⟨857⟩ ULTRAVIOLET-VISIBLE SPECTROSCOPY

INTRODUCTION

For the purposes of this chapter, an ultraviolet-visible (UV-Vis) spectrometer is defined as an optical system capable of producing monochromatic radiation in the range of 200–780 nm and as a device capable of detecting the optical transmittance, usually expressed in absorbance (*A*), whose primary function is to measure the stated absorbance/transmittance at defined wavelength(s). [NOTE—A UV-Vis spectrometer may also be referred to as a spectrophotometer or absorption spectrometer.]

This functionality may be extended to incorporate multiple channel measurements, either by instrumental design or with time, e.g., in dissolution or kinetic studies.

Chromatographic detectors are specifically excluded from this chapter.

For essential supporting information on the best practices and principles of measurements, see *Ultraviolet-Visible Spectroscopy—Theory and Practice* ⟨1857⟩.

The suitability of a specific instrument for a given procedure is ensured by a stepwise life cycle evaluation for the desired application from selection to instrument retirement: design qualification (DQ); installation qualification (IQ); an initial performance-to-specification qualification, also known as operational qualification (OQ); and an ongoing performance qualification (PQ). For more details, see *Analytical Instrument Qualification* ⟨1058⟩. *Acceptance criteria* for "fitness for purpose" are based on the uncertainty of the reference material and the performance specification of the instrument.

Principles of Measurement

UV-Vis spectra are derived when the interaction between incident radiation and the electron cloud in a chromophore results in an electronic transition involving the promotion of one or more of the outer shell or the bonding electrons from a ground state into a state of higher energy. The UV and visible spectral bands of substances generally are broad and do not possess a high degree of specificity for compound identification. Nevertheless, they are suitable for quantitative assays and, for many substances, are useful as an additional means of identification.

In the Beer–Lambert law the absorbance (A_λ) of a solution at given wavelength, λ, is defined as the logarithm to base 10 of the reciprocal of the transmittance (T_λ):

$$A_\lambda = \log_{10}\left(\frac{1}{T_\lambda}\right) \text{ and } T_\lambda = \frac{I_\lambda}{I_{\lambda 0}}$$

T_λ = transmittance
I_λ = intensity of the transmitted radiation at the same wavelength λ
$I_{\lambda 0}$ = intensity of the incident radiation at wavelength λ

In the absence of any other physical or chemical factors, A_λ is proportional to path length, *b*, through which the radiation passes, and to the concentration, *c*, of the substance in the solution in accordance with the following:

$$A_\lambda = \varepsilon_\lambda c b$$

ε_λ = molar absorptivity
c = solute concentration (mol/L)
b = path length (cm)

If the concentration, *c*, is expressed in g/L, the constant ε_λ becomes a_λ, which is called the absorptivity.

The expression $A^{1\,\%}_{1\text{ cm}}$, which represents the specific absorbance of a dissolved substance, refers to the absorbance of a 10-g/L solution (1%, m/v) in a 1-cm cell measured at a defined wavelength so that:

$$A^{1\,\%}_{1\text{ cm}} = 10a_\lambda = 10\varepsilon_\lambda / M$$

a_λ = absorptivity
ε_λ = molar absorptivity
M = molar concentration of the solution

When solutions are observed in 1-cm cells, concentrations of about 10 µg/mL often will produce absorbances of 0.2–0.8 in the UV or visible region.

Change to read:

QUALIFICATION OF UV-VIS SPECTROMETERS

The purpose of this section of the chapter is to provide test methodologies and acceptance criteria to ensure that the instrument is suitable for its intended use (OQ), and that it will continue to function properly over extended time periods as

part of PQ. As with any spectrometric device, a UV-Vis spectrometer must be qualified for both wavelength (*x*-axis) and photometric (*y*-axis, or signal axis) accuracy and precision, and the fundamental parameters of stray light and resolution must be established. OQ is carried out across the operational ranges required within the laboratory for both the absorbance and wavelength scales.

Installation Qualification

Documented proof of the IQ requirements provide evidence that the hardware and software are properly installed in the desired location.

