Application Note

Agilent
Trusted Answers

Pharmaceutical Analysis Using UV-Vis: Compliance with USP Chapter <857>, and European Pharmacopoeia (Ph. Eur. Chapter 2.2.25)

Meeting the requirements of the global pharmacopeias with the Agilent Cary 3500 UV-Vis

Introduction

UV-Vis spectroscopy is a widely used analytical technique in quality assurance/quality control (QA/QC) and pharmaceutical research. It is critical that any laboratory in such environments set up appropriate controls for laboratory access and ensure that Good Manufacturing Practice (GMP) documentation, including system suitability tests (SSTs) and standard operating procedures (SOPs) are available and followed. The United States pharmacopeia (USP) and the European pharmacopeia (Ph. Eur.) guidelines describe how to verify that the analytical performance of UV-Vis spectrophotometers is suitable for the intended operational range of the analysis.

Within Cary UV Workstation software for the Cary 3500, a range of system verification tests are available and automated. These tests align with the pharmacopeia requirements, while also allowing the flexibility to cover a limited custom test list. USP general chapter <857> and Ph. Eur. chapter 2.2.25 guide instrument operational qualification protocols for UV-Vis spectroscopy. Table 1 and 2 outline these system verification tests, along with a brief description. The system verification tests are designed such that successfully passing all tests will ensure the instrument is performing according to both the USP and the Ph. Eur. specifications.

Table 1. United State Pharmacopeia (USP) system verification tests recommended for the Cary 3500 UV-Vis system.

Test Category	USP Test Description and Limits	
Control of Wavelength Method	At least six replicate measurements reporting mean and standard deviation for each analysis wavelength.	
Control of Wavelength Recommended Reference Material	Holmium in perchloric acid solution.	200 – 400 nm ± 1 nm 400 – 780 nm ± 2 nm ≤ 0.5 nm standard deviation
	Cerium sulfate solution	200 – 400 nm ± 1 nm ≤ 0.5 nm standard deviation
	Didymium solution	400 – 900 nm ± 2 nm ≤ 0.5 nm standard deviation
	Holmium oxide glass filter	200 – 400 nm ± 1 nm 400 – 780 nm ± 2 nm ≤ 0.5 nm standard deviation
	Xenon lamp	Recommended atomic line at 260.6 nm
Control of Absorbance Method	At least six replicate measurements, reporting standard deviation for each analysis wavelength.	
Control of Absorbance Recommended Reference Material	Potassium dichromate ($K_2Cr_2O_7$) solutions	UV (200-400 nm) < 1 Abs, use 20 – 60 mg/L Accuracy: < ± 0.01 Abs Precision: < ± 0.005 Abs > 1 Abs, use 80 – 200 mg/L Accuracy: < ±1 % Abs Precision: < ±0.5 % Abs VIS (400-780 nm) < 1 Abs, 600 mg/L Accuracy: < ± 0.01 Abs Precision: < ± 0.005 Abs
	NIST neutral density standards	VIS (400-780 nm) Accuracy: < 1 Abs, < ± 0.008 Abs > 1 Abs, < ± 0.8 % Abs Precision: < 1 Abs, < ± 0.005 Abs > 1 Abs, < ± 0.5 % Abs

Table 1, continued. United State Pharmacopeia (USP) system verification tests recommended for the Cary 3500 UV-Vis system.

Test Category	USP Test Description and Limits	
Control of Photometric Linearity Method	At least three different absorbance levels appropriate to and spanning the required operational range are measured.	
Control of Photometric Linearity Recommended Reference Material	Suitable certified reference material e.g. Potassium Dichromate ($K_2Cr_2O_7$) solutions	UV 200 – 400 nm, all concentrations must meet accuracy of absorbance acceptance criteria.
Stray Light Method	Procedure A: Produce the differential spectrum resulting from the subtraction of a spectrum produced by a 5-mm path length cell from that of a 10-mm cell, both filled with the same filter solution.	
	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 % Transmittance (%T) at the recommended wavelength.	
Stray Light Recommended Reference Material	%T at 198 nm reported 190 – 210 nm	Aqueous potassium chloride (12 g/L), Procedure A: sλ ≤ 0.01 or Aλ ≥ 0.7 A Procedure B: Amax ≥ 2.0 A or %Tmin < 1%T
	%T at 220 nm reported 210 – 270 nm	Aqueous sodium iodide (10 g/L), Procedure A: sλ ≤ 0.01 or Aλ ≥ 0.7 A Procedure B: Amax ≥ 2.0 A or %Tmin < 1%T
	%T at 320 nm reported 250 – 330 nm	Acetone, Procedure A: sλ ≤ 0.01 or Aλ ≥ 0.7 A Procedure B: Amax ≥ 2.0 A or %Tmin < 1%T
	%T at 370 nm reported 300 – 400 nm	Aqueous sodium nitrite (50 g/L), Procedure A: sλ ≤ 0.01 or Aλ ≥ 0.7 A Procedure B: Amax ≥ 2.0 A or %Tmin < 1%T
Resolution Method and limits	Ratio of absorbance at 269 and 266 nm	Toluene in hexane, 0.02 % v/v

