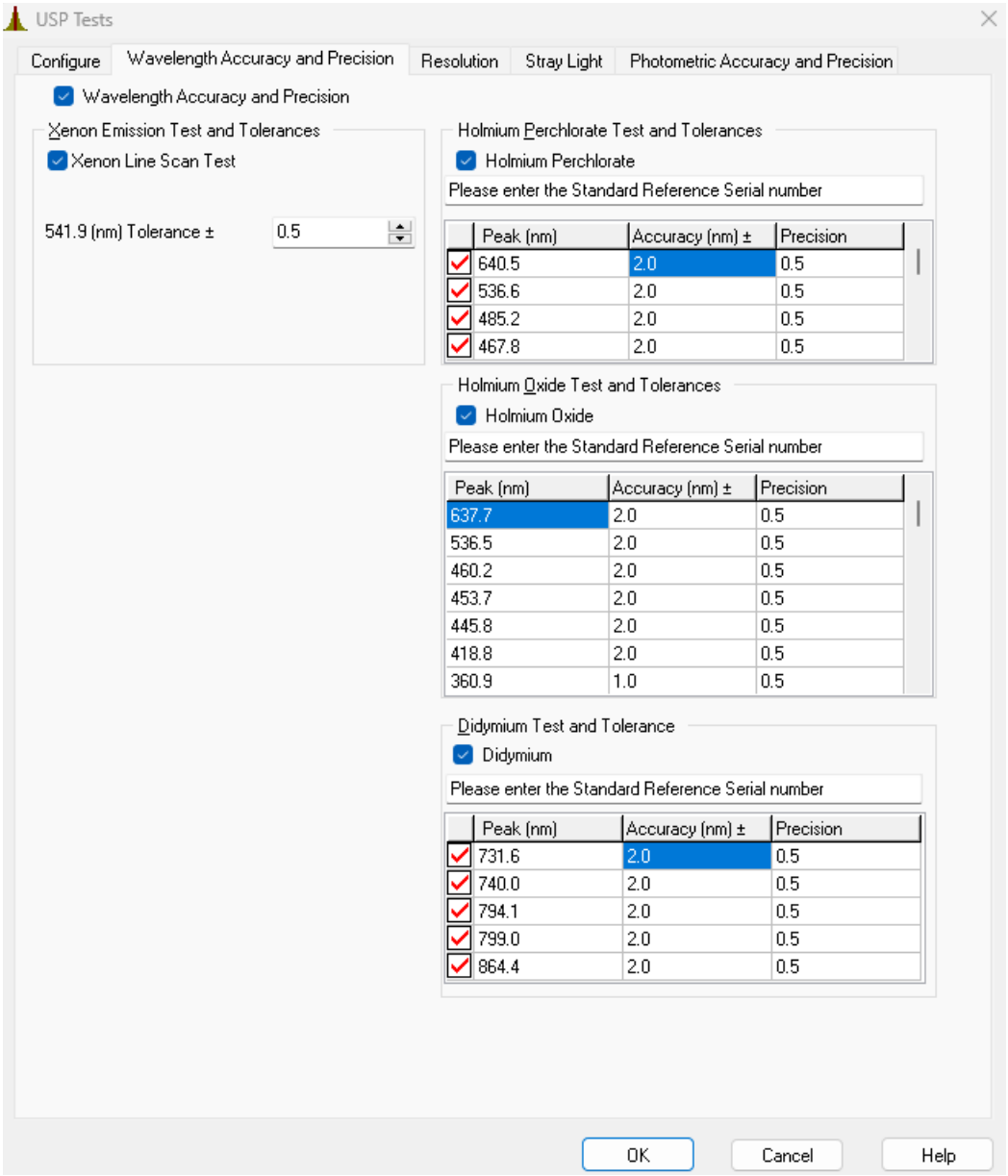


Figure 9. Once the appropriate tests have been selected, the reference values specific for the CRMs in use must be added.

Further automate the qualification process

The use of the 18-cell changer accessory (Figure 10) allows the user to automatically run USP tests by placing the required standards in the instrument following the easy-to-use loading guide (Figure 11). This means less operator time is required in the lab for routine instrument qualification, which can take 1 to 2 hours depending on operator familiarity.

Figure 10. The Agilent Cary 60 UV-Vis with the 18-cell changer streamlines instrument qualification and enhances efficiency.