Operational Qualification

Acceptance criteria for critical instrument parameters that establish "fitness for purpose" are verified during IQ and OQ. Specifications for particular instruments and applications can vary, depending on the analytical procedure used and the desired accuracy of the final result. OQ should establish suitable control over the operational range of wavelength, absorbance, and the evaluation of stray light and resolution, i.e., spectral bandwidth (SBW). Instrument vendors often have reference materials and protocols available as part of the IQ/OQ package.

Wherever possible in the procedures detailed as follows, certified reference materials (CRMs) are to be used in preference to laboratory-prepared solutions. These CRMs should be obtained from a recognized accredited source and include independently verified, traceable value assignments with associated calculated uncertainty. CRMs must be kept clean and free from dust. Recertification should be performed periodically to maintain the validity of the certification.

Control of Wavelengths

Ensure that the accuracy of the wavelength axis (*x*-axis) over the intended operational range is correct within acceptable limits. The control of wavelength OQ must include at least one method in each operational range where it is intended for use. The options and acceptance criteria are summarized in *Table 1*.

Table 1. Wavelength Ranges and Procedures for Control of Wavelengths

Method	UV (200–400 nm)	Vis (400–780 nm)	Vis/NIR (400–900 nm)
Mercury (Hg) emission lines	Accuracy ±1 nm Precision ≤0.5 nm	Accuracy ±2 nm Precision ≤0.5 nm	—
Deuterium (D_2) emission lines	—	Accuracy ±2 nm Precision ≤0.5 nm	—
Cerium oxide solutions	Accuracy ±1 nm Precision ≤0.5 nm	—	—
Holmium oxide solutions or glasses	Accuracy ±1 nm Precision ≤0.5 nm	Accuracy ±2 nm Precision ≤0.5 nm	—
Didymium solutions or glasses	—	—	Accuracy ±2 nm Precision ≤0.5 nm

[NOTE—Certified reference standards, traceable to the National Institute of Standards and Technology (NIST) (www.nist.gov) or to other recognized standards-setting organizations, are commercially available, and should be used where possible.]

For non-diode array instruments, wavelength accuracy and precision are determined over the operational range using at least six replicate measurements. For wavelength accuracy, the difference of the mean measured value to the certified value of the CRM must be within ±1 nm in the UV region (200–400 nm), and in the visible region (400–780 nm) and visible/NIR (near-infrared) region (400–900 nm) must be within ±2 nm.

For wavelength precision, the standard deviation of the mean value of the six wavelength measurements must not exceed 0.5 nm. For diode array instruments, only one wavelength accuracy measurement is required, and no precision determination needs to be performed, due to the non-mechanical design of the monochromator.

Establishment of Acceptance Limits (Wavelength)—Choice of Standards

For all *Control of Wavelengths* accuracy procedures, acceptance limits for adequate calibration are established by adding the expanded uncertainty of the CRM to the instrument vendor specification at the wavelength(s) required in a linear manner, and these values must lie within the values specified in *Table 1*. In the case where atomic line spectra are used, this expanded uncertainty of the CRM is deemed to be zero for this process, i.e., the limit simply becomes the instrument vendor specification.[1]

[1] NIST Special Publication 829—Use of NIST Standard Reference Materials for Decisions on Performance of Analytical Chemical Methods and Laboratories. www.nist.gov/sites/default/files/documents/mml/csd/inorganic/NIST_SpecialPub829.pdf.

ATOMIC LINE SPECTRA

This procedure is described as the primary application because the emission lines produced from a discharge lamp are characteristic of the source element and, as a fundamental physical standard, these wavelengths have been measured with an uncertainty of NMT ±0.01 nm. In solution spectrometry, the wavelength accuracy required rarely exceeds 1.0 nm. For these reasons, the atomic line standard values are cited without uncertainty.

The arc of the atomic emission source, or its image, needs to be located on the same optical path as the image of the primary light source of the spectrometer; thus, it can be used only in spectrometers that can be operated in a single-beam intensity mode and practically should be implemented only on a system designed to accommodate these sources, e.g., as an accessory.