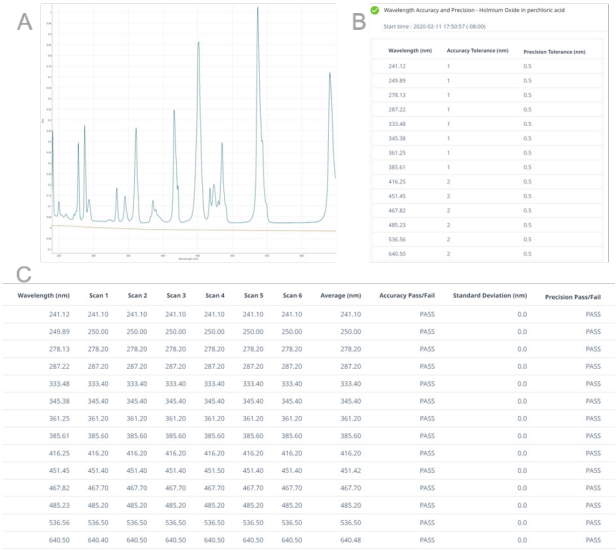
Table 2. European Pharmacopeia (Ph. Eur.) system verification tests recommended for the Cary 3500 UV-Vis system.

Test Category	Ph. Eur. Test Description and limits	
Control of Wavelength Method	It is recommended to test at least 2 wavelengths that bracket the intended spectral range using 1 or more certified reference materials.	
Control of Wavelength Recommended Reference Material	Holmium in perchloric acid solution	200 – 400 nm ± 1 nm 400 – 700 nm ± 3 nm
	Cerium Sulfate solution	200 – 400 nm ± 1 nm
	Didymium solution	400 – 700 nm ± 3 nm
	Holmium oxide glass filter	200 – 400 nm ± 1 nm 400 – 700 nm ± 3 nm
	Xenon lamp	200 – 400 nm ± 1 nm 400 – 700 nm ± 3 nm
Control of Absorbance Method	An appropriate number of wavelengths in the intended spectral range using suitable solid or liquid filters to check that the absorbance measured with the spectrometer matches the known absorbance of the filter measured at the intended wavelength. It is recommended to test absorbance accuracy at the same wavelength using several filters with different absorbance levels.	
Control of Absorbance Recommended Reference Material	Potassium dichromate solutions	235 nm 124.5 (specific absorbance) 122.9 to 126.2 max. tol. 257 nm 144.5 (specific absorbance) 142.8 to 146.2 max. tol. 313 nm 48.6 (specific absorbance) 47.0 to 50.3 max. tol. 350 nm 107.3 (specific absorbance) 105.6 to 109.0 max. tol. 430 nm 15.9 (specific absorbance) 15.7 to 16.1 max. tol The difference between the measured absorbance and the certified absorbance of the filter is ≤ 0.010 for each combination of wavelength and absorbance assessed (valid for absorbance values no greater than 2.0). Tolerances for higher absorbance values should be defined based on a risk assessment.
Control of Photometric Linearity Method and Limit	Using a suitable certified reference material, the photometric linearity is acceptable if the coefficient of determination (R^2) is not less than 0.999.	
Stray Light Method	Stray light is determined at an appropriate wavelength using suitable solid or liquid filters or solutions prepared in-house.	
Stray Light Recommended Reference Material	Abs at 198 nm reported	Aqueous potassium chloride (12 g/L), tolerance ≥ 2.0 A
	Abs at 220 nm reported	Aqueous sodium iodide (10 g/L), tolerance ≥ 3.0 A
	Abs at 250 nm reported	Aqueous potassium iodide (10 g/L), tolerance ≥ 3.0 A
	Abs at 340 and 370 nm reported	Aqueous sodium nitrite (50 g/L), tolerance ≥ 3.0 A
Resolution Method and limits	Ratio of absorbance at 269 and 266 nm	Toluene in Hexane, 0.02 % v/v

