

UV/Vis Pharmacopeia Performance Verification on Lunatic & Stunner

Introduction

Compliance with international standards for analytical testing is a must-have for drug manufacturing and quality assurance/quality control (QA/QC). The United States Pharmacopeia (USP) chapter <857> and European Pharmacopeia (Ph. Eur.) chapter 2.2.2.25 lay out test protocols and acceptance criteria to ensure the suitability of UV/Vis spectrometers as analytical tools.

Lunatic and Stunner's UV/Vis Performance Verification (PV) kit and software makes testing for compliance a snap. The kit contains 8 sealed cuvettes of certified reference materials (CRMs) that satisfy all the USP and Ph. Eur. verification tests. These cuvettes are placed in a custom metal frame so they can be read by Lunatic or Stunner. Software guides the user on which cuvettes to use for each test and analyzes the results automatically, giving a simple pass/fail report at the end. It maintains an audit history of when each test was done, whether the instrument passed, and reports detailed results for review after each test.

Lunatic is the next-gen UV/Vis spectrometer that makes quantification easy with a dynamic range from 0.02–200 mg/mL for monoclonal antibody and 1.5–13,750 ng/ μ L for DNA (Figure 1A). It maximizes your throughput with high-speed UV/Vis spectral analysis with just 2 μ L of sample and up to 96 samples at a time. The uniquely molded microfluidic circuits prevent sample evaporation and have fixed path-length microcuvettes that mean your pathlength isn't dependent on moving parts. Lunatic's plates are fully compatible with manual loading or with your favorite automation system, if you need even higher throughput. 21 CFR Part 11 compliant tools can be added next to USP and Ph. Eur. compliance for even stronger regulatory control.

Stunner takes protein characterization to the next level by combining the same high-speed UV/Vis spectral analysis from Lunatic with dynamic light scattering

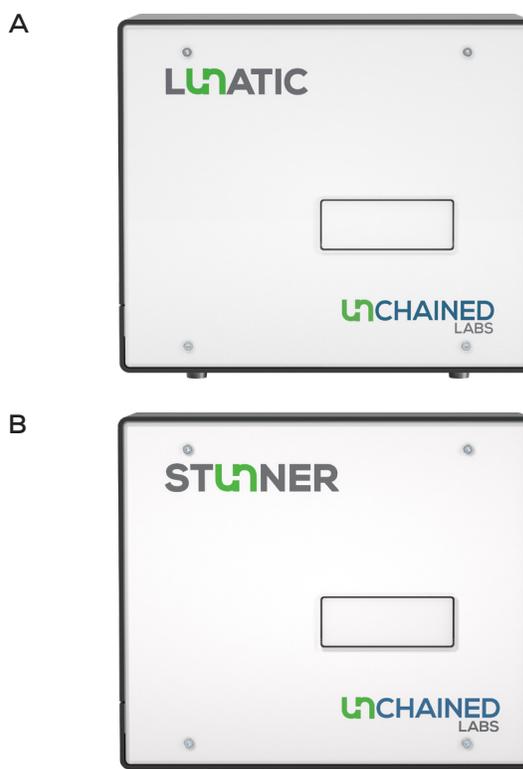


Figure 1: Lunatic: the Concentration Liberator (A). Stunner: the Quality Defender (B).

(Figure 1B). Using micro-volume Stunner plates, Stunner measures both the concentration, particle size, and polydispersity of 96 samples in just 1 hour. With this info, you can control how best to sync up sample quantity and quality. Like Lunatic, Stunner is automation-friendly and is USP and Ph. Eur. compliant, and can be souped-up with 21 CFR Part 11 tools.

This tech note provides an overview of USP 42 and the 10th edition Ph. Eur. guidelines for test procedures and acceptance criteria for UV/Vis spectrometers and shows how the UV/Vis PV tools on new Lunatic and Stunner instruments test for compliance with the guidelines.

With the UV/Vis PV and 21CFR11 tools available on instruments shipped after April 30, 2020, Lunatic and Stunner can fit anywhere from research and

development through to manufacturing and QC. Unify your entire biologics workflow with the best and easiest to use UV/Vis spectrometers on the market.

