

# Pharmaceutical Analysis using UV-Vis: Compliance with the 18th Edition of the Japanese Pharmacopoeia

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



## Introduction

Laboratories that are involved in the development, testing, or manufacture of pharmaceuticals must comply with the appropriate regulatory framework within their respective country or region. To ensure the quality, safety, and efficacy of pharmaceutical products, laboratories typically set up controls for laboratory access and adhere to Good Manufacturing Practice (GMP) guidelines. As part of GMP compliance, laboratories often need to have appropriate documentation in place. This documentation can include system suitability tests (SSTs), which are used to verify that analytical instruments or methods are suitable for their intended use, as well as standard operating procedures (SOPs), which provide detailed instructions for performing specific laboratory processes or tasks.

While GMP provides guidelines for pharmaceutical manufacturing, "pharmacopeias" provide specific quality standards and testing methods relating to the quality, purity, identity, and strength of pharmaceutical products, active ingredients, and excipients. Examples of global pharmacopeias include the United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), Chinese Pharmacopoeia (ChP), Japanese Pharmacopoeia (JP), British Pharmacopoeia (BP), and International Pharmacopoeia (Ph. Int.), which is published by the World Health Organization (WHO). Each pharmacopeia has its own specific standards and guidelines, although efforts are made to harmonize standards. The aim of the pharmacopeias is to ensure the quality and safety of pharmaceutical products for patients.

## Japanese Pharmacopoeia (JP)

Ultraviolet visible (UV-Vis) spectroscopy is a well-established analytical technique that is widely used in the pharmaceutical industry for routine, quality assurance/quality control (QA/QC) testing of raw materials and final products. It is also used in pharmaceutical research and development projects.

The JP was updated in 2021 to include guidelines on how to verify that the analytical performance of a UV-Vis spectrophotometer is suitable for the intended operational range of the analysis. The procedure is described in Supplement I to the JP Eighteenth Edition (JP18), General Tests, Processes and Apparatus, Section 2.24, which became official on June 7, 2021.<sup>1</sup>

JP18 also includes more reference spectra of substances that are used for identification or assay of samples by UV-Vis spectrophotometry.

Within the Agilent Cary UV Workstation software for the Agilent Cary 3500 UV-Vis spectrophotometer series, a range of automated system verification tests are available. These automated tests align with the requirements of the JP, while also allowing the flexibility to cover some custom tests.

Table 1 includes the system verification tests outlined in JP18 that are relevant to the Cary 3500 UV-Vis series of spectrophotometers. The system verification tests are designed in a way that successfully passing all tests will ensure that the instrument is performing in accordance with the specifications stated in the JP. The certified reference materials (CRMs) listed in Table 1 are mentioned and available from the Japan Quality Assurance Organization (JQA), Tokyo, Japan.

**Table 1.** System verification tests specified in JP of relevance to the Agilent Cary 3500 UV-Vis spectrophotometer series.

Test	Test Description and Limits	Reference Materials Used
Wavelength Calibration	When the measurement is repeated three times, the difference between the measured wavelength and the standard wavelength value should be within $\pm 0.5$ nm, and each value obtained should be within the mean $\pm 0.2$ nm.	JCRM 001 – Neodymium optical filter (400 to 750 nm) JCRM 002 – Holmium optical filter (250 to 600 nm)
Transmission Rate or Absorbance Calibration	<p><b>Photometric accuracy</b> The transmission rate of the certified optical filter is determined at the standard wavelength value given in the certificate.</p> <p>The difference between the measured transmission rate and the standard transmission rate value should be within the range from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the certificate provided.</p> <p><b>Photometric precision</b> When the measurement of the certified optical filter is repeated three times, each absorbance value (or calculated from the transmission rate) obtained should be within the mean <math>\pm 0.002</math> when the absorbance is not more than 0.500, and within the mean <math>\pm 0.004</math> when the absorbance is more than 0.500.</p>	<p><b>Visible wavelength range:</b> JCRM 101 to 1%T JCRM 110 to 10%T JCRM 120 to 20%T JCRM 130 to 30%T JCRM 140 to 40%T JCRM 150 to 50%T</p> <p><b>Ultraviolet wavelength range:</b> JCRM 210 A to 10%T JCRM 230 A to 30%T JCRM 250 A to 50%T</p> <p><b>Near-ultraviolet wavelength range:</b> JCRM 310 to 10%T JCRM 330 to 30%T JCRM 350 to 50%T</p>
Photometric Linearity	Linearity of transmission rate at the same wavelength should be confirmed using several optical filters with different transmission rates. Typically, filters used for calibration of transmission rate are used.	Same as transmission rate or absorbance calibration reference materials.