The multicell module of the Cary 3500 has no moving parts. This allows simultaneous measurements of a reference and up to seven samples with eight cuvette positions. As well as the benefits of simultaneity, this design allows for the sampling module to be optimized for the type of measurement being performed. The difference in the design of the Cary 3500 multicell module and engine (shown in Figure 1), compared with conventional spectrophotometers affects the rationale of operational qualification (OQ) testing for the instrument in two basic ways: The engine and multicell module are separate, and the multicell module has duplicated optics and electronics for each cuvette position. These differences can be considered in the context of testing and will be discussed below.

Figure 1. The Cary 3500 Multicell spectrophotometer consists of two parts: the engine (shown on the right) and the multicell module (on the left). The two parts are connected to create the complete spectrophotometer.

Alternatively, glass filters, prepared by fusing a rare earth, such as holmium, into a base glass matrix, can be used for wavelength verification tests. To assess the wavelength accuracy, the Cary 3500 UV-Vis spectrophotometer performs a wavelength scan across the relevant range for each material and identifies the wavelength position for the corresponding peak maximum (Figures 2, 3, 4, 5 and 6). The peak positions are then cross-checked with the certified data for that standard (or emission line). USP General Chapter <857> requires that wavelength accuracy in the UV and visible regions of the spectrum must be ± 1 nm and ± 2 nm, respectively.



Wavelength (nm)	Scan 1	Scan 2	Scan 3	Scan 4	Scan 5	Scan 6	Average (nm)	Accuracy Pass/Fail	Standard Deviation (nm)	Precision Pass/Fail
241.12	241.10	241.10	241.10	241.10	241.10	241.10	241.10	PASS	0.0	PASS
249.89	250.00	250.00	250.00	250.00	250.00	250.00	250.00	PASS	0.0	PASS
278.13	278.20	278.20	278.20	278.20	278.20	278.20	278.20	PASS	0.0	PASS
287.22	287.20	287.20	287.20	287.20	287.20	287.20	287.20	PASS	0.0	PASS
333.48	333.40	333.40	333.40	333.40	333.40	333.40	333.40	PASS	0.0	PASS
345.38	345.40	345.40	345.40	345.40	345.40	345.40	345.40	PASS	0.0	PASS
361.25	361.20	361.20	361.20	361.20	361.20	361.20	361.20	PASS	0.0	PASS
385.61	385.60	385.60	385.60	385.60	385.60	385.60	385.60	PASS	0.0	PASS
416.25	416.20	416.20	416.20	416.20	416.20	416.20	416.20	PASS	0.0	PASS
451.45	451.40	451.40	451.40	451.50	451.40	451.40	451.42	PASS	0.0	PASS
467.82	467.70	467.70	467.70	467.70	467.70	467.70	467.70	PASS	0.0	PASS
485.23	485.20	485.20	485.20	485.20	485.20	485.20	485.20	PASS	0.0	PASS
536.56	536.50	536.50	536.50	536.50	536.50	536.50	536.50	PASS	0.0	PASS
640.50	640.40	640.50	640.50	640.50	640.50	640.50	640.48	PASS	0.0	PASS

Figure 2. Wavelength accuracy test results for holmium oxide in perchloric acid. (A) Six repeated wavelength scans of holmium oxide in perchloric acid; (B) peak positions and tolerances applied; (C) raw peak positions for each individual scan tabulated with the average, standard deviation and the pass/fail result.

Control of wavelength

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. Confirmation of wavelength accuracy is recommended to be tested using atomic line spectra from xenon or deuterium light sources. Rare earth oxides that yield well characterized absorption bands, enabling the comparison of the UV-Vis spectrophotometer wavelength readings to the published values, can also be used. The rare earth oxide solutions: holmium oxide in perchloric acid (from 200 to 600 nm), didymium (from 700 to 860 nm), and cerium sulfate solution (200 –300 nm) are well established and widely available as certified reference materials (CRMs) that yield well-characterized peaks (Figure 2A.) across the range of the UV-Vis spectrum.

