

Molecular Spectroscopy

Molecular Spectroscopy USP Capabilities: Infrared, UV/Visible and Fluorescence Spectrometers

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Introduction

The instrumentation and software used in the analysis of materials within the pharmaceutical industry must adhere to comprehensive policies set out by different organizations. The requirements for the physical tests used in instrument validation are set out by the United States Pharmacopoeia (USP) with various sections pertaining to different spectroscopic techniques.

Qualification of Spectrometers (USP 1058)

USP <1058> sets out guidelines for Analytical Instrument Qualification (AIQ). There are four phases of AIQ:

The first phase of AIQ is design qualification (DQ), which defines the functional and operational specifications and intended purpose of the instrument. DQ may be performed by highly trained engineers at PerkinElmer or by the user. The next two phases, installation

qualification and operational qualification (IQ/OQ), are commonly grouped together. The testing required for IQ and OQ may be carried out by PerkinElmer OneSource service engineers as part of the UOQ program. The final phase, Performance Qualification (PQ), may be easily implemented by the user using PerkinElmer software such as Spectrum™ 10, UV WinLab™ and Spectrum FL for Infrared, UV/Vis and Fluorescence spectroscopy respectively.

Laboratory Universal Operation Qualification (UOQ)

The PerkinElmer OneSource® UOQ program offers a laboratory ongoing compliance with continuously evolving regulatory conditions. The UOQ program includes:

- A protocol which is customizable by the user, including options to test according to pharmacopoeial requirements.
- Instrument testing by a service engineer according to user defined specifications. Multiple instruments may be tested simultaneously or overnight to reduce downtime.
- The UOQ report provides an ultra-secure document including pass/fail results and built-in calculations.
- Digital Archiving of data making it easy to provide information required for audits.

The OneSource UOQ program is currently available for IR, UV-Vis, GC and LC instruments, regardless of manufacturer.

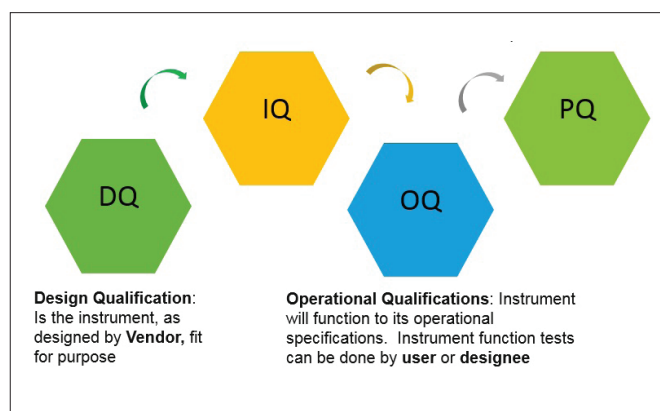


Figure 1. DQ/IQ/OQ/PQ life cycle.

Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared Spectroscopy is commonly used in the pharmaceutical industry as it is a primary analytical technique for the identification of pharmaceutical materials. A key part of pharmacopoeial compliance is the ability to perform an instrument validation in order to ensure that results obtained are reliable. There are two USP entries related to Infrared Spectroscopy as a technique, USP 854 (Mid-IR) and USP 1119 (Near-IR). These outline the procedure with which instrument performance should be validated.

Mid-Infrared Spectroscopy (USP 854)

USP defines the Mid-Infrared Region as between 4000 and 400 cm^{-1} (2.5 – 25 μm) but specifies all validation procedures take place between 3800-650 cm^{-1} . The first specified test is determination of the wavenumber accuracy which uses a 35 μm thick matte polystyrene film. The PerkinElmer Spectrum Two includes an inbuilt filter wheel including a polystyrene film of the specified thickness. This allows wavenumber accuracy tests to be easily performed without the need for an external standard. An example spectrum of polystyrene over the validation range (3800 – 650 cm^{-1}) is shown in Figure 2. The standard peaks used in instrument verification are: 3060.0, 2849.5, 1942.9, 1601.2, 1583.0, 1154.5 and 1028.3 cm^{-1} . A pass/fail limit of $\pm 1 \text{ cm}^{-1}$ is set for this test.