## The Cary 3500 UV-Vis spectrophotometer series

The Cary 3500 UV-Vis spectrophotometer series, shown in Figure 1, shares a common UV-Vis engine. The engine produces monochromatic light that is measured by various UV-Vis sample measurement modules. This design enables the development of custom modules for specific applications, maximizing the flexibility of the technique. The Cary 3500 Compact and Multicell UV-Vis modules are used for cuvette-based

measurements of liquid samples, while the Cary 3500 Flexible UV-Vis module provides measurements for both liquid and solid samples.

### Wavelength calibration

#### Wavelength accuracy

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 wavelength range.

Confirmation of wavelength accuracy is recommended to be

tested using rare earth oxide optical filters. Rare earth oxides yield well characterized absorption bands, enabling the comparison of the UV-Vis spectrophotometer wavelength readings to the published values. Optical filters that are prepared by fusing a rare earth, such as holmium, into a base glass matrix, are widely available as CRMs for UV-Vis.

To assess the wavelength accuracy of a UV-Vis spectrophotometer, a wavelength scan is performed across the relevant range for each CRM and the wavelength position for the corresponding peak maximum is identified.

## Agilent Cary 3500 UV-Vis spectrophotometers series



#### Cary 3500 Compact UV-Vis spectrophotometer\*

The Cary Compact UV-Vis is designed for measuring a single sample and reference. It is available in either an ambient or temperature-controlled configuration.



#### Cary 3500 Multicell UV-Vis spectrophotometer\*\*

The Cary 3500 Multicell UV-Vis is designed for measuring up to seven samples and a reference (or other combinations in the eight cell positions). It is available in either an ambient, temperature-controlled, or multiple-temperature-zone configuration.



#### Cary 3500 Flexible UV-Vis spectrophotometer

The Cary 3500 Flexible UV-Vis is designed for measuring a single sample and reference. It has a large sample compartment, in which liquid and solid samples can be measured.

\* Compact ambient and temperature-controlled

\*\* Multicell ambient, temperature-controlled or multiple-temperature-zone configuration

Figure 1. The Agilent Cary 3500 UV-Vis spectrophotometer series.