UV/Vis PV kit and tests

USP chapter <857> and Ph. Eur. chapter 2.2.2.25 specify that UV/Vis spectrometers should be verified for absorbance accuracy, precision, linearity, wavelength accuracy, resolution and stray light in the operational ranges for the instrument's intended use. The UV/Vis PV kit for Lunatic and Stunner contains CRMs chosen to test the wavelengths of light most absorbed by proteins and nucleic acids, and absorbances from 0–2 A in a 1 cm cuvette. USP and Ph. Eur. each contain their own acceptance criteria for each of the tests, but Lunatic and Stunner instruments now meet the stricter of the two for all tests, performing better than the minimum requirements (Table 1).

Control of absorbance

Acidic potassium dichromate ($K_2Cr_2O_7$) solutions in perchloric acid ($HClO_4$) are USP-appropriate references for UV/Vis spectrometry in the 200–400 nm wavelength range. They are suitable liquid filters with certified absorbances in the intended spectral range of operation of Lunatic and Stunner, as required by the Ph. Eur. The UV/Vis PV kit contains 3 concentrations of $K_2Cr_2O_7$ in $HClO_4$, each with certified absorbance values at 235, 257, 313, and 350 nm, and the appropriate blank.

For absorbance accuracy tests, the USP requires at least 1 absorbance level in the 0–2 A range while the Ph.Eur. requires absorbance levels at the 2 ends of the expected absorbance range. The acceptance limits are calculated by adding the expanded manufacturer uncertainty (MU) of the certified value to the acceptance criteria.

The 10th edition Ph. Eur. 2.2.2.25 does not contain requirements for absorbance precision. USP 42 <857> states that the standard deviation (SD) of 6 replicate absorbance measurements of the CRMs should be equal to or less than 0.005 A, if the certified values are less than 1 A. If they are greater than or equal to 1 A the relative standard deviation (RSD) of the 6 replicates must be less than or equal to 0.5%.

To meet the linearity criteria of USP 42 <857>, CRMs with 3 different absorbance levels spanning the operational range must all pass the accuracy and precision tests. The photometric linearity test in 10th edition Ph. Eur. 2.2.2.25 requires 2 absorbance levels and a coefficient of determination (R^2) greater than 0.999.

The UV/Vis PV kit for Lunatic and Stunner uses the same set of CRMs to perform absorbance accuracy, precision, and photometric linearity tests, in compliance with USP <857> and Ph. Eur. 2.2.2.25. These CRMs cover the operational wavelength and absorbance ranges suitable for determining protein and nucleic acid concentrations with the highest degree of accuracy and precision. With the UV/Vis PV kit, you can prove for yourself how good Lunatic and Stunner really are.

Test	Standard	Blank	Acceptance criteria
Absorbance accuracy	20 mg/L $K_2Cr_2O_7$ in $HClO_4$ (UV20), 60 mg/L $K_2Cr_2O_7$ in $HClO_4$ (UV60), 120 mg/L $K_2Cr_2O_7$ in $HClO_4$ (UV120)	$HClO_4$ (UV14)	<1 A: ± 0.01 A 1–2 A: $\pm 1\%$
Absorbance precision			<1 A: SD ≤ 0.005 A 1–2 A: RSD $\leq 0.5\%$
Absorbance linearity			$R^2 \geq 0.999$
Wavelength accuracy	Holmium oxide in $HClO_4$ (UV5)	$HClO_4$ (UV14)	<400 nm: ± 1 nm 400–750 nm: ± 2 nm
Resolution	0.02% (v/v) toluene in hexane (UV6)	Hexane (UV9)	Ratio ≥ 1.3
Stray light	Acetone (UV19)	Air	≥ 3.0 A

Table 1: The UV/Vis PV kit contains CRMs appropriate for each of the tests as laid out by USP 42 chapter <857> and the 10th edition Ph. Eur. 2.2.2.25. Lunatic and Stunner now meet the acceptance criteria for each test.