All instrument components that determine the wavelength of light are in the engine. This design means that any one of the cuvette positions can be used to determine the wavelength accuracy of the instrument. Only one cuvette position of the module needs to be tested because the module has no capability to change the wavelength of the light.

Wavelength precision

Wavelength precision is tested by calculating the standard deviation of at least six replicate measurements of the absorbance peaks (Figure 2, 3, 4, 5 and 6). USP <857> requires that the precision of UV-Vis instruments is better than 0.5 nm across the operational range of the instrument. The wavelength precision test assesses how reproducibly a scanning UV-Vis spectrophotometer can measure at each specific wavelength in the wavelength range.

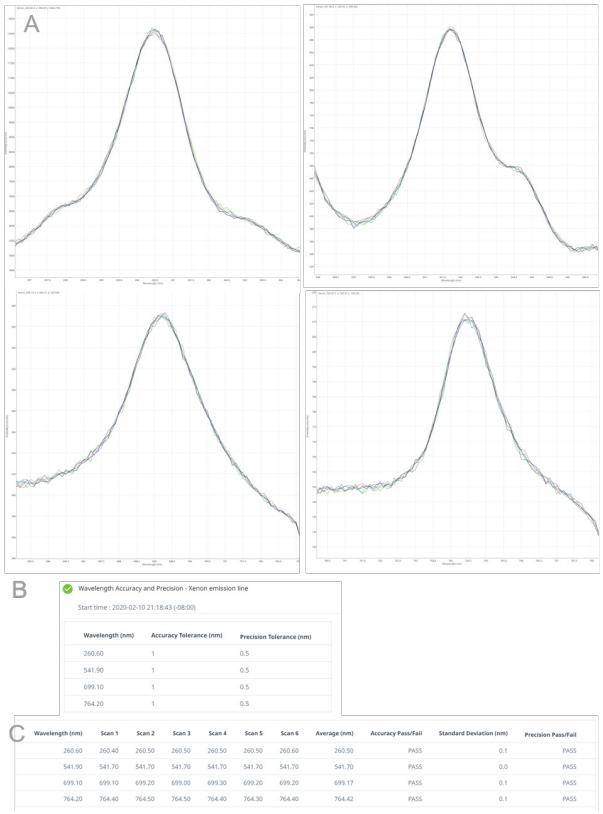


Figure 3. Xenon emission line test results for wavelength accuracy. (A) Six repeated wavelength scans of xenon lamp plotting intensity (counts) against four specified wavelengths; (B) peak positions and tolerances; (C) raw peak positions for each individual scan tabulated with the average, standard deviation and the pass/fail result.

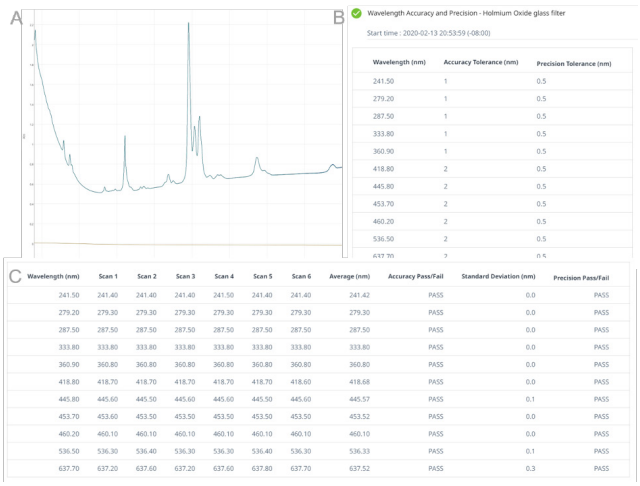


Figure 4. Wavelength accuracy test results for the holmium oxide glass filter. (A) Six repeated wavelength scans of a holmium oxide glass filter; (B) peak positions and tolerances; (C) raw peak positions for each individual scan tabulated with the average, standard deviation and the pass/fail result.

