White Paper

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Pharmaceutical Analysis using UV-Vis: Compliance with Supplement I to the Japanese Pharmacopoeia 18th Ed., Section 2.24

Meeting the requirements of the global pharmacopeias with the 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 Japanese Pharmacopeia (JP) guidelines describe how to verify that the analytical performance of UV-Vis spectrophotometers is suitable for the intended operational range of the analysis. This is described in Supplement I to the Japanese Pharmacopoeia Eighteenth Edition, General Tests, Processes and Apparatus, Section 2.24 revised on 7 June 2021.

Within Cary UV Workstation software for the Cary 3500 UV-Vis system, shown in Figure 1, a range of system verification tests are available and automated. These tests align with the JP requirements, while also allowing the flexibility to cover a limited custom test list.

Figure 1. The Cary 3500 Multicell UV-Vis spectrophotometer.

Table 1 outlines 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 the JP specifications.

Table 1. JP system verification tests recommended for the Cary 3500 UV-Vis system.

Test	Test description and limits	Reference materials used
Control of Wavelength	When the measurement is repeated three times, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm and each value obtained should be within the mean ± 0.2 nm.	Rare earth oxide liquid filters: - Holmium oxide in perchloric acid - Didymium - Cerium oxide in sulfuric acid Rare earth oxide glass filters: - Holmium oxide
Control of Absorbance	When the measurement is repeated three times, each absorbance obtained should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500.	Liquid filters: - Potassium dichromate Glass filters: - National Institute of Standards and Technology (NIST) traceable filters (930E)

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.

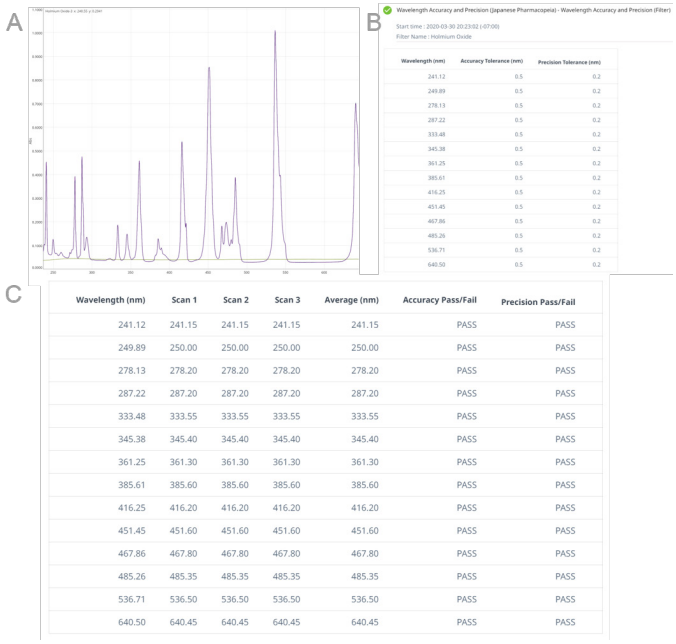
Control of wavelength

The wavelength accuracy tests are 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 rare earth oxide liquid or glass filters. Rare earth oxides yield well characterized absorption bands, enabling the comparison of the UV-Vis spectrophotometer wavelength readings to the published values. The rare earth oxide solutions: holmium oxide in perchloric acid (from 240 to 650 nm, Figure 2), didymium (from 290 to 870 nm, Figure 3), and cerium oxide in sulfuric acid (from 200 to 300 nm, Figure 4) are well established and widely available as certified reference materials (CRMs) that yield well-characterized peaks across the UV-Vis spectrum.

Alternatively, glass filters, prepared by fusing a rare earth, such as holmium, into a base glass matrix, can be used for wavelength verification tests (Figure 5).