Control of wavelength accuracy and resolution

UV/Vis spectrometers determine the absorbance of a sample at multiple wavelengths in the UV and visible light range. USP and Ph. Eur. prescribe that all UV/Vis spectrometers, like Lunatic and Stunner, must be verified for absorbance and wavelength accuracy and resolution.

Wavelength accuracy of a UV/Vis spectrometer is a metric of how close the wavelength reported by the instrument is to a certified value. This can be determined by measuring the absorbance spectrum of a traceable CRM with well-defined peaks at multiple wavelengths and comparing the measured and the known peak maxima. Lunatic and Stunner's UV/Vis PV kit contains holmium oxide in perchloric acid as its wavelength accuracy CRM, which has certified peaks at 241, 287, 361, 537, and 641 nm. Holmium oxide in perchloric acid is an internationally accepted liquid intrinsic wavelength standard, identified in both USP 42 <857> and the 10th edition Ph. Eur. 2.2.2.25.

Lunatic and Stunner are diode array polychromatic spectrometers, so only a single wavelength accuracy measurement at each peak is required by USP and Ph. Eur. No precision determination is required of diode array spectrometers. The acceptance limits of the measured peak maxima are calculated by adding the MU to the acceptance criteria. According to USP and Ph. Eur. guidelines, the acceptance criteria for the peak wavelengths between 200–400 nm is within 1 nm, or within 2 nm if the certified value is >400 nm.

Spectral resolution refers to the ability of a UV/Vis spectrometer to correctly distinguish two adjacent details in an absorbance spectrum. The spectral resolution of new Lunatics and Stunners is better than 2 nm. Thus, if an absorbance minimum and maximum are at least 2 nm apart, Lunatic or Stunner can resolve the features. The UV/Vis PV test for spectral resolution is performed by measuring the absorbance spectrum of toluene in hexane and determining a ratio between the absorbance maximum at about 269 nm and an absorbance minimum at about 266 nm. The acceptance criteria for this test is a ratio not less than 1.3, according to both USP and Ph. Eur.

Due to Lunatic and Stunner's autocalibration before every measurement, they always have excellent wavelength accuracy and resolution.

With the UV/Vis PV kit you can prove the quality of the instrument for yourself and show it is fully in compliance with international guidelines.

Limit of stray light

Stray light is any light measured by a UV/Vis detector that is not of the identified wavelength. Stray light tends to have the biggest influence in the UV range, which is also the most important range for nucleic acid and protein quantification. Lunatic and Stunner maximize linearity by minimizing the influence of common sources of stray light, like internal reflections and 2nd order diffraction. UV/Vis spectrometers must define their stray light limits to comply with USP and Ph. Eur. guidelines.

The UV/Vis PV kit contains acetone to test stray light, as recommended by USP for monitoring stray light between 250–330 nm. Ph. Eur. specifies using sodium iodide, potassium iodide, or sodium nitrite solutions to test stray light, but also allows for other tests. Since none of the solutions specified by the Ph. Eur. cover the absorbance maxima of nucleic acids and proteins (260 and 280 nm, respectively), acetone is a more appropriate reference material for the Lunatic and Stunner operational range.

Acetone in a 10 mm cuvette absorbs most of the light from 250–330 nm. Therefore, any light in this range transmitted through the cuvette, as measured by a UV/Vis spectrometer, is likely stray light. Absorbance is calculated from transmitted light based on the following equation:

$$\text{Absorbance} = -\log\left(\frac{I_{\text{filled}}}{I_{\text{empty}}}\right)$$

In this equation, I_{filled} is the intensity of light transmitted through the filled cuvette and I_{empty} is the intensity of light through the empty cuvette.

The stray light test on Lunatic and Stunner consists of determining the absorbance at 260, 280, and 300 nm of the acetone cuvette. The USP acceptance criteria for this test are absorbances greater than or equal to 2 A, while the Ph. Eur. requires absorbances ≥ 3 A. Absorbances greater than 3 A are equivalent to less than 0.1% stray light.