Figure 5. Wavelength accuracy test results for the didymium filter. (A) Six wavelength scans of didymium; (B) peak positions and tolerances; (C) raw peak positions for each individual scan tabulated along with the average, standard deviation and the pass/fail result.

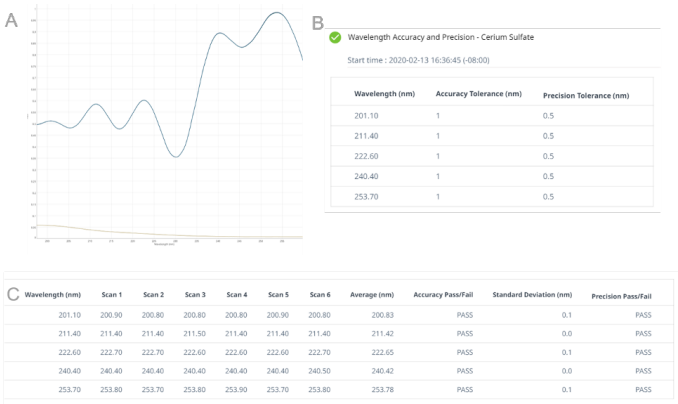


Figure 6. Wavelength accuracy test results for the cerium sulfate filter. (A) Six wavelength scans of cerium sulfate; (B) peak positions and tolerances; (C) raw peak positions for each individual scan tabulated along with the average, standard deviation and the pass/fail result.

Control of absorbance

Photometric accuracy

Photometric accuracy and precision tests are used to confirm the photometric performance of a UV-Vis spectrophotometer across the operational absorbance range of the instrument. 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 CRMs of potassium dichromate, which has absorbance peaks in the UV region of

the spectrum; National Institute of Standards and Technology (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 ($K_2Cr_2O_7$) in dilute perchloric acid is measured, and the absorbance intensity at 235 nm, 257 nm, 313 nm, and 350 nm is determined (Figure 7). In addition, the absorbance at 430 nm of a 600 mg/L solution is performed (Figure 8). With the Cary 3500, it is possible to measure up to three potassium dichromate solution concentrations (Figure 7). 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 to 635 nm. The Cary UV Workstation System Verification Tests 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 (Figure 9).

A

$K_2Cr_2O_7$ 40mg/L

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
235.00	0.490	0.490	0.490	0.490	0.490	0.490	0.490	Pass	0.000	Pass
257.00	0.568	0.568	0.568	0.568	0.568	0.568	0.568	Pass	0.000	Pass
313.00	0.190	0.190	0.190	0.190	0.190	0.190	0.190	Pass	0.000	Pass
350.00	0.423	0.423	0.423	0.423	0.423	0.423	0.423	Pass	0.000	Pass

$K_2Cr_2O_7$ 60mg/L

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
235.00	0.746	0.746	0.746	0.746	0.746	0.746	0.746	Pass	0.000	Pass
257.00	0.865	0.865	0.865	0.865	0.865	0.865	0.865	Pass	0.000	Pass
313.00	0.292	0.292	0.292	0.292	0.292	0.292	0.292	Pass	0.000	Pass
350.00	0.643	0.643	0.643	0.643	0.643	0.643	0.643	Pass	0.000	Pass

$K_2Cr_2O_7$ 120mg/L

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
235.00	1.491	1.491	1.491	1.491	1.491	1.491	1.491	Pass	0.007	Pass
257.00	1.728	1.728	1.728	1.729	1.729	1.729	1.728	Pass	0.010	Pass
313.00	0.574	0.574	0.574	0.574	0.574	0.574	0.574	Pass	0.000	Pass
350.00	1.271	1.271	1.272	1.272	1.272	1.272	1.272	Pass	0.026	Pass

B

Linearity ($r^2 \geq 0.999$)

Wavelength (nm)	$K_2Cr_2O_7$ 40mg/L	$K_2Cr_2O_7$ 60mg/L	$K_2Cr_2O_7$ 120mg/L	r^2	Pass/Fail
235.00	0.490	0.746	1.491	1.000	Pass
257.00	0.568	0.865	1.728	1.000	Pass
313.00	0.190	0.292	0.574	1.000	Pass
350.00	0.423	0.643	1.272	1.000	Pass

Figure 7. (A) Potassium dichromate photometric accuracy and precision results for the 40, 60 and 120 mg/L standards. The wavelengths measured, the tolerances for accuracy and precision as well as the raw absorbance data, the averages, standard deviation and the pass/fail determination are all presented herein. (B) Potassium dichromate photometric linearity (R^2) calculated at three different concentration and four wavelengths, and the pass/fail determination.