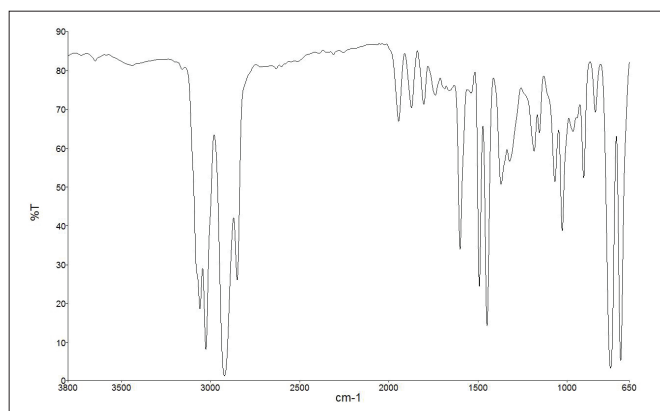


Figure 2. An example spectrum of 35 μm thickness polystyrene measured using the PerkinElmer Spectrum Two FT-IR spectrometer.

This test, along with others required in USP 854 and other common pharmacopoeias may be easily setup using the PerkinElmer Spectrum 10 software (Figure 3).

The software also allows the user to select different pharmacopoeias to test an instrument against. This will, in some cases, alter the parameters required for the instrument to pass a specific test. When using Generic (USP) MIR Tests, the following checks will be conducted to verify the instrument performance:

- Wavenumber Verification
- Resolution Performance
- Repeatability

Wavenumber Accuracy

Wavenumber accuracy of the instrument is checked using the internal matte polystyrene sample (35 μm) and measuring the wavenumber at which specific peaks occur. The reference and threshold values for this test are shown in Table 1.

If other pharmacopoeias are selected, the limits may be changed. For example, in the Chinese pharmacopoeia (PPRC), the limits are set to $\pm 1.5 \text{ cm}^{-1}$. Spectrum 10 allows any of these standard peaks to be included, as well as extra peaks which may be input by the user. It is also possible to select the spectral resolution used for the measurement of these peaks.

Table 1. Reference wavenumber values for peaks given by polystyrene standard.

Peak Number	Limits		
	Nominal (cm^{-1})	Lower (cm^{-1})	Upper (cm^{-1})
1	3060.00	3059.00	3061.00
2	2849.50	2848.50	2850.50
3	1942.90	1941.90	1943.90
4	1601.20	1600.20	1602.20
5	1583.00	1582.00	1584.00
6	1154.50	1153.50	1155.50
7	1028.30	1027.30	1029.30

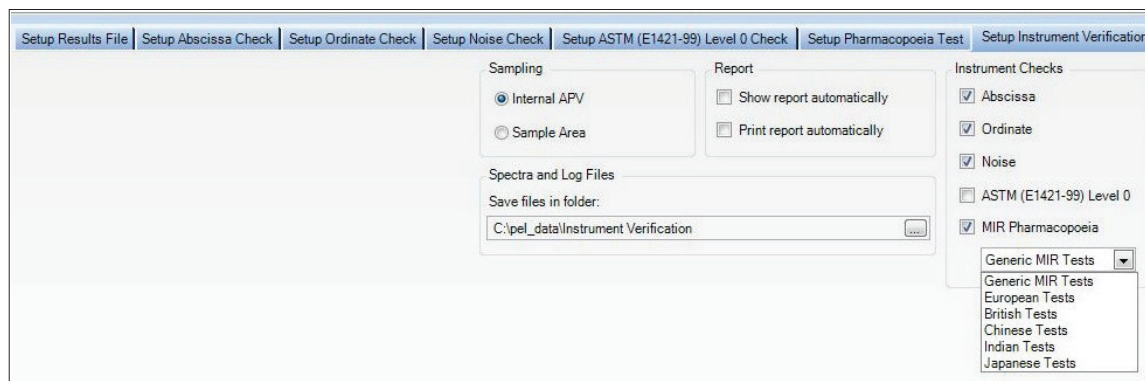


Figure 3. Setup Instrument Verification tab in Spectrum 10.