A commonly employed low-pressure mercury lamp has a number of intense lines that cover a large part of the UV and visible spectra. Two deuterium lines from the source at 486.0 and 656.1 nm often are used by manufacturers as an internal calibration check and can be used for diagnostic purposes, as can the Xe line at 260.6 nm, if appropriate (see *Table 2*).[2]

Table 2. Recommended Atomic Lines from Low-Pressure Mercury (Hg) and Deuterium (D_2) Lamps for Wavelength Calibration Purposes

Element	nm
Hg	253.7
Xe	260.6
Hg	296.7
Hg	313.2
Hg	365.0
Hg	404.7
Hg	435.8
D_2	486.0
Hg	546.1
Hg	577.0
Hg	579.1
D_2	656.1

[NOTE—Select the nearest wavelength(s) spanning the operational range required.]

RARE EARTH OXIDE SOLUTIONS

This procedure uses solutions of rare earth oxides prepared by dissolution in acid media. The most frequently used is holmium oxide in perchloric acid.[3] Holmium oxide solution has been internationally accepted as an intrinsic wavelength standard, and suitable CRMs are available commercially.[4]

The observed peak maxima are determined using the normal scan mode on the spectrometer. The peak maxima for a 4% (m/v) solution of holmium oxide in perchloric acid at 1.0-nm SBW and a path length of 1 cm are shown in *Table 3*.

Table 3. Recommended Peak Maxima from a 4% Solution of Holmium Oxide in Perchloric Acid for Wavelength Calibration Purposes

nm
241.1
249.9
278.1
287.2
333.5
345.4
361.3
385.6
416.3
451.4

[2] The rounded values are taken from ASTM Standard E275.

[3] Travis JC, Acosta JC, Andor G, Bastie J, Blattner P, Chunnilall CJ, et al. Intrinsic wavelength standard absorption bands in holmium oxide solution for UV/visible molecular absorption spectrophotometry. *J Phys Chem Ref Data.* 2005;34(1):41–57.

[4] CRMs produced under appropriate control, which may be demonstrated by accreditation to ISO/EC 17025 and/or ISO Guide 34 (ISO 17034).

Table 3. Recommended Peak Maxima from a 4% Solution of Holmium Oxide in Perchloric Acid for Wavelength Calibration Purposes *(continued)*

nm
467.8
485.2
536.6
640.5

[NOTE—Select the nearest wavelength(s) spanning the operational range required.]

If the operational range of the spectrometer lies outside the range 240–650 nm, other certified rare earth oxides or other solutions can be used if they are traceable to a national or international standard.

Cerium is available as a traceable solution CRM in acidic media, e.g., in sulfuric acid. It has useful peak characteristics in the 200–300 nm region at approximately 201.1, 211.4, 222.6, 240.4, and 253.7 nm.

Didymium (a mixture of neodymium and praseodymium) is available as a traceable CRM both in solution and as a glass. Didymium is similar in preparation to the holmium materials and has useful peak characteristics in the 730–870-nm region, which may vary from melt to melt of the glass. Useful peaks are found in the didymium solution at approximately 731.6, 740.0, 794.1, 800.0, and 864.4 nm.

RARE EARTH GLASSES

This procedure uses glasses manufactured by fusing the appropriate rare earth oxide in a base glass matrix. The most frequently used is holmium, for which the reference wavelengths have been well defined. Although manufacturing can cause batch variation in these glasses, traceable CRMs are commercially available and can be used. Typical values for a holmium glass using a 1.0-nm SBW are the following: 241.5, 279.2, 287.5, 333.8, 360.9, 418.8, 445.8, 453.7, 460.2, 536.5, and 637.7 nm.

Control of Absorbance

To establish the transmittance accuracy, precision, and linearity of a given system, it is necessary to verify the absorbance accuracy of a system over its intended operational range by selection and use of the following procedures as appropriate for the wavelength and absorbance ranges required.

The control of absorbance OQ must include at least one assessment at each wavelength range in the 0–2.00 *A* region. If absorbance >2.00 *A* is to be used for quantitation, then control of absorbance must also be evaluated in the >2.00 *A* range.

Establishment of Acceptance Limits (Absorbance)—Choice of Standards

For all *Control of Absorbance* procedures, acceptance limits for adequate calibration are established by adding the expanded uncertainty of the CRM to the instrument vendor specification at the wavelength(s) and absorbance levels required in a linear manner, and these values must lie within the values specified in *Table 4*.

[NOTE—CRMs, traceable to NIST Standard Reference Materials (SRMs), or other international or national standards, are commercially available and should be used where possible.]