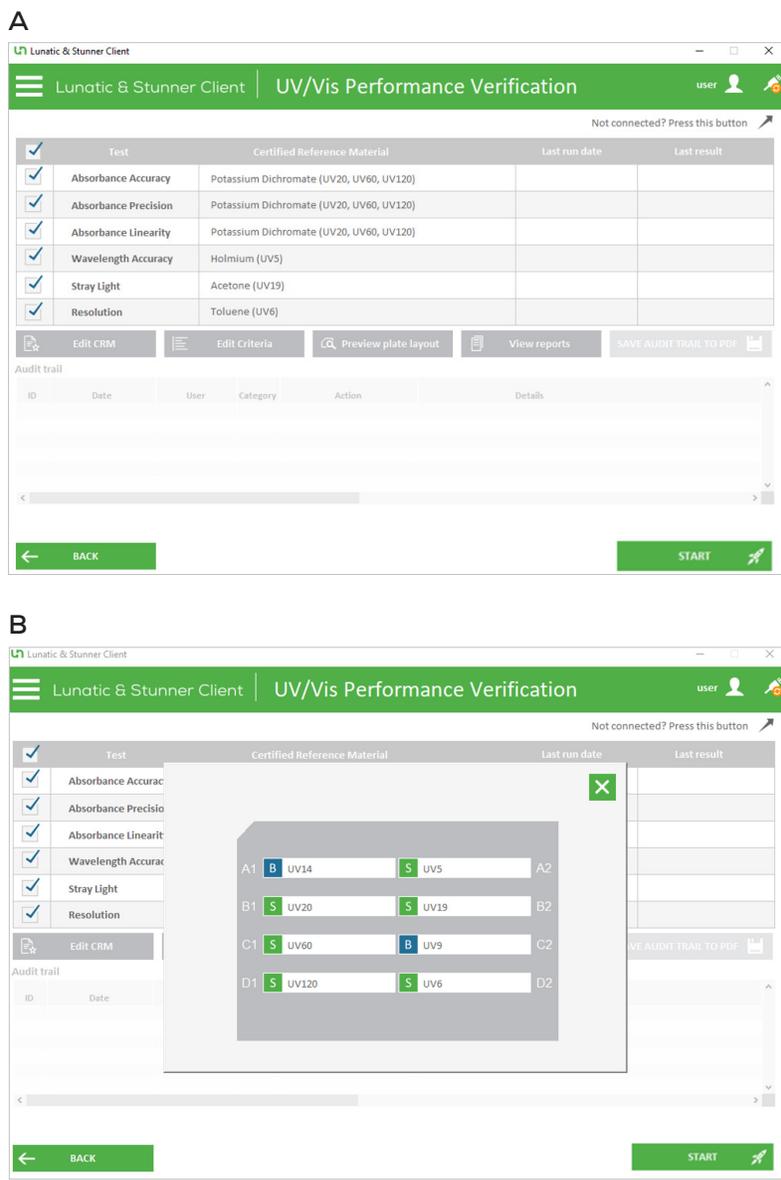


Figure 2: The Lunatic & Stunner Client UV/Vis Performance Verification tool allows for the user to select which tests to run and records when they were last run and the results (A). It also allows the user to edit and save the CRM values and testing criteria, and to view previous reports. With the 21CFR11 tools activated, it keeps track of changes in the audit trail. Clicking on “Preview plate layout” displays how to load the PV kit cuvettes to perform the selected verification tests (B).

Methods

The 8 sealed CRM cuvettes of the UV/Vis PV kit (Table 1, Hellma part number 667EP10-UL2) were equilibrated to room temperature. The UV/Vis Performance Verification tool was selected in Lunatic & Stunner Client (Figure 2A) on a Lunatic and the CRM values edited to include the certified values and MUs. All tests were selected, and the plate layout was previewed (Figure 2B) to determine the location of each cuvette in the metal Lunatic & Stunner PV

cuvette plate (Figure 3). The tests were then started and ran automatically. This protocol was repeated in 7 other Lunatics or Stunners.