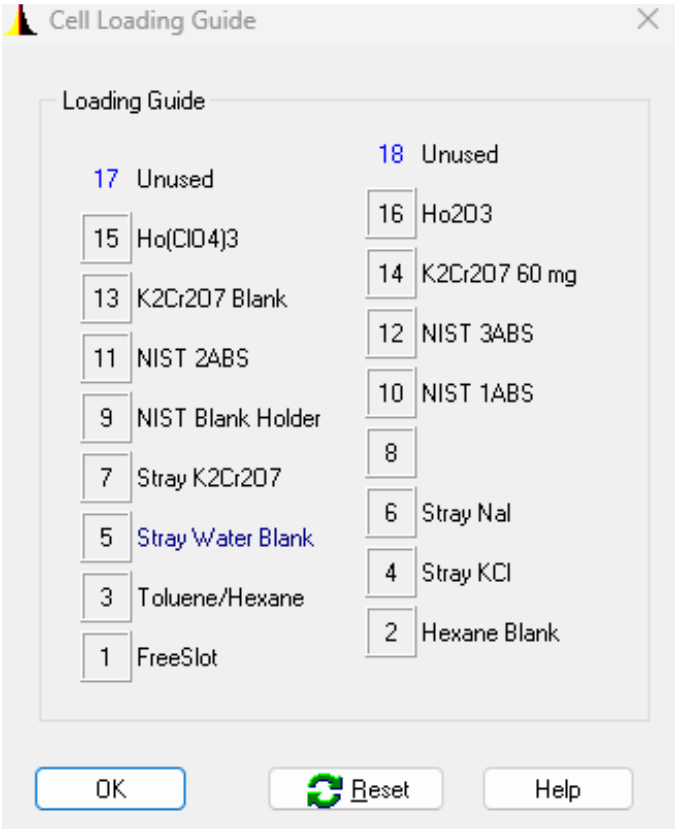


Figure 11. USP test suite loading guide for the 18-cell changer.

Qualification report: What happens when all tests are passed?

Once all selected tests have been performed, the Cary WinUV Validate application will generate a report that includes individual test results and a summary of Pass/Fail results for all tests (Figure 12). This report is saved with the batch file and can also be saved to a chosen location as a PDF file. If using the Cary WinUV Pharma software, the file can be signed by operators and supervisors who possess the required privileges.