Photometric Accuracy and Precision - Potassium Dichromate 600 mg/L

Start time : 2020-02-12 16:09:53 (+11:00)

Wavelength (nm)	Abs	Accuracy Tolerance (Abs)	Precision Tolerance (Abs)
430.000	0.9564	0.010	0.005

$K_2Cr_2O_7$ 600mg/L

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
430.00	0.954	0.954	0.954	0.954	0.954	0.954	0.954	Pass	0.000	Pass

Figure 8. Potassium dichromate photometric accuracy and precision results for 600 mg/L standard at 430 nm. The wavelengths measured, the tolerances for accuracy and precision as well as the raw absorbance data, the averages, standard deviation and the pass/fail determination are all presented herein.

A

Filter 1 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
440.00	1.050	1.051	1.051	1.051	1.051	1.051	1.051	Pass	0.009	Pass
465.00	0.969	0.969	0.969	0.969	0.969	0.969	0.969	Pass	0.000	Pass
546.10	0.979	0.980	0.980	0.980	0.980	0.980	0.980	Pass	0.000	Pass
590.00	1.018	1.019	1.019	1.019	1.019	1.020	1.019	Pass	0.071	Pass
635.00	0.973	0.973	0.973	0.973	0.973	0.974	0.973	Pass	0.000	Pass

Filter 2 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
440.00	0.729	0.730	0.730	0.730	0.730	0.730	0.730	Pass	0.000	Pass
465.00	0.674	0.674	0.674	0.674	0.674	0.674	0.674	Pass	0.000	Pass
546.10	0.684	0.684	0.684	0.684	0.684	0.684	0.684	Pass	0.000	Pass
590.00	0.713	0.713	0.713	0.713	0.713	0.713	0.713	Pass	0.000	Pass
635.00	0.682	0.682	0.682	0.682	0.682	0.682	0.682	Pass	0.000	Pass

Filter 3 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Read 4	Read 5	Read 6	Accuracy	Pass/Fail	Precision	Pass/Fail
440.00	0.549	0.549	0.549	0.549	0.549	0.549	0.549	Pass	0.000	Pass
465.00	0.499	0.499	0.499	0.499	0.499	0.499	0.499	Pass	0.000	Pass
546.10	0.514	0.514	0.514	0.514	0.514	0.514	0.514	Pass	0.000	Pass
590.00	0.543	0.543	0.543	0.543	0.543	0.543	0.543	Pass	0.000	Pass
635.00	0.528	0.528	0.528	0.529	0.529	0.529	0.528	Pass	0.000	Pass

B

Linearity ($r^2 \geq 0.999$)

Wavelength (nm)	Filter 1 - NIST glass filter	Filter 2 - NIST glass filter	Filter 3 - NIST glass filter	r^2	Pass/Fail
440.00	1.051	0.730	0.549	1.000	Pass
465.00	0.969	0.674	0.499	1.000	Pass
546.10	0.980	0.684	0.514	1.000	Pass
590.00	1.019	0.713	0.543	1.000	Pass
635.00	0.973	0.682	0.528	1.000	Pass

Figure 9. (A) Photometric accuracy and precision results for the NIST glass filters showing the wavelengths measured, the tolerances for accuracy and precision as well as the raw absorbance data, the averages, standard deviation and the pass/fail determination. The absorbance is measured by the instrument detectors, and there is a detector in every cuvette position. (B) NIST glass filters photometric linearity (R^2) calculated at three different concentration and five wavelengths, and the pass/fail determination.

Photometric precision

The photometric precision of the system is determined similarly to the wavelength precision test. The results of six replicate measurements are required and assess how reproducibly the UV-Vis determines photometric absorbance. The results are shown in Figures 7, 8 and 9.

Due to this, the absorbance accuracy, precision and linearity must be confirmed for the intended operational range for every cuvette position.

Recently, the USP and Ph. Eur. have described the use of metal-on-quartz filters for verifying photometric accuracy, since the Cary 3500 sample modules (CTM and MCM) are designed to handle liquid sample analysis, therefore, this filter is not suitable on the Cary 3500 due to internal reflection.