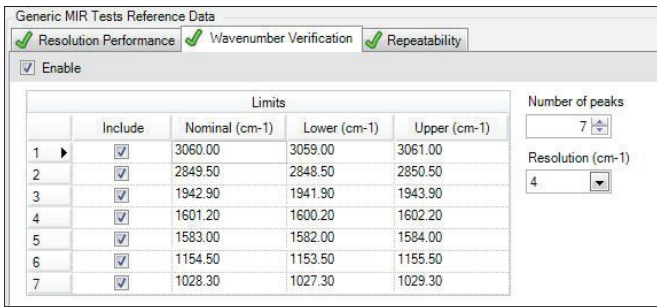


Figure 4. Wavenumber Verification setup tab in Spectrum 10.

Resolution Performance

The resolution performance test checks the spectral resolution of the instrument by measuring the difference in absorbance between a peak and a trough at specific wavenumbers in the spectrum of the polystyrene standard. The standard requirements used for USP 854 tests are that the difference between the trough at 2870 cm⁻¹ and peak at 2849 cm⁻¹ must be greater than 0.33 A; and the difference between the trough at 1589 cm⁻¹ and the peak at 1583 cm⁻¹ must be greater than 0.08 A.

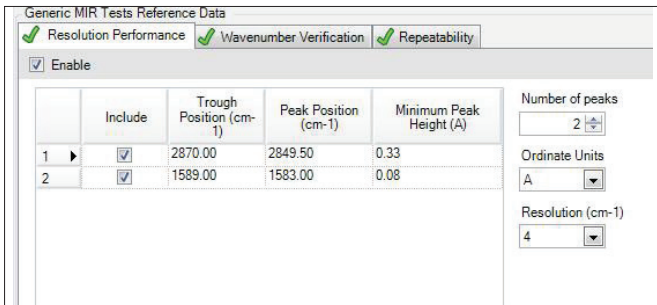


Figure 5. Resolution Performance test setup tab in Spectrum 10.

Repeatability

The USP 854 repeatability checks involve measuring the internal polystyrene sample multiple times and looking at the variation in the wavenumber and %T. Spectrum 10 allows the user to specify the number of repeats, which is set to two by default. The allowed variability for both the abscissa and ordinate values may also be specified by the user.

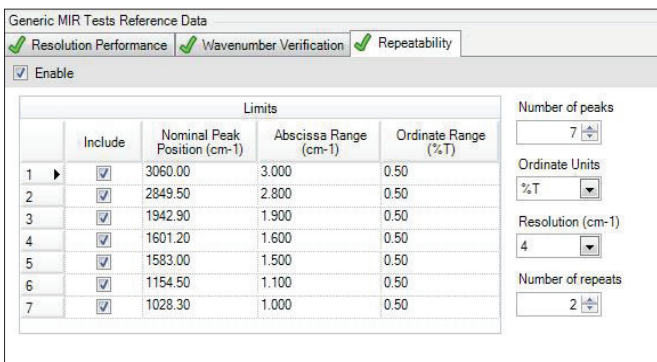


Figure 6. Repeatability test setup tab in Spectrum 10.

Near-Infrared Spectroscopy (FT-NIR)

The United States Pharmacopeia defines the near-infrared region as between 750 and 2500 nm (13,333 – 4,000 cm⁻¹). The standards used for performance qualification of a NIR instrument depend on whether the instrument is being used in reflectance or transmission. In both cases, the standard will usually contain some combination of rare earth oxides. The standard reference material (SRM) used for reflectance measurements is NIST SRM 2036 which is a combination of glass and sintered PTFE (acting as a diffuse reflector) containing a mixture of rare earth oxides. For transmission measurements, the standard is NIST SRM 2035b which contains the same rare earth oxides, in the same proportions, in a glass filter. Both standards 2036 and 2035b have an optical filter which is 25 mm in diameter and 1.5 mm thick. The pharmacopeia tests for FT-NIR are:

- Wavelength Accuracy
- Linearity
- Noise
- Repeatability

Wavenumber Accuracy

The standard peak positions in Spectrum 10 for the wavelength/wavenumber test are referred to in Table 2. This uses a certified rare earth reflectance multi-wavelength calibration standard.