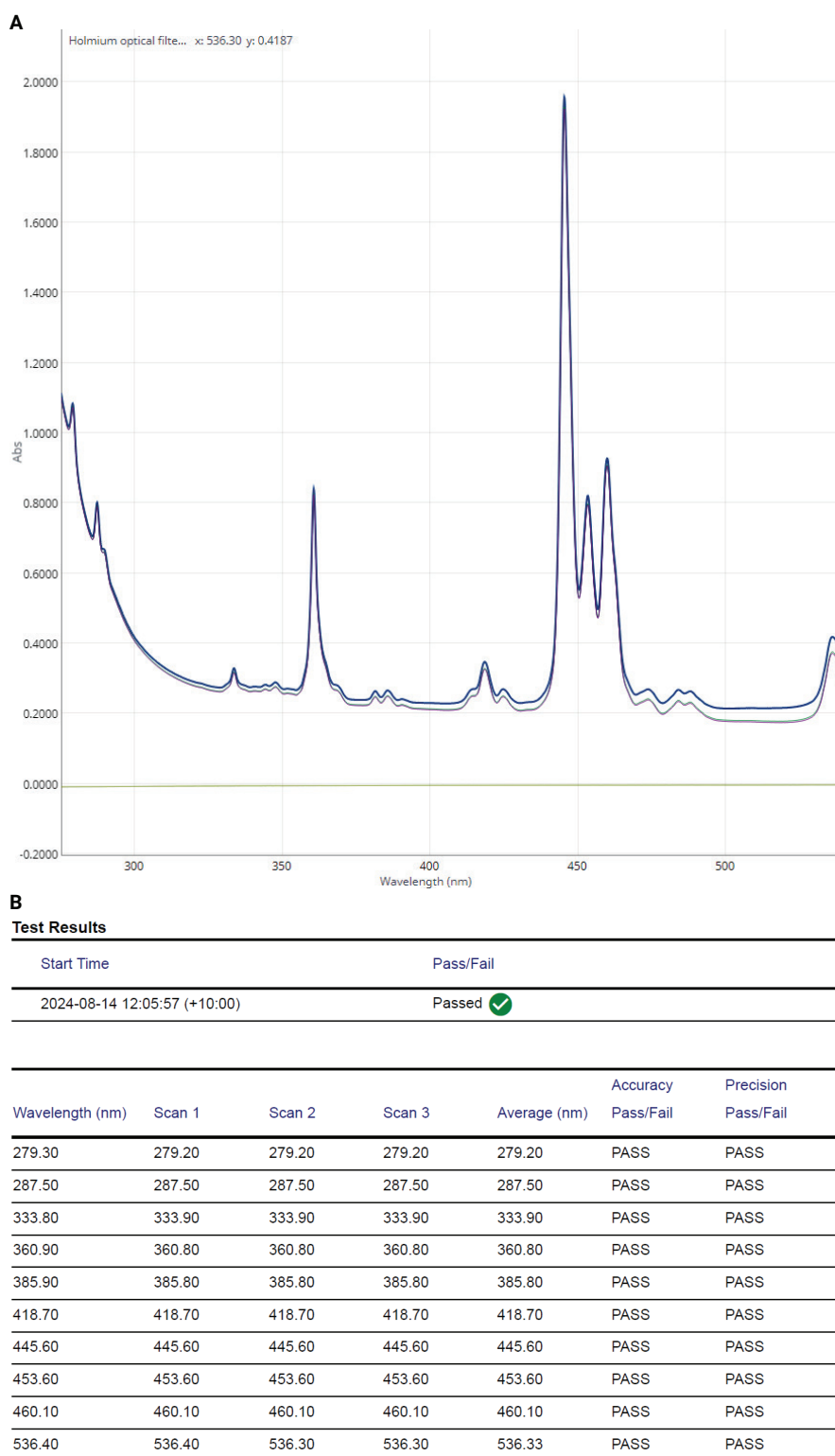
To demonstrate the wavelength accuracy test, the Cary 3500 acquired wavelength scan data for the holmium glass filter (JQA, catalogue number: JCRM002). The peak positions were then cross-checked with the certified data for the standard (Figure 2). JP chapter 2.24 on UV-Vis spectrophotometry requires that wavelength accuracy in the UV and visible regions of the spectrum must be  $\pm 0.5$  nm across the operational range of the instrument.

The engine of the Cary 3500 includes all instrument components that are used to determine the wavelength of light. For the Multicell module of the 3500, any one of the cuvette positions can be used to determine the wavelength accuracy of the instrument. Also, only one cuvette position of the Multicell module needs to be tested since the module cannot change the wavelength of the light.

### Wavelength precision

The wavelength precision was determined by repeating the measurement peaks of the holmium glass filter three times (Figure 2). The JP requires that each measurement should be within  $\pm 0.2$  nm of the mean.

As shown in Figure 2B, the Cary 3500 UV-Vis spectrophotometer passed the accuracy and precision wavelength tests across the wavelength range.