Control of Photometric Response (Linearity)

Verification of photometric response (linearity) is required, and it should be evaluated using a standard type appropriate for the wavelength(s) required, where at least three different absorbance levels appropriate to and spanning the required operational range are measured. The options and acceptance criteria are summarized in *Table 4*.

[NOTE—Given the above requirement, it is not necessary to calculate the correlation coefficient of the standard response to demonstrate linearity; just demonstrate that at the different absorbance levels selected the acceptance limits have been met.]

Table 4. Available CRMs for the Control of Absorbance

Absorbance Range	Standard	UV (200–400 nm)	Vis (400–780 nm)
0–1 *A*	Certified nicotinic acid solution	6–24 mg/L Evaluate at 213 and 261 nm. Accuracy ±0.010 A_λ Precision $\blacktriangle \leq_{\blacktriangle\ (ERR\ 1\text{-}Jan\text{-}2020)} 0.005\ A_\lambda$	—
0–1 *A*	Certified potassium dichromate ($K_2Cr_2O_7$) solution	20–60 mg/L Evaluate at 235, 257, 313, and 350 nm. Accuracy ±0.010 A_λ Precision $\blacktriangle \leq_{\blacktriangle\ (ERR\ 1\text{-}Jan\text{-}2020)} 0.005\ A_\lambda$	600 mg/L Evaluate at 430 nm. Accuracy ±0.010 A_λ Precision $\blacktriangle \leq_{\blacktriangle\ (ERR\ 1\text{-}Jan\text{-}2020)} 0.005\ A_\lambda$
0–1 *A*	Certified neutral density glass filters	—	Evaluate at 440, 465, 546.1, 590, and 635 nm. Accuracy ±0.008 A_λ Precision $\blacktriangle \leq_{\blacktriangle\ (ERR\ 1\text{-}Jan\text{-}2020)} 0.005\ A_\lambda$

Table 4. Available CRMs for the Control of Absorbance *(continued)*

Absorbance Range	Standard	UV (200–400 nm)	Vis (400–780 nm)
0–1 *A*	Certified metal-on-fused-silica filters	Evaluate at 250, 280, 340, 360, and 400 nm. Accuracy ±0.010 A_λ Precision ▲≤▲ (ERR 1-Jan-2020)0.005 A_λ	Evaluate at 465, 500, 546.1, 590, and 635 nm. Accuracy ±0.008 A_λ Precision ▲≤▲ (ERR 1-Jan-2020)0.005 A_λ
1–3 *A*	Certified potassium dichromate ($K_2Cr_2O_7$) solution	80–200 mg/L Evaluate at 235, 257, 313, and 350 nm. Accuracy ±1% Precision ▲≤▲ (ERR 1-Jan-2020)0.5%	—
1–3 *A*	Certified neutral density glass filters	—	Evaluate at 440, 465, 546.1, 590, and 635 nm. Accuracy ±0.8% Precision ▲≤▲ (ERR 1-Jan-2020)0.5%
UV Photometric linearity	Certified nicotinic acid solutions	All concentrations meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified potassium dichromate ($K_2Cr_2O_7$) solutions	All concentrations meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified metal-on-fused-silica filters	At least three certified filters evaluated at 250, 280, 340, 360, or 400 nm. All filters meet accuracy of absorbance acceptance criteria.	—
Photometric linearity	Certified neutral-density glass filters	—	At least three certified filters evaluated. All filters meet accuracy of absorbance acceptance criteria.

[NOTE—Select the appropriate absorbance CRMs spanning the operational range(s) required.]

ACIDIC NICOTINIC ACID SOLUTIONS IN 0.1 N HYDROCHLORIC ACID

In the 0–24 mg/L range, nicotinic acid solutions provide reference values of up to 1.0 *A* at one of the certified values of 213 or 261 nm. These solutions are available as CRMs, in the 0–60 mg/L range, providing reference values up to 2.5 *A*. Using nicotinic acid solutions, the absorbance accuracy must be ± ▲0.010▲ (ERR 1-Jan-2020) A_λ ▲(for values below 1.00 A_λ).▲ (ERR 1-Jan-2020)

The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. These sets of measurements should be performed, i.e., repeated at both the upper and lower absorbances values of the operational range. In each case, absorbance deviations from the mean must not exceed ± 0.005 A_λ (for values below 1.0 A_λ).