Table 2. Reference wavenumber values for NIR wavenumber accuracy test.

Peak Number	Limits		
	Nominal (cm ⁻¹)	Lower (cm ⁻¹)	Upper (cm ⁻¹)
1	7925.00	7917.00	7933.00
2	5947.00	5943.00	5951.00
3	5167.00	5163.00	5171.00

NIR wavelength accuracy tests have variable tolerances for the peak positions with the thresholds being +/- 8, 4 and 4 cm⁻¹ for peaks 1, 2 and 3 respectively. Spectrum 10 allows the user to include any of these peaks, as well as add others if required.

There is also the option to change the way in which the software calculates the position of the peak. The 'Interpolated Peak' option defines the peak position as the point at which the intensity is the highest whereas the 'Center of Gravity' option finds the center of the peak shape and will therefore give a slightly different result for an asymmetric peak.

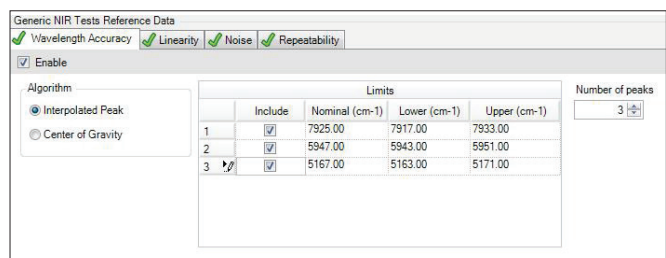


Figure 7. Wavelength Accuracy setup tab in Spectrum 10.

Linearity

The NIR linearity test essentially involves producing a calibration curve using at least 4 reference materials with known concentration of the analyte. Typically the concentration of the analyte will range between 10 % and 90 % and calibrations are constructed based on peaks at wavelengths/wavenumbers specified by the user. The calibration is assessed on how close the slope of the curve is to +/- 1 and how close the intercept is to +/- 0, with the default threshold in both cases being +/- 0.05. The Spectrum 10 Pharmacopoeia tests allow for all the parameters to be defined by the user.

Noise Test

The NIR noise test involves using high reflectance and low reflectance (high and low flux) materials, usually 99 % and 10 % reflectance standards, to measure RMS and Max noise levels in segments of specified size (usually 300 cm⁻¹). The standard range over which this test is conducted is 8350 – 4450 cm⁻¹. Spectrum 10 software allows the user to set the limits, define segment size and range over which the noise is tested.

Repeatability

The repeatability test for USP 1119, as with USP 854 for FT-MIR, involves measuring several user-defined peaks multiple times and assessing the variation between replicates. The key difference with USP 1119 is that the difference is assessed based on the standard deviation of the abscissa (cm⁻¹) and ordinate (%T) values as opposed to USP 854 which uses absolute values as the threshold. Spectrum 10 allows the user to define peak positions and standard deviation thresholds for this test.

Summary of Consumables Available for IR USP Compliance

For FT-IR and FT-NIR spectroscopy, Instrument Performance Verification (IPV) kits are available, containing all reference materials required for pharmacopoeial tests. The kits include a traceable polystyrene reference material for abscissa calibration valid for three years. IPV upgrade kits are available for replacement of the polystyrene TRM once the validity period has expired. Table 3 shows the PerkinElmer consumables available for instrument verification.

Table 3. Consumables available for IR instrument verification.

Part Number	Description	Pharmacopoeia Test(s)
L1250404	Mid Infrared IPV kit for Spectrum 10 software	MIR Wavelength Accuracy, Resolution, Repeatability
L1250474	Mid Infrared IPV upgrade kit for Spectrum 10 software	MIR Wavelength Accuracy, Resolution
L1202057	Polystyrene Test Film – 0.038 mm thickness (not traceable)	MIR Wavelength Accuracy, Resolution
L1281928	NIR Reflectance Abscissa Standard, 60 mm, 8 mm x 58 mm, Calibrated	NIR Reflectance Wavelength Accuracy
L1281929	NIR Reflectance Abscissa Standard, 100 mm, 8 mm x 98 mm, Calibrated	NIR Reflectance Wavelength Accuracy
L1281926	Reflectance Standards, 60 mm, 8 mm x 58 mm, Calibrated, Set of 4	NIR Linearity
L1281927	Reflectance Standards, 100 mm, 8 mm x 98 mm, Calibrated, Set of 4	NIR Linearity
PELA9012	Spectralon™ Calibrated Diffuse Reflectance Standards, 1.25 in, Set of 8	NIR Linearity, Noise
L1281930	Reflectance Standard Recalibration Service	-

Figure 8. NIR Linearity test setup tab in Spectrum 10.