Results

The 8 Lunatics and Stunners all passed the UV/Vis PV tests and were fully compliant with both USP and Ph. Eur. guidelines (Figure 4).

Representative $K_2Cr_2O_7$ absorbance spectra show the impressive accuracy of Lunatic’s unique optical design and show the acceptance limits of the UV/Vis PV absorbance accuracy test (Figure 5). Since all 3 samples are different concentrations of the same solution, they have the same spectral shape with different absorbance values. UV20, UV60, and UV120 were all measured 6 times and the means and SD were computed at each of the 4 test wavelengths, which were within the USP and Ph. Eur. acceptance limits.

A linear regression of the means of the 6 replicate measurements at each tested wavelength of UV20, UV60, and UV120, plotted through 0, have $R^2 \geq 0.999$ (Figure 6).

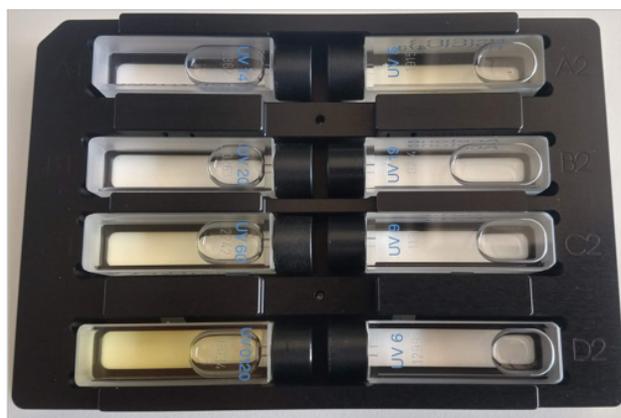


Figure 3: UV/Vis PV cuvette plate holds the sealed cuvettes in place during testing. Lunatic and Stunner automatically identifies the air bubble in each cuvette and selects a “clear” region for the verification measurements.

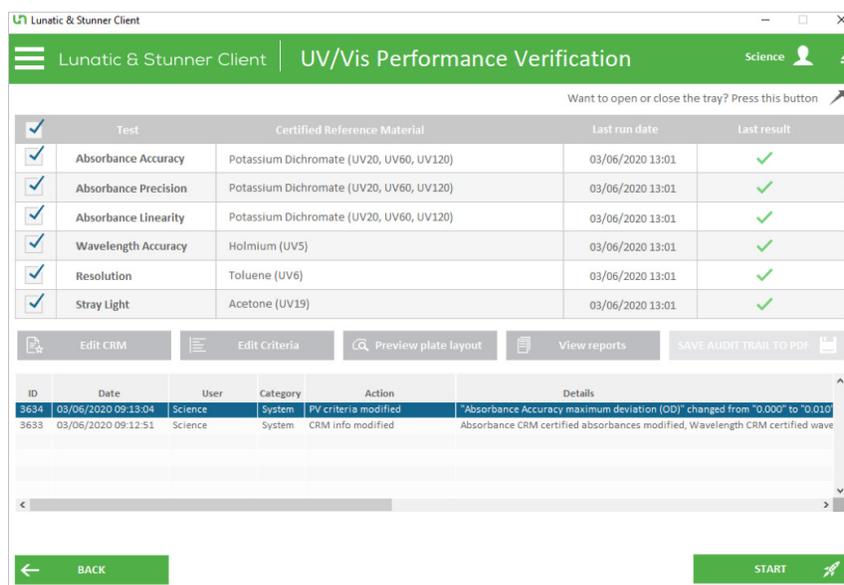


Figure 4: When Lunatic or Stunner passes the UV/Vis PV tests a green check mark is displayed next to the test in Lunatic & Stunner Client, along with the test date.