Photometric linearity

Photometric linearity affects how accurately an instrument measures absorbance with increasing optical density or concentration. Poor photometric linearity will produce incorrect results and cause calibrations to become non-linear. To test photometric linearity according to Ph. Eur., the filters recommended for photometric accuracy can be used. At least three concentrations should be measured and the coefficient of determination (R^2) should not be less than 0.999. With the Cary 3500 and the Cary UV Workstation System Verification application, photometric linearity can be calculated automatically from three different concentrations of solutions or filters (Figure 7B and 9B) as recommended by global pharmacopeias.

Limit of stray light

The test for stray light quantifies light that is detected by the UV-Vis spectrophotometer, that is 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 incident 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. As shown in Table 1. the pharmacopeia tests require four cutoff filters to be measured to qualify the stray light levels within an instrument, potassium chloride (KCl), sodium iodide (NaI), sodium nitrite ($NaNO_2$) and acetone. Figure 10 shows the scan of each stray light test solution. Another procedure specified in the USP Chapter <857> to measure the limit of stray light is to produce a differential spectrum resulting from the subtraction of a spectrum produced by a 5-mm path length cell from that of a 10-mm cell, both filled with the same filter solution (e.g. KCl, NaI, $NaNO_2$ and acetone). The stray light ratio test is implemented in Cary UV Workstation system verification tests which will automatically verify if the instrument fall within the tolerance limit as shown in Figure 11.

Stray light is light of wavelengths outside the specified bandwidth. The significant causes of stray light in the instrument system are defects and contamination of optical components in the monochromator section of the engine. These defects would affect all cuvette positions equally, so it is only necessary to test one cuvette position of the multicell module.

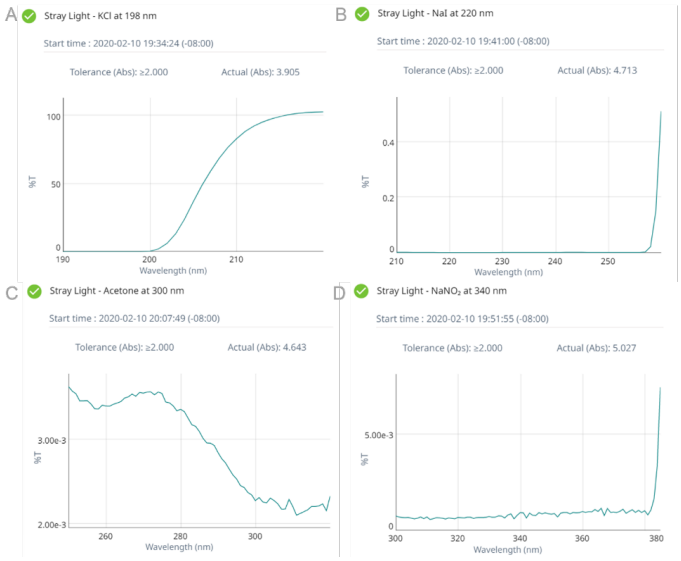


Figure 10. Stray light results for (A) KCl; (B) NaI; (C) acetone; and (D) $NaNO_2$ showing the filter scans in %T and the corresponding absorbance values at the specified wavelengths.