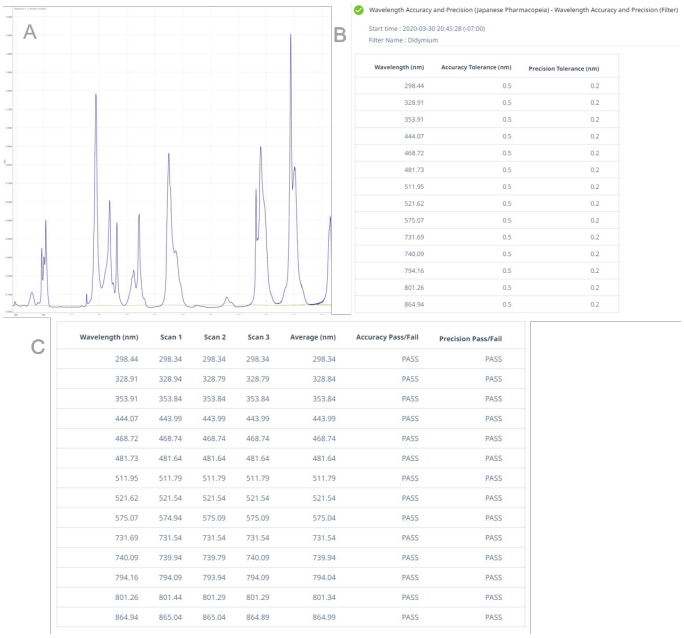
To assess the wavelength accuracy, the Cary 3500 UV-Vis spectrophotometer performs a wavelength scan across the relevant range for each material and identifies the wavelength position for the corresponding peak maximum (Figures 2, 3, 4, 5 and 6). The peak positions are then cross-checked with the certified data for that standard. JP chapter 2.24 on UV-Vis spectrophotometry requires that wavelength accuracy in the UV and visible regions of the spectrum must be ± 0.5 nm. While the Cary 3500 has a variable spectral bandwidth from 0.1 to 5.0 nm that allows for the optimal parameters for all sample types, all these tests use a spectral band width (SBW) of 1.0 nm.



Wavelength (nm)	Accuracy Tolerance (nm)	Precision Tolerance (nm)
241.12	0.5	0.2
249.89	0.5	0.2
278.13	0.5	0.2
287.22	0.5	0.2
333.48	0.5	0.2
345.38	0.5	0.2
361.25	0.5	0.2
385.61	0.5	0.2
416.25	0.5	0.2
451.45	0.5	0.2
467.86	0.5	0.2
485.26	0.5	0.2
536.71	0.5	0.2
640.50	0.5	0.2

Wavelength (nm)	Scan 1	Scan 2	Scan 3	Average (nm)	Accuracy Pass/Fail	Precision Pass/Fail
241.12	241.15	241.15	241.15	241.15	PASS	PASS
249.89	250.00	250.00	250.00	250.00	PASS	PASS
278.13	278.20	278.20	278.20	278.20	PASS	PASS
287.22	287.20	287.20	287.20	287.20	PASS	PASS
333.48	333.55	333.55	333.55	333.55	PASS	PASS
345.38	345.40	345.40	345.40	345.40	PASS	PASS
361.25	361.30	361.30	361.30	361.30	PASS	PASS
385.61	385.60	385.60	385.60	385.60	PASS	PASS
416.25	416.20	416.20	416.20	416.20	PASS	PASS
451.45	451.60	451.60	451.60	451.60	PASS	PASS
467.86	467.80	467.80	467.80	467.80	PASS	PASS
485.26	485.35	485.35	485.35	485.35	PASS	PASS
536.71	536.50	536.50	536.50	536.50	PASS	PASS
640.50	640.45	640.45	640.45	640.45	PASS	PASS