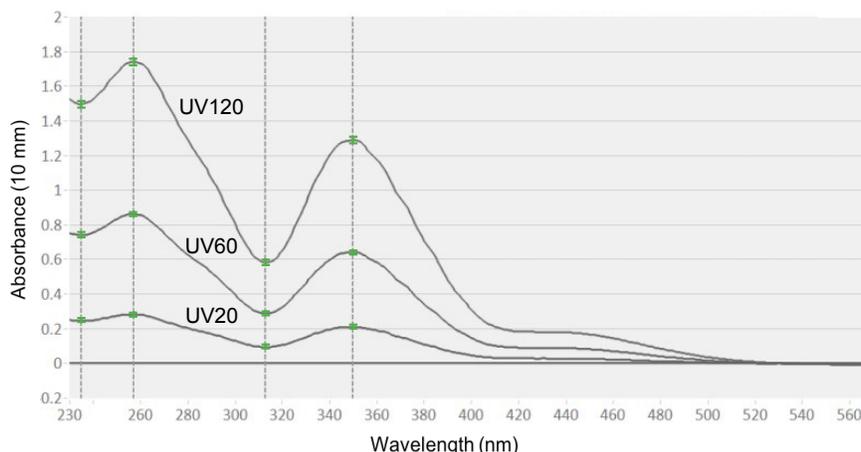


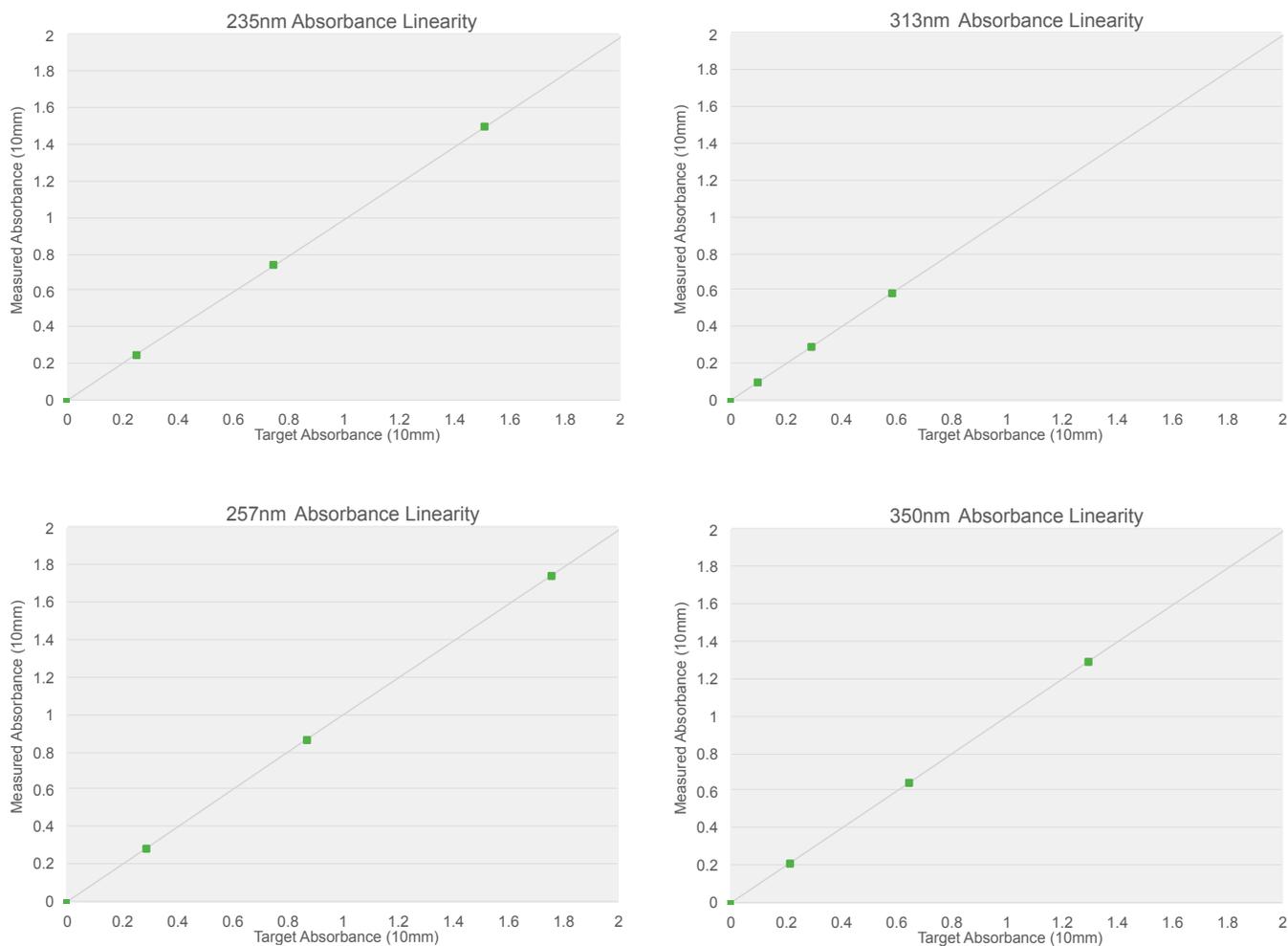
Figure 5: Representative absorbance spectra from UV20, UV60, and UV120. The dotted gray lines indicate the test wavelengths and the green bars on each spectrum mark the upper and lower absorbance acceptance limits.