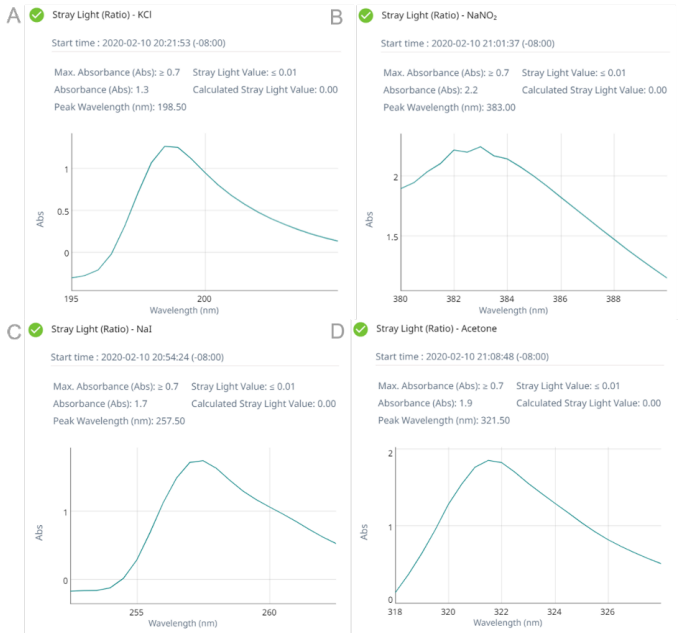


Figure 11. Stray light (ratio) results for (A) KCl; (B) $NaNO_2$; (C) NaI; and (D) acetone with calculated stray light values.

Control of Resolution

The resolution of a UV-Vis spectrophotometer is the narrowest spectral bandwidth that the instrument can achieve. This parameter is important when measuring samples that have complex spectra or spectra that have multiple, near overlapping absorbance peaks. 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. Alternatively, benzenoid compounds, or other compounds with sharp absorption bands (natural half-bandwidths of less than 15 nm). A wavelength scan of toluene in hexane is shown in Figure 12.

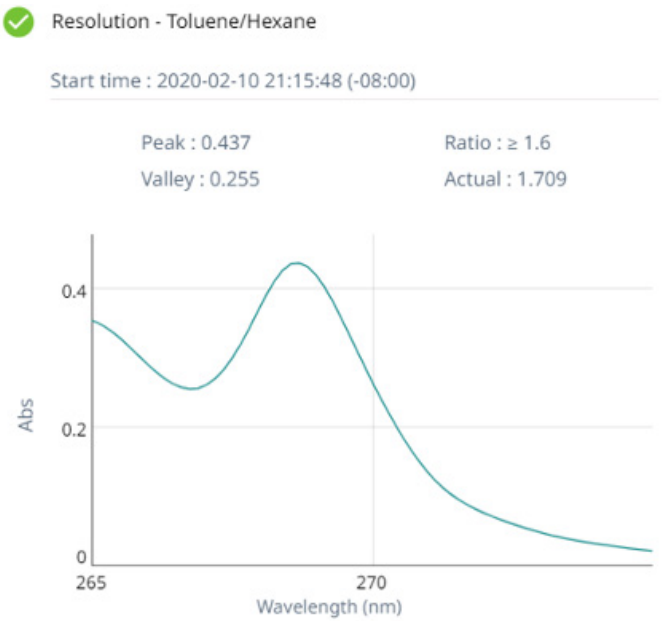


Figure 12. Resolution test results for toluene/hexane.

The engine determines the spectral bandwidth of the monochromatic light that is coupled into the module and thus the resolution of the instrument. All the parts of the instrument system that determine the bandwidth of the light are in the engine. Any of the cuvette positions in the module can be used to determine the resolution of the instrument system as long as sufficient light is present (this is tested in other tests). Only one cuvette position of the module needs to be tested because the module has no capability to change the resolution (spectral bandwidth) of the light.

Conclusion

It is critical for pharmaceutical laboratories to ensure that their UV-Vis spectrophotometer is performing as required to meet the QA/QC measurement needs it is being used for. The global pharmacopeia prescribes how to test the intended operational range of the instrument, and these tests are conveniently automated in the Cary UV Workstation software. By simply and easily performing the system verification tests with the Cary 3500, a laboratory can readily confirm their instrument performance.

The Agilent Cary 3500 UV-Vis spectrophotometer provides unique simultaneous measurement capabilities for the pharmaceutical industry to improve data quality and integrity. This benefit comes from a unique optical design and this technical note demonstrates how to ensure qualification of the Cary 3500 as per global pharmacopeias' requirements. In addition, the instrument is manufactured according to a quality management system certified to ISO 9001 and meets or exceeds all the global pharmacopeia performance requirements. The Cary 3500 UV-Vis spectrophotometer also offers a comprehensive software package to help achieve compliance with 21 CFR Part 11 and EU Annex 11. This software product helps achieve data integrity and traceability for all electronic records associated with the operation of the Cary 3500 UV-Vis spectrophotometer, including the system verification application used to perform the instrument performance tests described in this technical note.

www.agilent.com/chem

Printed in the USA, March 30, 2020
5994-1188EN
DE.698275463

Agilent
Trusted Answers