**Figure 2.** Wavelength accuracy test results for holmium oxide optical filter obtained using the Agilent Cary 3500 UV-Vis. (A) Three repeated wavelength scans of holmium glass filter with 100%T baseline. (B) Tabulated wavelength data of the raw peak positions for each individual scan and the average reading. The pass/fail result for wavelength accuracy and precision is also shown.

## Transmission rate or absorbance calibration

### 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 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.

According to the JP, photometric accuracy tests can be conducted using optical filters for transmission rate (Table 1), which have absorbance peaks in the near-ultraviolet, ultraviolet, and visible wavelength range of the spectrum.

Photometric accuracy is performed by measuring the transmission rate of certified optical filters at the standard wavelength value given in the certificate. The difference between the measured transmission rate and the standard transmission rate value should be within the range of +1% of the upper limit to -1% of the lower limit of the relative accuracy shown in the certificate.

The Cary UV Workstation system verification tests allow for the details of up to four optical filter CRMs to be entered, so that each filter can then be automatically measured sequentially. The user is able to enter the expected transmission value and relative uncertainty into the software, and the required accuracy and precision will be calculated automatically, as described in the workflow section of this paper.

The Cary 3500 was used to measure the JCRM 110, 130, and 150 standards. The results shown in Figure 3 demonstrate the high level of photometric accuracy

(%T) of the instrument, passing the criteria specified in the JP.

JCRM110 - Accuracy (%T)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (%T)	Accuracy Pass/Fail
440.00	7.14	7.14	7.14	7.14	Pass
465.00	9.76	9.76	9.76	9.76	Pass
546.10	9.95	9.95	9.94	9.95	Pass
590.00	8.56	8.56	8.56	8.56	Pass
635.00	8.97	8.97	8.97	8.97	Pass

JCRM130 - Accuracy (%T)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (%T)	Accuracy Pass/Fail
440.00	24.18	24.18	24.18	24.18	Pass
465.00	29.43	29.43	29.43	29.43	Pass
546.10	30.02	30.02	30.02	30.02	Pass
590.00	27.56	27.57	27.56	27.57	Pass
635.00	27.31	27.31	27.31	27.31	Pass

JCRM150 - Accuracy (%T)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (%T)	Accuracy Pass/Fail
440.00	43.70	43.70	43.70	43.70	Pass
465.00	50.07	50.07	50.07	50.07	Pass
546.10	50.72	50.73	50.73	50.73	Pass
590.00	48.95	48.96	48.96	48.96	Pass
635.00	49.41	49.41	49.40	49.40	Pass

**Figure 3.** Photometric accuracy results for JCRM 110, 130, and 150 standards using an Agilent Cary 3500 UV-Vis spectrophotometer. The tables show the wavelengths measured, three reads of the transmission values, %T averages, and the pass/fail determination of photometric accuracy per the JP criteria.

## Photometric precision

The test used to determine the photometric precision of a UV-Vis spectrophotometer is similar to the wavelength precision test. Three replicate measurements of a certified optical filter are taken to determine the difference between the measured and certified absorbance values (or calculated from transmission rate). When the absorbance is not more than 0.500, the absorbance precision must be within the mean  $\pm 0.002$  Abs, and when the absorbance is more than 0.500, the absorbance precision must be within the mean  $\pm 0.004$  Abs.

The Cary 3500 was used to measure the JCRM 101, 120, and 140 standards. The results shown in Figure 4 demonstrate the high level of photometric precision (Abs) of the instrument, passing the criteria specified in the JP.

## 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 nonlinear. According to the JP, it is desirable to confirm the linearity of absorbance at the same wavelength using different optical densities or concentrations.

With the Cary 3500 UV-Vis spectrophotometer series and the Cary UV Workstation System Verification application, photometric linearity can be calculated automatically by measuring three to four optical filters of different concentrations. Figure 5 shows the excellent linearity ( $R^2$  of 1.000) of the Cary 3500 for the measurement of three CRMs at five different wavelengths.

For the Multicell module of the Cary 3500, the photometric (absorbance) accuracy, precision, and linearity must be confirmed for the intended operational range for all cuvette positions.