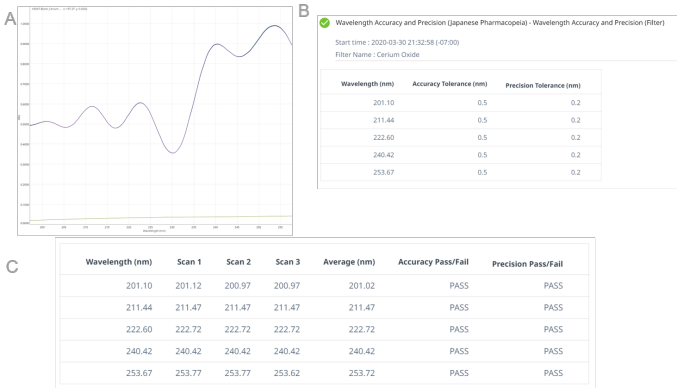
Figure 2. Wavelength accuracy test results for holmium oxide in perchloric acid. (A) Three repeated wavelength scans of holmium oxide in perchloric acid with 100%T baseline; (B) peak positions and tolerances applied; (C) raw peak positions for each individual scan tabulated with the average, and the pass/fail result for accuracy and precision.



Wavelength (nm)	Accuracy Tolerance (nm)	Precision Tolerance (nm)
298.44	0.5	0.2
328.91	0.5	0.2
353.91	0.5	0.2
444.07	0.5	0.2
468.72	0.5	0.2
481.73	0.5	0.2
511.95	0.5	0.2
521.62	0.5	0.2
575.07	0.5	0.2
731.69	0.5	0.2
740.09	0.5	0.2
794.16	0.5	0.2
801.26	0.5	0.2
864.94	0.5	0.2

Wavelength (nm)	Scan 1	Scan 2	Scan 3	Average (nm)	Accuracy Pass/Fail	Precision Pass/Fail
298.44	298.34	298.34	298.34	298.34	PASS	PASS
328.91	328.94	328.79	328.79	328.84	PASS	PASS
353.91	353.84	353.84	353.84	353.84	PASS	PASS
444.07	443.99	443.99	443.99	443.99	PASS	PASS
468.72	468.74	468.74	468.74	468.74	PASS	PASS
481.73	481.64	481.64	481.64	481.64	PASS	PASS
511.95	511.79	511.79	511.79	511.79	PASS	PASS
521.62	521.54	521.54	521.54	521.54	PASS	PASS
575.07	574.94	575.09	575.09	575.04	PASS	PASS
731.69	731.54	731.54	731.54	731.54	PASS	PASS
740.09	739.94	739.79	740.09	739.94	PASS	PASS
794.16	794.09	793.94	794.09	794.04	PASS	PASS
801.26	801.44	801.29	801.29	801.34	PASS	PASS
864.94	865.04	865.04	864.89	864.99	PASS	PASS

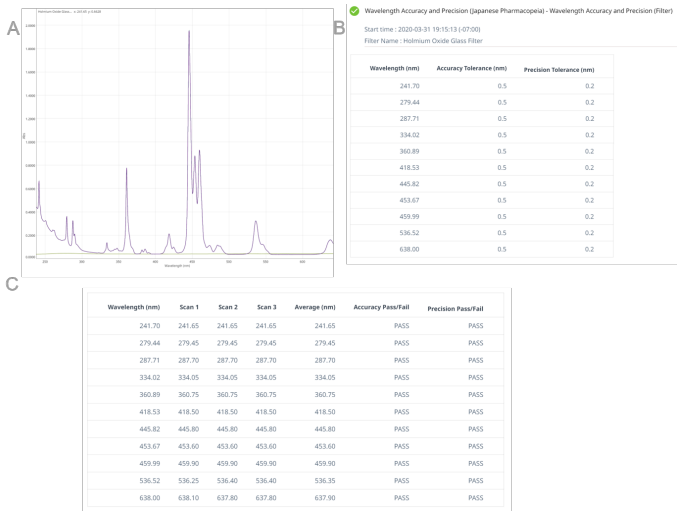
Figure 3. Wavelength accuracy test results for didymium. (A) Three repeated wavelength scans of didymium with 100%T baseline; (B) peak positions and tolerances applied; (C) raw peak positions for each individual scan tabulated with the average, and the pass/fail result for accuracy and precision.