Figure 9. NIR Noise test setup tab in Spectrum 10.

Figure 10. Repeatability test setup tab in Spectrum 10.

Ultraviolet-visible (UV-Vis) Spectroscopy (USP 857)

The United States Pharmacopeia defines a UV-Vis spectrum as that produced when incident radiation (with wavelength anywhere in the range 175 – 3300 nm) interacts with the electron cloud in a chromophore. This results in an electronic transition involving the promotion of one or more of the outer shell or bonding electrons from a ground state into a state of higher energy.

The PerkinElmer LAMBDA™ series UV-Vis spectrophotometers may be validated to USP and other pharmacopoeial specifications. There are several parameters which must be monitored in order to maintain compliance with USP chapter 857:

- Wavelength Accuracy
- Absorbance Accuracy
- Stray Light
- Resolution

Wavelength Accuracy

There are multiple methods by which the wavelength accuracy of a UV-Vis spectrophotometer may be assessed. The first, most simple method is to measure the position of the spectral lines produced by either a Deuterium (D₂) or Mercury (Hg) lamp. The Mercury emission source provides a larger number of spectral lines (Table 4) however it is less practical as it needs to be positioned in the instrument lamp compartment. An example of a deuterium peak at 656.1 nm is shown in Figure 11. USP 857 does not specify confidence limits for these measurements as the wavelength accuracy of spectrometry in solution rarely exceeds 0.5 nm. All deuterium lamp spectral lines shown in Figure 11 fall between 656.12 and 656.14 nm showing a good level of precision and accuracy.

The second way in which the wavelength accuracy of a UV-Visible spectrophotometer may be validated is using a rare earth oxide solution prepared in acid media. The standard solution for this test in the range 240 – 650 nm is 4% holmium oxide in perchloric acid. In order to test wavelength accuracy in the range 730 – 870 nm, a Didymium (mixture of neodymium and praseodymium) may be used. The wavelength position of the spectral lines in these solutions is shown in Table 5 and a UV-Vis spectrum of Holmium Oxide is shown in Figure 12.

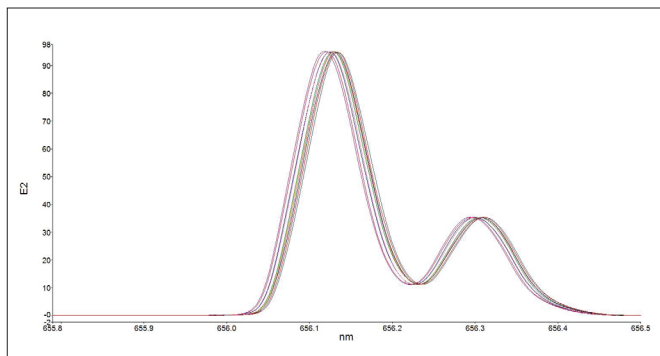


Figure 11. Replicate (n=10) measurements of the deuterium lamp peak at 656.1 nm.

Table 4. Spectral line positions of mercury and deuterium lamps.

Lamp	Spectral Lines (nm)
Mercury (Hg)	253.7
	296.7
	365.0
	404.7
	435.8
	546.1
	577.0
Deuterium (D ₂)	486.0
	656.1

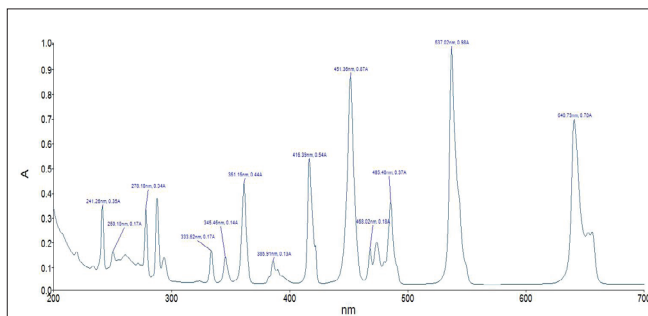


Figure 12. UV-Vis spectrum of holmium oxide in perchloric acid.