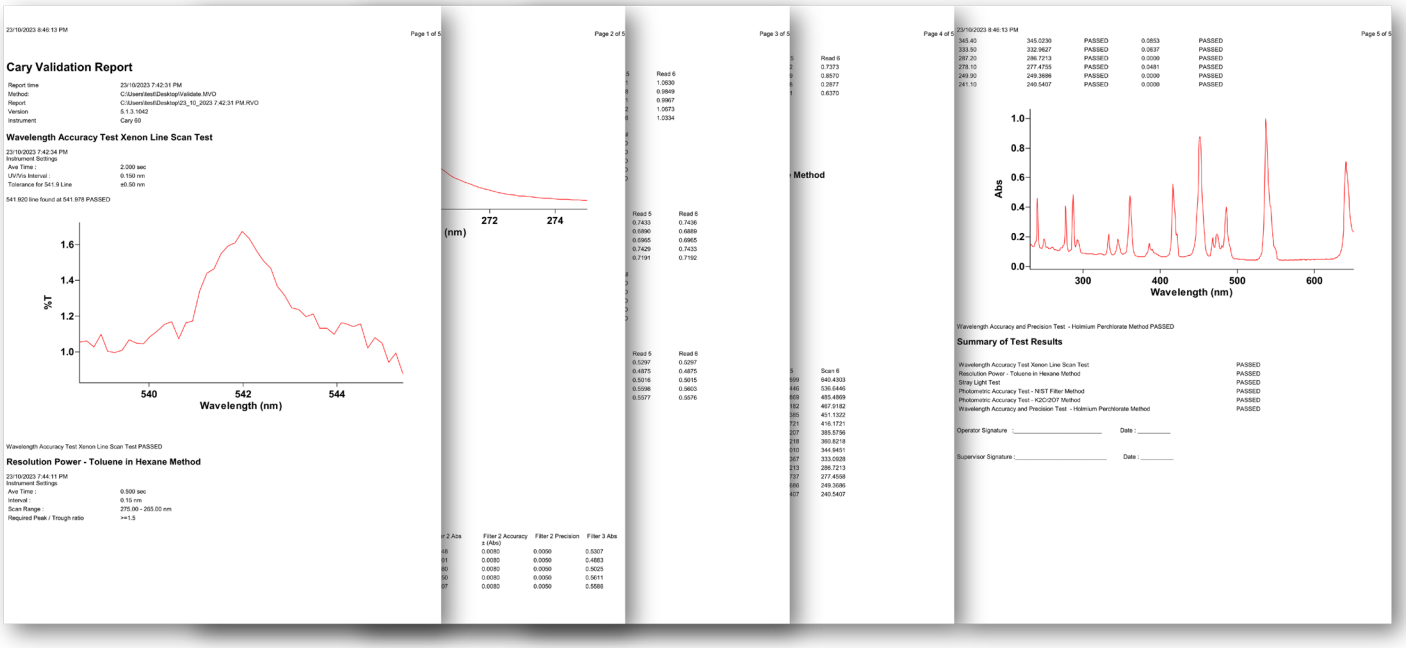


Figure 12. The Agilent Cary WinUV Validate application can automatically generate a PDF report following the completion of the tests according to the requirements of the USP.

Conclusion

Laboratories operating in pharmaceutical research, manufacturing, or quality control require their instruments to be qualified regularly to ensure they are fit for purpose. Regulatory bodies, such as the USP, provide detailed methodology and limits, which qualify key instrument characteristics such as wavenumber accuracy, wavenumber precision, and photometric accuracy.

This white paper has demonstrated that the Agilent Cary 60 UV-Vis spectrophotometer can successfully meet the requirements outlined in USP chapter <857>. Qualifying the instrument in this way helps the user ensure that it is suitable for its intended use.

Agilent recommends that the full suite of tests be regularly undertaken to qualify the instrument and help build a database of instrument operating parameters that will aid in troubleshooting and streamline maintenance procedures. This is easy to do with the Validate application of Agilent Cary WinUV software, which provides a fully automated method for qualifying that your instrument is performing according to the specifications prescribed in USP 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 Agilent Cary 60 UV-Vis spectrophotometer meeting the requirements specified by the USP <857>.

Agilent also offers a comprehensive portfolio of service plans and regulatory compliance services to help achieve qualification of the Cary 60 UV-Vis spectrophotometer, software, and its components to ensure the highest levels of reliability and performance.

- The Agilent Cary 60 UV-Vis spectrophotometer is part of the Agilent analytical tools suite manufactured under the ISO 9001 quality management system, meeting global pharmacopeia requirements.
- The Agilent Cary 60 features a unique Xenon flash lamp source for high-quality data across the UV-Vis range. Long-lasting Xenon flash lamps with 10-year replacement warranty reduce replacement and revalidation costs.
- The Agilent Cary 60 offers room light immunity, no warm-up time, and fast scanning for improved productivity.
- The Agilent Cary 60 UV-Vis has been independently audited for its environmental impact, helping laboratories achieve their sustainability goals.
- The optional Agilent Cary WinUV Pharma software supports compliance as defined by US FDA 21 CFR Part 11, EU Annex 11, and similar national electronic records regulations. The Cary WinUV Pharma software ensures data integrity and traceability for all electronic records associated with the Cary 60 UV-Vis spectrophotometer's operation, including the Validate application for instrument tests.
- The Cary WinUV Validate application within the Cary WinUV software simplifies the process of instrument qualification.
- The 18-cell changer enables automatic running of USP tests by loading all required standards, reducing operator time in routine instrument qualification.

References

1. USP Chapter <857> Ultraviolet-Visible Spectroscopy.
2. Support for Title 21 CFR Part 11 and Annex 11 Compliance: Agilent Cary WinUV Pharma, *Agilent Technologies white paper*, publication number 5991-7268EN, **2023**.

Further information

Agilent Cary 60 UV-Vis Spectrophotometer

Agilent Cary WinUV Software

Find Out Which Agilent UV-Vis Is Best for Your Needs

Agilent UV-Vis Spectrophotometer Uses and Applications

Agilent UV-Vis Spectroscopy and Spectrophotometer FAQs

www.agilent.com

DE19461867

Printed in the USA, December 7, 2023
5994-6869EN

Agilent
Trusted Answers