ACIDIC POTASSIUM DICHROMATE SOLUTIONS IN 0.001 M PERCHLORIC ACID

In the 0–200 mg/L range, potassium dichromate solutions provide reference values of up to 3.0 absorbance units at one of the certified values of 235, 257, 313, or 350 nm. These solutions are available as CRMs or can be prepared according to NIST from appropriate available SRMs, e.g., SRM 935*x*, or SRM 136*x* (where *x* can be any letter from "a" to "z"). Using potassium dichromate solutions, the absorbance accuracy must be ±1% A_λ (for values above 1.00 A_λ) or ±0.010 A_λ (for values below 1.00 A_λ).

The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. These sets of measurements should be performed, i.e., repeated at both the upper and lower absorbance values of the operational range. In each case, absorbance deviations from the mean must not exceed ±0.5% A_λ (for values above 1.0 A_λ) or ±0.005 A_λ (for values below 1.0 A_λ).

NEUTRAL-DENSITY GLASS FILTERS

These gray glass filters are manufactured from doped glass and have a nominally flat spectrum in the region of the calibration wavelengths. They provide reference values of up to 3.5 absorbance units at the certified values of 440, 465, 546.1, 590, and 635 nm. These filters are available as CRMs that are traceable to NIST SRM 930e, 1930, and 2930. Other certified standard solutions or optical filters can be used if they are traceable to a national or international standard. Using gray glass filters, the absorbance accuracy must be ±0.8% A_λ (for values above 1.00 A_λ) or ±0.008 A_λ (for values below 1.00 A_λ). The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements. The absorbance deviation from the mean must not exceed ±0.5% *A* (for values above 1.0 *A*) or ±0.005 A_λ (for values below 1.0 *A*).

Metal-on-Fused-Silica Filters

These neutral density filters are manufactured by deposition of a metallic film on a silica substrate and have a substantially flat spectrum in the region of the calibration wavelengths. They provide reference values of up to 2 *A* at the usually certified values of 250, 280, 340, 360, and 400 nm in the UV range, and reference values of 465, 500, 546.1, 590, and 635 nm in the visible range. These filters are available as CRMs that are traceable to NIST SRM 2031*x* (where *x* = "a" to "z"), or to other national or international standards. In the UV range, the absorbance accuracy must be ±1% A_λ (for values above 1.00 A_λ) or

±0.01 A_λ (for values below 1.00 A_λ). In the visible range, the absorbance accuracy must be ±0.8% A_λ (for values above 1.00 A_λ) or ±0.008 A_λ (for values below 1.00 A_λ). The absorbance precision can be determined as the standard deviation from the mean of at least six replicate measurements at the upper and lower absorbance values of the operational range. The standard deviation from the mean must not exceed ±0.5% A_λ (for values above 1.00 A_λ) or ±0.005 A_λ (for values below 1.00 A_λ) in both the UV and visible ranges.

Estimation of the Limit of Stray Light (Stray Radiant Energy)

Although the measurement of absorbance or transmittance is a ratio measurement of intensities and therefore theoretically is independent of monochromatic source intensity, practical measurements are affected by the presence of unwanted radiation called "stray radiant energy" or "stray light". In addition, the adverse effect of stray light increases with aging of optical components and lamps in a spectrometer. The effects are greater at the extremes of detector and lamp operational ranges. The limit of stray light OQ must include evaluation at one or more UV wavelengths, by selection of appropriate material(s) shown in *Table 5* to span the UV operational range required. In the visible region, i.e., above 400 nm, stray light does not need to be evaluated.

[NOTE—Published stray light specifications for a given spectrometer must be verified in the OQ.]

Analysts must choose and use the appropriate reference(s) to monitor the level of stray light as part of PQ. Stray light can be detected at a given wavelength with a suitable liquid filter by either of the two procedures (*A* or *B*) detailed below.

[NOTE—If measurements are being performed in the 250–330 nm region, on a spectrometer using individual sources for the UV and visible regions of the spectrum, then an additional PQ verification using acetone should be performed.]

These solutions are available as CRMs or can be prepared at the concentrations shown in *Table 5* by using reagent-grade materials.