Wavelength (nm)	Accuracy Tolerance (nm)	Precision Tolerance (nm)
201.10	0.5	0.2
211.44	0.5	0.2
222.60	0.5	0.2
240.42	0.5	0.2
253.67	0.5	0.2

Wavelength (nm)	Scan 1	Scan 2	Scan 3	Average (nm)	Accuracy Pass/Fail	Precision Pass/Fail
201.10	201.12	200.97	200.97	201.02	PASS	PASS
211.44	211.47	211.47	211.47	211.47	PASS	PASS
222.60	222.72	222.72	222.72	222.72	PASS	PASS
240.42	240.42	240.42	240.42	240.42	PASS	PASS
253.67	253.77	253.77	253.62	253.72	PASS	PASS

Figure 4. Wavelength accuracy test results for cerium oxide in sulfuric acid. (A) Three repeated wavelength scans of cerium oxide in sulfuric acid with 100%T baseline; (B) peak positions and tolerances applied; (C) raw peak positions for each individual scan tabulated with the average, and the pass/fail result for accuracy and precision.



Wavelength (nm)	Accuracy Tolerance (nm)	Precision Tolerance (nm)
241.70	0.5	0.2
279.44	0.5	0.2
287.71	0.5	0.2
334.02	0.5	0.2
360.89	0.5	0.2
418.53	0.5	0.2
445.82	0.5	0.2
453.67	0.5	0.2
459.99	0.5	0.2
536.52	0.5	0.2
638.00	0.5	0.2

Wavelength (nm)	Scan 1	Scan 2	Scan 3	Average (nm)	Accuracy Pass/Fail	Precision Pass/Fail
241.70	241.65	241.65	241.65	241.65	PASS	PASS
279.44	279.45	279.45	279.45	279.45	PASS	PASS
287.71	287.70	287.70	287.70	287.70	PASS	PASS
334.02	334.05	334.05	334.05	334.05	PASS	PASS
360.89	360.75	360.75	360.75	360.75	PASS	PASS
418.53	418.50	418.50	418.50	418.50	PASS	PASS
445.82	445.80	445.80	445.80	445.80	PASS	PASS
453.67	453.60	453.60	453.60	453.60	PASS	PASS
459.99	459.90	459.90	459.90	459.90	PASS	PASS
536.52	536.25	536.40	536.40	536.35	PASS	PASS
638.00	638.10	637.80	637.80	637.90	PASS	PASS

Figure 5. Wavelength accuracy test results for holmium oxide glass filter. (A) Three repeated wavelength scans of holmium oxide glass filter with 100%T baseline; (B) peak positions and tolerances applied; (C) raw peak positions for each individual scan tabulated with the average, and the pass/fail result for accuracy and precision.

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 three replicates of the absorbance peaks (Figure 5). JP requires that the precision of UV-Vis system is better than 0.2 nm across the operational range of the instrument.

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 photometric accuracy according to the JP, can be conducted on CRM solutions of potassium dichromate (Figure 6), which has absorbance peaks in the UV region of the spectrum; National Institute of Standards and Technology (NIST) traceable filters (930E, Figure 7) can be similarly used for the visible region of the spectrum.

Photometric accuracy is performed by taking three replicates using a certified optical filter to determine the difference between the measured and certified absorbance values. When the absorbance is not more than 0.500, the absorbance accuracy must be within the mean ± 0.002 Abs, and when the absorbance is more than 0.500, the absorbance accuracy must be within the mean ±0.004 Abs.

The Cary UV Workstation system verification tests allows for up to three potassium dichromate solution concentrations or NIST-traceable filters to be measured automatically. The user is able to enter the expected absorbance and required accuracy, and precision (Figure 7).

Photometric precision

The photometric precision of the system is determined similarly to the wavelength precision test. The results of three replicate measurements are required and assess how reproducibly the UV-Vis determines photometric absorbance. The results are shown in Figures 6, and 7.