Table 5. Spectral peak positions of holmium oxide and didymium.

Lamp	Spectral Lines (nm)
Mercury (Hg)	241.1
	249.9
	278.1
	333.5
	345.4
	361.3
	385.6
	416.3
	451.4
	467.8
	485.2
	536.6
	640.5
	Deuterium (D ₂)
740.0	
794.1	
799.0	
864.4	
	656.1

The third way in which the wavelength accuracy of a UV-Visible spectrophotometer may be evaluated is using a glass filter containing a rare earth oxide. The most common example of this is a holmium glass filter with a spectral bandwidth of 1 nm.

Photometric (Absorbance) Accuracy

The photometric accuracy of a UV-Vis spectrophotometer may be measured using either a solution or glass/quartz filter. The solution used for this test is acidified potassium dichromate with a concentration in the range 0 – 200 mg/L. Over this range, the solution should have peaks of up to three absorbance units at 235, 247, 313 or 350 nm. Photometric accuracy is evaluated by comparing the absorbance value with the certified reference value. This may be obtained by either purchasing a certified reference material or preparing the solution according to NIST SRM 935a. The allowed threshold for these measurements is $\pm 1\%$ A for values above 1.0 A or ± 0.01 A for values below 1.0 A, whichever is larger. Furthermore, the photometric precision may also be determined by using the standard deviation of at least six replicate measurements over at least two certified peaks. The standard deviation of these measurements may not exceed $\pm 0.5\%$ A for values above 1.0 A or ± 0.005 A for values below 1.0 A, whichever is larger.

The other method by which photometric accuracy may be tested is using glass or quartz filters. These filters may be obtained as CRMs which provide reference values up to 3.0 A, at 440.0, 465.0, 546.1, 590.0 and 635.0 nm. The measured absorbance must be $\pm 0.8\%$ A for values over 1.0 A and ± 0.008 A for values under 1.0 A. The photometric precision may be measured using a glass or quartz filter with the same parameters as those used for solutions.

Stray Light

Stray light is defined as the amount of unwanted radiation reaching the detector. This issue is larger in single monochromator instruments as the monochromator is between source and sample. Stray light may be reduced by using either a premonochromator or double monochromator instrument, or by diluting samples to reduce the maximum absorbance value.

Stray light may be tested using solutions which may be prepared or purchased as CRMs. Each of these solutions will provide stray light at the wavelengths shown in Table 6.

The stray light value may be calculated by first measuring a 5 mm cell (as a reference) and then measuring a 10 mm cell filled with the same certified solution over the required spectral range. The pass threshold for this test is a stray light value of less than 0.01 at an absorbance of greater than 0.7 A. Examples of two spectra of stray light solutions, acetone and sodium iodide, are shown in Figures 13a and 13b respectively.

Table 6. Spectral regions used for each stray light solution.

Spectral Range (nm)	Solution
190 – 205	Potassium Chloride (12 g/L)
210 – 259	Sodium Iodide or Potassium Iodide (10 g/L)
250 – 320	Acetone (pure liquid)
300 – 385	Sodium Nitrite (50 g/L)