Table 5. Spectral Ranges of Selected Materials for Monitoring Stray Light

Recommended Wavelength (nm)	Spectral Range (nm)	Liquid or Solution
198	190–210	Aqueous potassium chloride (12 g/L)
220	210–270	Aqueous sodium iodide or potassium iodide (10 g/L)
300	250–330	Acetone
340	300–400	Aqueous sodium nitrite (50 g/L)

Procedure A

The aim of this procedure is to 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. This spectrum will contain a peak maximum absorbance value, and analysts can calculate the stray light value from the observed maximum absorbance using the formula:

$$s_\lambda = 0.25 \times 10^{-2A\lambda}$$

s_λ = stray light value, in transmittance (T)
A_λ = observed maximum absorbance

This procedure requires the 10-mm cell measurement to be subtracted from the 5-mm cell, both filled with the same cut-off solution filter. This measurement can be achieved by either using the physical capabilities of the spectrometer, i.e., using the double-beam capability, with the 5-mm cell as the reference, or by mathematically subtracting the spectra of the 10-mm cell from the 5-mm cell, chronologically produced by sequential measurement of both the 5- and 10-mm cells, using the single-sample beam, with the spectrometer referenced to air (blank holder).

ACCEPTANCE CRITERIA: $s_\lambda \leq 0.01$ or $A_\lambda \geq 0.7\ A$

Procedure B

Analysts can measure the absorbance of the cut-off solution filters specified in *Table 5* against a 10-mm cell filled with an appropriate reference, and record the maximum absorbance value (A_{max}) or the minimum % Transmittance ($\%T_{min}$) at the recommended wavelength listed in *Table 5*.

ACCEPTANCE CRITERIA: $A_{max} \geq 2.0\ A$ or $\%T_{min} < 1\%T$

Control of Resolution

If accurate absorbance measurements must be made on benzenoid compounds or other compounds with sharp absorption bands (natural half-bandwidths of <15 nm), the SBW of the spectrometer used should not be greater than 1/8th the natural half-bandwidth of the compound's absorption; i.e., this equates to a spectrometer with a SBW of 2 nm or less.

Determine the resolution of the spectrometer in the UV region by using the following procedure. Using UV-grade *n*-hexane as the reference, measure the absorbance of a 0.020% (v/v) solution of toluene in UV-grade *n*-hexane at the maximum, about

269 nm, and minimum, about 266 nm. The ratio of the absorbance at the maximum to the absorbance at the minimum will typically fall in the 1.0–2.6 range.

For most compendial quantitative purposes, a SBW of 2 nm or less is sufficient, and the *Acceptance criteria* for the ratio is NLT 1.3.

Performance Qualification

The purpose of PQ is to determine that the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement and to ensure that it will function properly over extended periods of time.

PROCEDURE

With few exceptions, compendial spectrometric tests and assays call for comparison against a USP Reference Standard. This helps ensure measurement under identical conditions for the test specimen and the reference substance. These conditions could include wavelength setting, SBW selection, cell placement and correction, and transmittance levels. Cells that exhibit identical transmittance at a given wavelength may differ considerably in transmittance at other wavelengths. Appropriate cell corrections should be established and used where required.

Comparisons of a test specimen with a reference standard are best made at a peak of spectral absorption for the compound concerned. Assays that prescribe spectrometry give the commonly accepted wavelength for peak spectral absorption of the substance in question. Different spectrometers may show minor variation in the apparent wavelength of this peak. Good practice demands that comparisons be made at the wavelength at which peak absorption occurs. Should this differ by >±1 nm (in the range 200–400 nm) or ±2 nm (in the range 400–800 nm) from the wavelength specified in the individual monograph, recalibration of the instrument may be indicated.

The expressions "similar preparation" and "similar solution" as used in tests and assays involving spectrometry indicate that the reference comparator, generally a USP Reference Standard, should be prepared and observed in an identical manner for all practical purposes to that used for the test specimen. Usually when analysts make up the solution of the specified reference standard, they prepare a solution of about (i.e., within 10%) of the desired concentration, and they calculate the absorptivity on the basis of the exact amount weighed out. If a previously dried specimen of the reference standard has not been used, the absorptivity is calculated on the anhydrous basis. The expressions "concomitantly determine" and "concomitantly measure" as used in tests and assays involving spectrometry indicate that the absorbances of both the solution containing the test specimen and the solution containing the reference specimen, relative to the specified test blank, must be measured in immediate succession.