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

A

PDC 40

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
350.00	0.4240	0.4238	0.4238	0.4239	Pass	Pass
313.00	0.1917	0.1917	0.1917	0.1917	Pass	Pass
257.00	0.5696	0.5696	0.5696	0.5696	Pass	Pass
235.00	0.4918	0.4918	0.4917	0.4918	Pass	Pass

PDC 80

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
350.00	0.8531	0.8530	0.8531	0.8531	Pass	Pass
313.00	0.3857	0.3856	0.3856	0.3857	Pass	Pass
257.00	1.1514	1.1514	1.1515	1.1514	Pass	Pass
235.00	0.9939	0.9939	0.9939	0.9939	Pass	Pass

PDC 120

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
350.00	1.2708	1.2706	1.2709	1.2708	Pass	Pass
313.00	0.5729	0.5730	0.5729	0.5729	Pass	Pass
257.00	1.7284	1.7285	1.7284	1.7284	Pass	Pass
235.00	1.4902	1.4900	1.4901	1.4901	Pass	Pass

B

Linearity ($r^2 \geq 0.999$)

Wavelength (nm)	PDC 40	PDC 80	PDC 120	r^2	Pass/Fail
350.00	0.4239	0.8531	1.2708	1.0000	Pass
313.00	0.1917	0.3857	0.5729	1.0000	Pass
257.00	0.5696	1.1514	1.7284	1.0000	Pass
235.00	0.4918	0.9939	1.4901	1.0000	Pass

Figure 6. (A) Potassium dichromate (PDC) photometric accuracy and precision results for the 40, 80 and 120 mg/L standards. The tables show the wavelengths measured, tolerances for accuracy and precision as well as the raw absorbance data, the averages, and the pass/fail determination. (B) Potassium dichromate photometric linearity (r^2) calculated at three different concentrations and four wavelengths, and the pass/fail determination.

A

Filter 1 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
440.00	1.0400	1.0399	1.0399	1.0399	Pass	Pass
465.00	0.9575	0.9575	0.9575	0.9575	Pass	Pass
546.10	0.9680	0.9688	0.9684	0.9684	Pass	Pass
590.00	1.0078	1.0079	1.0081	1.0079	Pass	Pass
635.00	0.9615	0.9616	0.9616	0.9616	Pass	Pass

Filter 2 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
440.00	0.7199	0.7198	0.7200	0.7199	Pass	Pass
465.00	0.6643	0.6643	0.6644	0.6644	Pass	Pass
546.10	0.6742	0.6740	0.6742	0.6741	Pass	Pass
590.00	0.7038	0.7036	0.7037	0.7037	Pass	Pass
635.00	0.6719	0.6717	0.6718	0.6718	Pass	Pass

Filter 3 - NIST glass filter

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Accuracy Pass/Fail	Precision Pass/Fail
440.00	0.5397	0.5397	0.5396	0.5396	Pass	Pass
465.00	0.4902	0.4903	0.4903	0.4903	Pass	Pass
546.10	0.5043	0.5044	0.5044	0.5044	Pass	Pass
590.00	0.5331	0.5332	0.5330	0.5331	Pass	Pass
635.00	0.5182	0.5182	0.5181	0.5182	Pass	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	1.0399	0.7199	0.5396	1.0000	Pass
465.00	0.9575	0.6644	0.4903	0.9999	Pass
546.10	0.9684	0.6741	0.5044	1.0000	Pass
590.00	1.0079	0.7037	0.5331	1.0000	Pass
635.00	0.9616	0.6718	0.5182	1.0000	Pass

Figure 7. (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, and the pass/fail determination. (B) NIST glass filters photometric linearity (r^2) calculated at three different concentration and five wavelengths, and the pass/fail determination.

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. According to the JP it is desirable to confirm the linearity of absorbance at the same wavelength using different optical densities. 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 (Figures 6B and 7B).