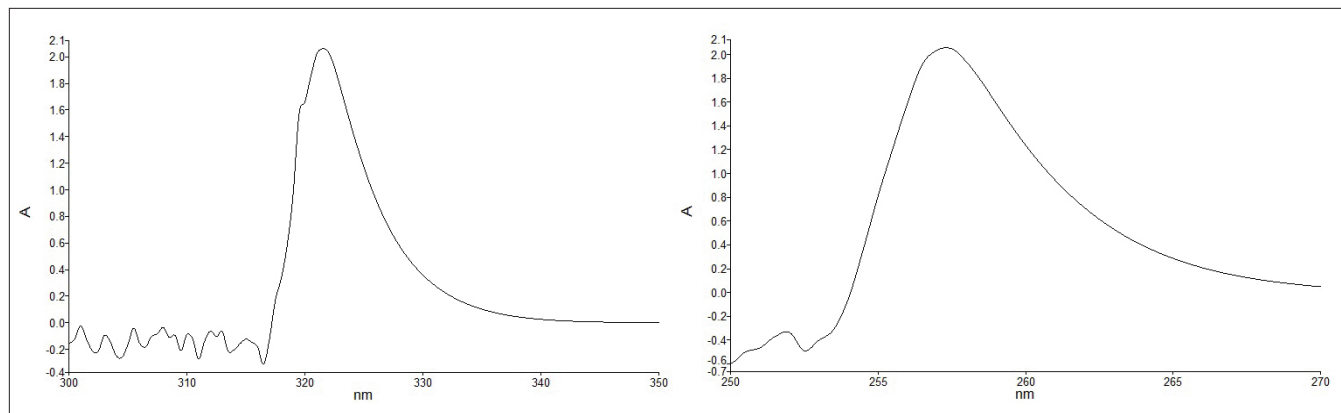


Figure 13. UV-Vis spectra of a) Acetone stray light solution and b) Sodium Iodide stray light solution.

Resolution

The resolution of a UV-Visible Spectrophotometer may be determined by measuring a 0.02% toluene in hexane solution. The ratio of the absorbance maximum at 269 nm and minimum at 266 nm must be above 1.3. A UV-Vis spectrum of toluene in hexane is shown in Figure 14.

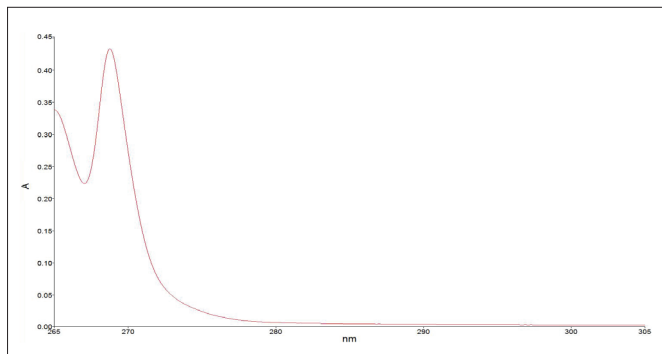


Figure 14. UV-Vis spectrum of toluene in hexane used to test spectral resolution.

Summary of Consumables Available for UV-Vis USP Compliance

For UV-Visible Spectrophotometry, there are a wide variety of reference samples and kits required to perform USP tests.

Table 7. Consumables available for UV-Vis instrument verification.

Part Number	Description	Pharmacopoeial Test
L6022728	Deuterium Lamp for LAMBDA 2X/3X/4X/6XX/7XX/8XX/9XX/10XX	Wavelength Accuracy
B2500100	Pharmacopoeia Solutions Reference Material Set	Wavelength Accuracy Photometric Accuracy Stray Light Resolution
N1010539	Recertification Program for UV/Vis Calibration Reference Materials	-

The PerkinElmer pharmacopoeia solutions reference material set contains the following solutions:

- Holmium oxide in perchloric acid – Wavelength accuracy
- Potassium dichromate in perchloric acid – Photometric accuracy
- Potassium chloride – Stray light
- Toluene in hexane – Resolution

Fluorescence Spectroscopy (USP 853)

Fluorescence is defined as a two-step process which involves absorption of light at a specific wavelength (excitation) followed by emission of light at a higher wavelength. The key parameters which may be measured in order to validate the performance of a Fluorescence spectrometer are wavelength accuracy and the signal-to-noise.

Wavelength Accuracy

The first method by which wavelength accuracy may be validated is by using the spectral lines produced by source lamps, shown in Table 8.

Table 8. Spectral lines produced by mercury and xenon lamps.

Lamp	Spectral Lines (nm)
Mercury (Hg)	253.7
	296.7
	365.0
	404.7
	435.8
	546.1
	577.0
	579.1
Xenon (Xe)	260.5
	541.9

The PerkinElmer FL6500 and FL8500 Fluorimeters use a pulse xenon and continuous xenon source, respectively. This allows the xenon spectral lines to be used as an internal validation for the instrument.