Sample Solution Preparation

For determinations using UV or visible spectrometry, the specimen generally is dissolved in a solvent. Unless otherwise directed in the monograph, analysts make determinations at room temperature using a path length of 1 cm. Many solvents are suitable for these ranges, including water, alcohols, lower hydrocarbons, ethers, and dilute solutions of strong acids and alkalis. Precautions should be taken to use solvents that are free from contaminants that absorb in the spectral region under examination. For the solvent, analysts typically should use water-free methanol or alcohol or alcohol denatured by the addition of methanol but without benzene or other interfering impurities. Solvents of special spectrometric quality, guaranteed to be free from contaminants, are available commercially from several sources. Some other analytical reagent-grade organic solvents may contain traces of impurities that absorb strongly in the UV region. New lots of these solvents should be checked for their transparency, and analysts should take care to use the same lot of solvent for preparation of the test solution, the standard solution, and the blank. The best practice is to use solvents that have NLT 40% transmittance (39.9% $T = 0.399$ A) at the wavelength of interest.

Assays in the visible region usually call for concomitantly comparing the absorbance produced by the assay preparation with that produced by a standard preparation containing approximately an equal quantity of a USP Reference Standard. In some situations, analysts can omit the use of a reference standard (e.g., when spectrometric assays are made with routine frequency) when a suitable standard curve is available and is prepared with the appropriate USP Reference Standard, and when the substance assayed conforms to the Beer–Lambert law within the range of about 75%–125% of the final concentration used in the assay. Under these circumstances, the absorbance found in the assay may be interpolated on the standard curve, and the assay result can be calculated. Such standard curves should be confirmed frequently and always when a new spectrometer or new lots of reagents are put into use.

VERIFICATION AND VALIDATION

Verification

Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of analytical procedures described in *USP–NF* are not required to validate these procedures if provided in a monograph. Instead, they simply must verify their suitability under actual conditions of use.

The objective of a UV-Vis procedure verification is to demonstrate the suitability of a test procedure under actual conditions of use. Performance characteristics that verify the suitability of a UV-Vis procedure are similar to those required for any analytical procedure. A discussion of the applicable general principles is found in *Verification of Compendial Procedures* ⟨1226⟩. Verification is usually performed using a reference material and a well-defined matrix. Verification of compendial UV-Vis procedures includes

at minimum the execution of the validation parameters for specificity, accuracy, precision, and quantitation limit, when appropriate, as indicated in *Validation*.

Validation

Validation is required when a UV-Vis method is intended for use as an alternative to the official procedure for testing an official article or when no official procedure exists in the current *USP–NF*.

The objective of UV-Vis method validation is to demonstrate that the measurement is suitable for its intended purpose, including quantitative determination of the main component in a drug substance or a drug product (Category I assays), quantitative determination of impurities or limit tests (Category II), and identification tests (Category IV). For dissolution procedures, see *The Dissolution Procedure: Development and Validation* ⟨1092⟩. Depending on the category of the test (see *Validation of Compendial Procedures* ⟨1225⟩, *Table 2*), the analytical method validation process for UV-Vis requires testing for linearity, range, accuracy, specificity, precision, detection limit, or quantitation limit. These analytical performance characteristics apply to externally standardized procedures and those that use standard additions.

Chapter ⟨1225⟩ provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for this technology. For each particular application, tighter criteria may be needed in order to demonstrate suitability for the intended use.

ACCURACY

For Category I, II, and III procedures, accuracy can be determined by conducting recovery studies with the appropriate matrix spiked with known concentrations of the analyte. Analysts also can compare assay results obtained using the UV-Vis procedure under validation to those from an established analytical procedure.

Validation criteria: 98.0%–102.0% mean recovery for the drug substances, 95.0%–105.0% mean recovery for the drug product assay, and 80.0%–120.0% mean recovery for the impurity analysis. These criteria are met throughout the intended range.

Precision

REPEATABILITY

The repeatability of the analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at 100% of the assay test concentration. Alternatively, it can be assessed by measuring the concentrations of three replicates of three separate sample solutions at different concentrations. The three concentrations should be close enough so that the repeatability is constant across the concentration range. If this is done, the repeatability at the three concentrations is pooled for comparison to the acceptance criteria.