The wavelength accuracy test, which compares the measured wavelengths of 5 absorbance peaks of holmium oxide in HClO₄ to their certified values, showed Lunatic determines wavelengths to within 1 nm across its entire range from 230–750 nm (Figure 7).

In order to pass USP and Ph. Eur. guidelines for wavelength resolution as a quantitative analysis tool, a UV/Vis spectrometer should have a spectral band width (SBW) less than 2 nm and will therefore determine an maximum absorbance/minimum absorbance ratio for toluene in hexane that is ≥ 1.3 .

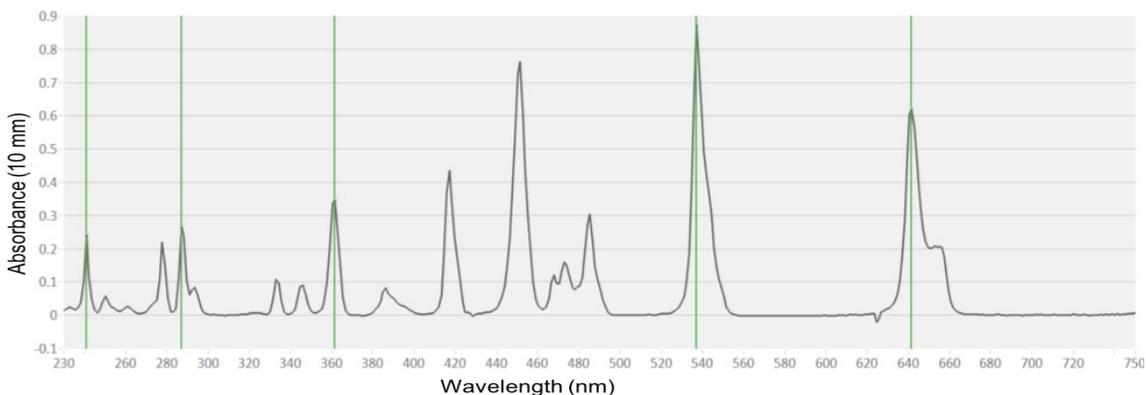
Shown is a graph where Lunatic determined a ratio of 1.32 (Figure 8).

The absorbances of the UV/Vis PV kit acetone cuvette at 260, 280, and 300 nm were greater than 3.0 and stray light was less than 0.1% at all 3 tested wavelengths (Figure 9). These results meet the acceptance criteria for both USP and Ph. Eur.