The second method for validating the wavelength accuracy of a fluorescence instrument is using a rare earth oxide solution with a diffuse reflector in the sample position. This test is carried out using the same solutions and wavelengths as the wavelength accuracy test for UV-Visible spectrophotometry, with the addition of a diffuse reflector in the sample position.

The final method by which wavelength accuracy may be validated in fluorescence spectroscopy is by using polymethylacrylate-doped reference standards. These standards have well defined excitation and emission wavelengths shown in Table 9. A fluorescence spectrum of Ovalene is shown in Figure 15.

Table 9. Excitation and emission wavelengths for commonly used fluorescence standards.

Dopant	Excitation Wavelength (nm)	Emission Wavelength (nm)
p-Terphenyl	295	338
Ovalene	342	482
Tetraphenylbutadiene	348	422
Anthracene	360	402

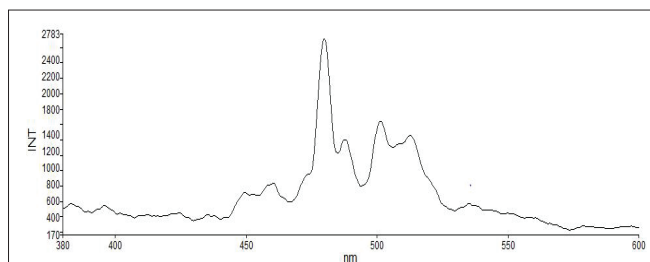


Figure 15. Fluorescence spectrum of Ovalene.

Signal-to-noise

The signal-to-noise of a fluorimeter is measured using low-conductivity (18-M Ω) water. This is because water produces a Raman band which is consistently red-shifted 3382 cm⁻¹ relative to the excitation wavelength. This Raman band is usually measured using an excitation wavelength of 350 nm which gives a low-level Raman peak at 397 nm. This Raman band is inherently reproducible and provides a good peak with which the signal-to-noise may be found by finding the ratio between the peak and the baseline of the spectrum. An example spectrum showing the Raman peak from a sealed water cell is shown in Figure 16.

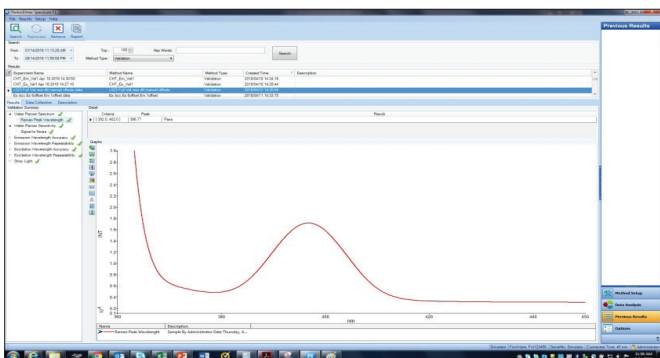


Figure 16. Raman peak of low conductivity water shown in PerkinElmer Spectrum FL software.

Summary of Consumables Available for Fluorescence USP Compliance

Table 10. Consumables available for Fluorescence instrument verification.

Part Number	Description	Pharmacopoeial Test
L2251157	Xenon Source Lamp for LS30/40/45/50/50B/55	Wavelength Accuracy
52019600	Luminescence Sample Blocks (Set of 6)	Wavelength Accuracy
L2251293	Sealed Water Sample	Signal-to-noise

The luminescence sample blocks set contains the following blocks listed in Table 11.

Table 11. Wavelength range for each block material.

Block No.	Standard	Wavelength Range (nm)
1	Anthracene/Naphthalene	300 – 400
2	Ovalene	420 – 600
3	<i>p</i> -Terphenyl	300 – 400
4	Tetraphenylbutadiene	400 – 600
5	Europium	600 – 640
6	Rhodamine B	540 – 640

Summary

PerkinElmer offer a wide variety of resources, from the OneSource UOQ program to consumables and software, which allow the user to easily ensure compliance with various Pharmacopeia. Options are available for verification of Infrared, UV-Visible and Fluorescence spectrometers.