JCRM101 – Precision (Abs)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Precision Pass/Fail
440.00	2.1346	2.1349	2.1344	2.1346	Pass
465.00	1.9429	1.9429	1.9435	1.9431	Pass
546.10	1.9360	1.9359	1.9361	1.9360	Pass
590.00	1.9932	1.9927	1.9947	1.9935	Pass
635.00	1.8440	1.8440	1.8449	1.8443	Pass

JCRM120 – Precision (Abs)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Precision Pass/Fail
440.00	0.8238	0.8238	0.8239	0.8238	Pass
465.00	0.7101	0.7102	0.7101	0.7101	Pass
546.10	0.6979	0.6979	0.6978	0.6979	Pass
590.00	0.7448	0.7450	0.7449	0.7449	Pass
635.00	0.7279	0.7280	0.7281	0.7280	Pass

JCRM140 – Precision (Abs)

Wavelength (nm)	Read 1	Read 2	Read 3	Average (Abs)	Precision Pass/Fail
440.00	0.4741	0.4741	0.4741	0.4741	Pass
465.00	0.4015	0.4015	0.4015	0.4015	Pass
546.10	0.3940	0.3941	0.3939	0.3940	Pass
590.00	0.4110	0.4109	0.4109	0.4110	Pass
635.00	0.4028	0.4029	0.4028	0.4028	Pass

**Figure 4.** Photometric precision results for JCRM 101, 120, and 140 standards using an Agilent Cary 3500 UV-Vis spectrophotometer. The tables show the wavelengths measured, three reads of absorbance values, absorbance averages, and the pass/fail determination of photometric precision per the JP criteria.

Linearity ( $r^2 \geq 0.999$ )

Wavelength (nm)	Filter 1	Filter 2	Filter 3	$r^2$	Pass/Fail
440.00	0.73	15.00	33.57	1.000	Pass
465.00	1.14	19.49	39.67	1.000	Pass
546.10	1.16	20.05	40.36	1.000	Pass
590.00	1.01	17.99	38.82	1.000	Pass
635.00	1.43	18.71	39.55	1.000	Pass

**Figure 5.** Photometric linearity results for JCRM 101, 120, and 140 standards using an Agilent Cary 3500 UV-Vis spectrophotometer. The table shows the photometric linearity ( $r^2$ ) calculated at three different concentrations and five wavelengths, and the pass/fail determination of photometric linearity per the JP criteria.

## Workflow

The system verification application of the Cary UV Workstation provides users with an automated method to verify that the performance of the Cary 3500 is in compliance with the recommended guidelines provided in JP section 2.24.<sup>1</sup>

The user selects the test (e.g., wavelength and/or photometric accuracy and precision) in the software, enters the serial number, name, and values from the CRM certificate for the reference filter (Figures 6 and 7). An on-screen sample loading guide then

indicates when and where to place each reference filter into the instrument. All collected results are displayed in the system verification report with pass/fail information recorded for each test.

**Setup**

Detector module: Multicell Peltier UV-Vis | Detector cell: Cell 1

Stray Light  
 Stray Light (Ratio)  
 Resolution  
 Wavelength Accuracy and Precision  
 Photometric Accuracy and Precision

**A**  Wavelength Accuracy and Precision (Japanese Pharmacopeia)

Test name	Standard Serial Number
<input checked="" type="checkbox"/> Holmium optical filter	<b>B</b> JCRM002 - SN 51997

<b>C</b> Wavelength (nm)	Accuracy Tolerance (nm)	Precision Tolerance (nm)
279.30	0.5	0.2
287.50	0.5	0.2
333.80	0.5	0.2
360.90	0.5	0.2
385.90	0.5	0.2
418.70	0.5	0.2
445.60	0.5	0.2
453.60	0.5	0.2
460.10	0.5	0.2
536.40	0.5	0.2

**Figure 6.** Wavelength accuracy and precision test using the Agilent Cary UV Workstation for the Agilent 3500 Cary UV-Vis according to the JP requirements. (A) Select the test. (B) Enter the standard (reference filter) serial number. (C) Manually enter the wavelengths specified in the certified reference material certificate. (D) Press the "play" icon and follow instructions on the screen.