Validation criteria: The relative standard deviation is NMT 1.0% for the drug substance assay, NMT 2.0% for the drug product assay, and NMT 15.0%–20.0% for the impurity analysis.

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the method must be established. Typical variables include performing the analysis on different days, using different instrumentation, and/or having the method performed by two or more analysts. At a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Validation criteria: The relative standard deviation is NMT 1.5% for the drug substance assay, NMT 3.0% for the drug product assay, and NMT 15.0%–25.0% for the impurity analysis.

SPECIFICITY

In UV-Vis measurements, specificity is ensured by the use of a reference standard wherever possible and is demonstrated by the lack of interference from other components present in the matrix.

DETECTION LIMIT

The detection limit (DL) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank solution and multiplying by 3.3. Alternatively, the standard deviation can be determined from the error of the intercept from a calibration curve or by determining that the signal-to-noise ratio is >3.3. The estimated DL must be confirmed by analyzing samples at the calculated concentration.

QUANTITATION LIMIT

The quantitation limit (QL) can be estimated by calculating the standard deviation of NLT 6 replicate measurements of a blank solution and multiplying by 10. Alternatively, the standard deviation can be determined from the error of the intercept from a calibration curve or by determining that the signal-to-noise ratio is >10.

Measurement of a test solution prepared from a representative sample matrix spiked at the required QL concentration must be performed to confirm sufficient sensitivity and adequate precision. The observed signal-to-noise ratio at the required QL

should be >10. [NOTE—A suitable procedure for measuring the signal-to-noise ratio is given in ASTM 1657-98 (2011) *Standard Practice for the Testing of Variable-Wavelength Photometric Detectors Used in Liquid Chromatography*.]
Validation criteria: For the estimated limit of quantitation to be considered valid, the measured concentration must be accurate and precise at a level ≤50% of the specification.

LINEARITY

A linear relationship between the analyte concentration and UV-Vis response must be demonstrated by preparation of NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve is then evaluated using appropriate statistical methods such as a least-squares regression. Deviation from linearity results from either instrumental or sample factors, or both, and can be reduced to acceptable levels by reducing the analyte concentration and thereby the associated absorbance values.

The (Pearson) correlation coefficient, *r*, measures the strength and direction of the association between two variables (*x* and *y*), in this instance, concentration and absorbance.

The coefficient of determination, r^2, is a measure of the fraction of the data's variation that is adequately modeled and not a measure of linearity. Linearity depends on the standard error of the calibration equation (and hence the reference procedure) and on the range of the calibration data. Thus, although values very near 1.00, such as 0.99 or greater, typically indicate a linear relationship, lower values do not distinguish between nonlinearity and variability.
Validation criteria: The coefficient of determination, r^2, must be NLT 0.995 for Category I assays and NLT 0.99 for Category II quantitative tests. Visual inspection of the residual plots should not reveal any significant pattern.

RANGE

The operational range of an analytical instrument (and the analytical procedure as a whole) is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the instrumental response function has a suitable level of precision, accuracy, and linearity.
Validation criteria: For Category I tests, the validation range for 100.0% centered acceptance criteria is 80.0%–120.0%. For non-centered acceptance criteria, the validation range is 10.0% below the lower limit to 10.0% above the upper limit. For content uniformity, the validation range is 70.0%–130.0%. For Category II tests, the validation range covers 50.0%–120.0% of the acceptance criteria.

ROBUSTNESS

This parameter is evaluated during method development.

The reliability of an analytical measurement is demonstrated by deliberate changes to experimental parameters. For UV-Vis, this can include measuring the stability of the analyte under specified storage conditions, varying pH, and adding possible interfering species, to list a few examples. Robustness is determined concurrently using a suitable design for the experimental procedure.

INDIRECT MEASUREMENT REQUIREMENTS

For certain UV-Vis procedures, chromogenic reactions are employed. Generally, the requirements for the analytical performance characteristics are used. In some instances, the required accuracy and precision criteria for the direct measurements may not be achievable. Under these circumstances, the accuracy and precision requirements can be widened by as much as 50%. Any such widening must be justified on scientific grounds and with documented evidence. It may be necessary to increase the amount of replication required to produce a scientifically sound reportable value.