Workflow

The system verification application of the Cary UV Workstation provides an automated method for verifying that your instrument is performing to the recommendations in Supplement I to the Japanese Pharmacopoeia Seventeenth Edition, General Tests, Processes and Apparatus, Section 2.24. The user selects the test required (e.g. wavelength and/or photometric accuracy and precision), then selects the certified reference filter, followed by entering values from the CRM certificate. An on-screen sample loading guide indicates when and where to place each reference into the instrument (Figures 8 and 9). All collected results are displayed in the system verification report with Pass/Fail recorded for each test.

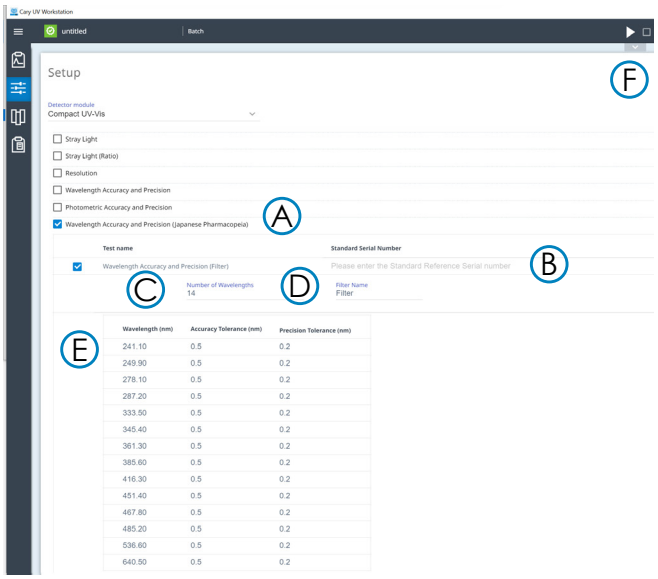


Figure 8. Wavelength accuracy and precision test using Cary UV Workstation according to the Japanese pharmacopeia requirements. (A) Select the test; (B) enter the standard reference serial number; (C) select number of wavelength entries; (D) enter name of the filter; (E) manually enter the wavelengths specified in the certified reference material certificate and; (F) press play and follow instructions on the screen.

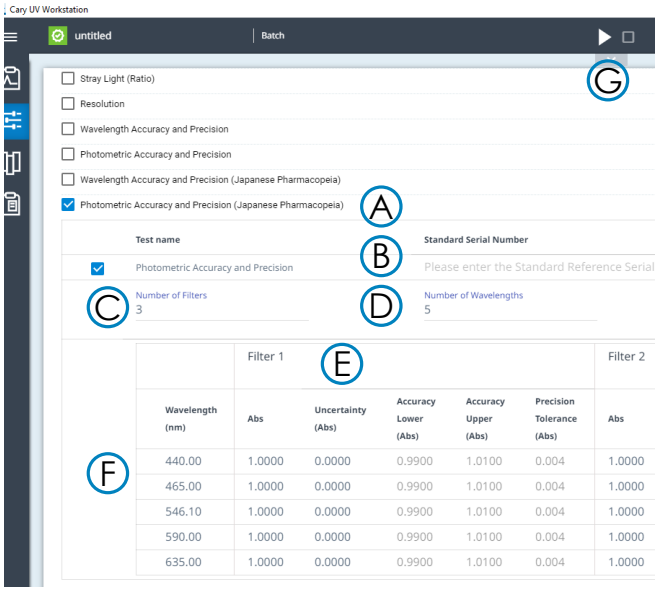


Figure 9. Photometric accuracy and precision test using Cary UV Workstation according to the Japanese pharmacopeia requirements. (A) Select the test; (B) enter the standard reference serial number; (C) select number of filters to be tested; (D) select number of wavelengths to be tested for each filter; (E) enter name of the filter; (F) manually enter the wavelengths, absorbance and uncertainties specified in the certified reference material certificate and; (G) press play and follow instructions on the screen.

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 JP describes 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 white paper demonstrates how to ensure qualification of the Cary 3500 as per JP 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 spectrophotometer, including the system verification application used to perform the instrument performance tests described in this white paper.

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