Cary UV Workstation

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### Setup

Detector module: Multicell Peltier UV-Vis | Detector cell: Cell 1

- Stray Light
- Stray Light (Ratio)
- Resolution
- Wavelength Accuracy and Precision
- Photometric Accuracy and Precision
- Wavelength Accuracy and Precision (Japanese Pharmacopeia)
- A** Photometric Accuracy and Precision (Japanese Pharmacopeia)

Test name	Standard Serial Number
<input checked="" type="checkbox"/> Photometric Accuracy and Precision	<b>B</b> 10381, 12331, 14246
<b>C</b> Number of Filters: 3	<b>D</b> Number of Wavelengths: 5

	<b>E</b> JCRM 101						JCRM 120	
	Wavelength (nm)	Expected Value (%T)	Relative Uncertainty (%)	Accuracy Lower (%T)	Accuracy Upper (%T)	Precision Tolerance (Abs)	Expected Value (%T)	Relative Uncertainty (%)
<b>F</b>	440.00	0.73	3.00	0.70	0.76	0.004	14.97	0.58
	465.00	1.14	3.00	1.09	1.19	0.004	19.44	0.48
	546.10	1.15	3.00	1.10	1.20	0.004	19.98	0.55
	590.00	1.01	3.00	0.97	1.05	0.004	17.96	0.58
	635.00	1.42	3.00	1.36	1.48	0.004	18.66	0.56

**Figure 7.** Photometric accuracy and precision test using the Agilent Cary UV Workstation for the Agilent 3500 Cary UV-Vis according to the JP requirements. (A) Select the test. (B) Enter the standard (reference filter) serial number. (C) Select number of filters to be tested (up to 4). (D) Select number of wavelengths to be tested for each filter. (E) Enter the name of the filter. (F) Manually enter the wavelengths, %T values, and uncertainties specified in the certified reference material certificate. (G) Press the "play" icon and follow instructions on the screen.



## Conclusion

Pharmaceutical laboratories must ensure that their UV-Vis spectrophotometer is performing as required to meet regulatory requirements for drug analysis. The Japanese Pharmacopoeia (JP) describes how to test the intended operational range of a UV-Vis instrument, such as the Agilent Cary 3500 UV-Vis. For convenience, the relevant tests for the Cary 3500 UV-Vis spectrophotometer series are automated in the Agilent Cary UV Workstation software. By performing these system verification tests for the Cary 3500 UV-Vis, a laboratory can easily confirm if their instrument meets the performance criteria specified in the JP.

This paper has demonstrated that the Cary 3500 UV-Vis successfully met the JP requirements for wavelength calibration, transmission rate or absorbance calibration, and photometric linearity. Each model of the Cary 3500 UV-Vis series shares a common UV-Vis engine that uses the same optical design, so the performance of the instruments is consistent across all models in the series.

The Cary 3500 UV-Vis series of instruments is manufactured according to a quality management system certified to ISO 9001. It also meets or exceeds all other global pharmacopoeia performance requirements.

Agilent offers a comprehensive software package to assist users of the Cary 3500 UV-Vis series to comply with 21 CFR Part 11 and EU Annex 11 regulatory guidelines. The software facilitates 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 paper.

## References

1. Japanese Pharmacopoeia Eighteenth Edition, *Supplement I*, Section 2.24, Official from June 7, 2021, accessed July 2023, <https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0029.html>

## Further information

- Cary 3500 Flexible UV-Vis Spectrophotometer
- Cary 3500 Multicell UV-Vis Spectrophotometer
- Cary 3500 Compact UV-Vis Spectrophotometer
- Cary UV Workstation software
- Data Integrity Options for GMP Facilities for the Agilent Cary 3500 UV-Vis Spectrophotometer Series
- UV-Vis Spectroscopy & Spectrophotometer FAQs

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