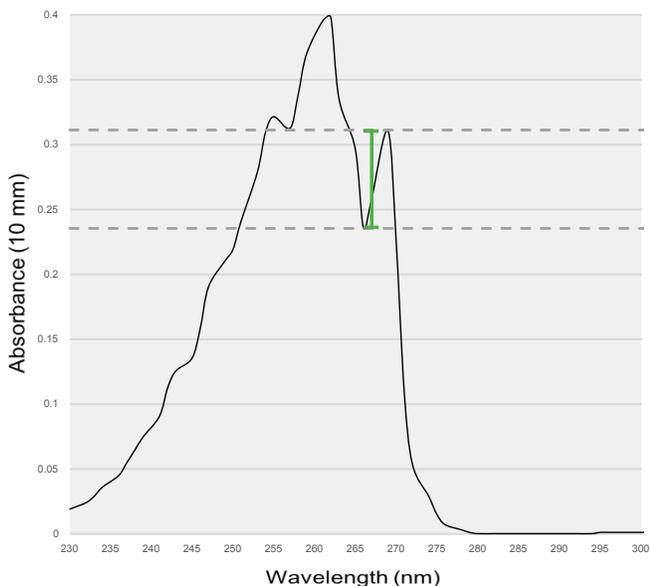
Wavelength (nm)	R ²	Results
235	0.999996	PASS
257	0.999992	PASS
313	0.999984	PASS
350	0.999985	PASS

Figure 6: Representative linearity results of the 3 CRMs were within the acceptance limits at each of the 4 test wavelengths.



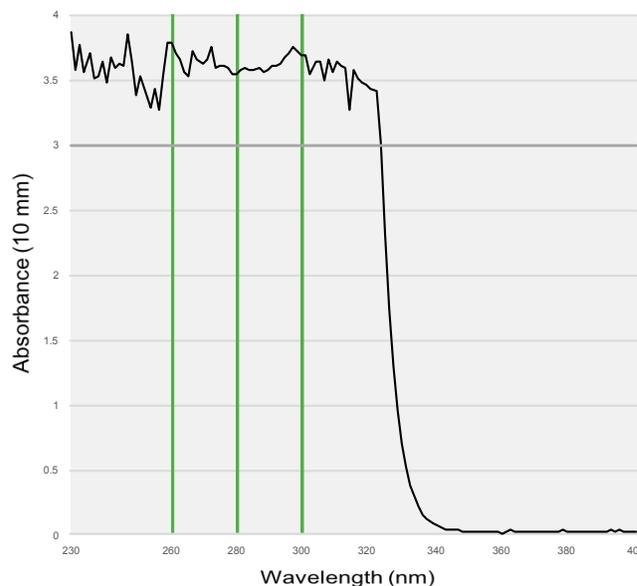
Certified wavelength (nm)	Measured peak position (nm)	Results
241	240.84	PASS
287	287.31	PASS
361	360.79	PASS
537	537.62	PASS
641	641.79	PASS

Figure 7: Representative absorbance of holmium oxide in HClO₄ with the certified peak positions depicted by vertical green lines. The peak positions of the measured absorbance spectrum were within the acceptance limits.



Min. (A)	Max. (A)	Ratio	Result
0.236	0.311	1.32	PASS

Figure 8: Representative absorbance of 0.02% (v/v) toluene in hexane with the minimum at approximately 266 nm and maximum at approximately 269 nm shown by the gray dashed lines. The ratio of the measured absorbances of the two wavelengths was 1.32, which indicates a SBW less than 2 nm.



Wvl. (nm)	Stray light	Abs. (A)	Result
260	0.02%	3.8	PASS
280	0.03%	3.6	PASS
300	0.02%	3.7	PASS

Figure 9: Representative absorbance curve of acetone. The minimum acceptable absorbance by Ph. Eur. is shown by the gray line. The tested wavelengths are depicted by the green vertical lines. Acceptance limits are less than 0.1% stray light and ≥ 3.0 A at each wavelength.

Conclusion

Adding UV/Vis Performance Verification to Lunatic and Stunner creates the perfect system to unify biologics quantification across an entire workflow from research to manufacturing to QA/QC. The high-performance UV/Vis spectrometers of Lunatic and Stunner make quantifying protein or nucleic acids easy, and their software features a range of applications that gives valuable quality and purity information on samples. Accuracy and precision you can prove for yourself, in full compliance with USP and Ph. Eur. standards, mean the results from Lunatic and Stunner are ready for any environment.



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