Reference Material Standardization Guidelines for Quality Control and Validation of UV/VIS Absorption Spectrophotometers

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SpectroStandards™ Analytical

UV/VIS absorption spectrophotometers are intricate laboratory measurement systems for chemical analysis and testing whose accuracy and comparability of analytical data can be adversely impacted by several important instrumental parameters . Reference material standardization, the underlying concept of analytical measurement science, is the practical laboratory means to investigate potential instrument-related systematic errors and, if present, to evaluate their impact on the quality and reliability of the data. The scientific defensibility of the validation of a UV/VIS spectrophotometer, whether conducted in-house or out-sourced, can be no better than the accuracy of the reference material standards and the relevance of the test procedure or protocol used for the validation. Reference material standardization must be the scientific cornerstone to UV/VIS analytical procedures and validation/qualification protocols if quality control benefits and regulatory compliance are to be achieved in a timely and cost-effective manner.

Ultraviolet/visible (UV/VIS) spectroscopic analysis is a vital laboratory means for obtaining important chemical information about products and processes in various industrial sectors. The UV/VIS spectrophotometer is a highly versatile laboratory instrument that is commercially available in a multitude of optical designs, specifications, spectral measurement ranges and capabilities, and operational configurations. While such instrumental versatility is advantageous, it also necessitates a high level of user comprehension to ensure that the chosen spectrophotometer is appropriate for the specified analytical task and also to verify that the spectrophotometer is functioning properly.

When performing a UV/VIS method on a given spectrophotometer, the laboratory analyst and manager should be concerned about data comparability and its consequences. Data comparability in UV/VIS analysis refers to whether different spectrophotometers are capable of producing equivalent measurement results for a given analytical method. From a quality control perspective, the logical question that should always be asked is "Would I be acquiring equivalent measurement data for this method if I was using a different UV/VIS spectrophotometer?" Reference material standardization is the analytical means to answer this question and to provide the necessary documented evidence.

Reference material standardization is central to the laboratory standard operating procedure or validation/qualification protocol, which must include assessment of stray radiant energy (stray light) and meaningful verification of the wavelength and photometric scales of the spectrophotometer. [1] Scientific defensibility is paramount in ensuring that the UV/VIS procedure or protocol will withstand the intense scrutiny of knowledgeable auditors in quality assurance reviews and regulatory inspections. By adopting the following important UV/VIS standardization guidelines, which are predicated on the principles of analytical measurement science, the laboratory will achieve the highest level of scientific defensibility in the validation/qualification and daily quality control of their UV/VIS instrumentation.

1. Verify the UV/VIS spectrophotometer in the spectral wavelength range of the analytical method for which it is being used.

Because of the wide spectral measurement range of most UV/VIS spectrophotometers, the operational performance and data quality of any given spectrophotometer may be optimal in certain spectral regions, but compromised in other spectral regions. If the UV/VIS procedure or protocol does not include validation tests relevant to instrument performance for the specified method, any insidious errors which will impact the integrity of the resulting measurement data may go undetected. For example, if the analytical method calls for the spectrophotometer to measure a sample at a UV wavelength such as 280 nm, the goal of the validation must be to identify any potential systematic errors which may occur in that particular wavelength region.

If conducted in the visible spectral region only, the validation tests may indeed verify proper functioning of the spectrophotometer in the visible spectrum, but the tests will be irrelevant and inconclusive because they may not identify the presence of any systematic errors that may be in the specified ultraviolet wavelength region. It is imperative, therefore, that the quality control and validation procedures specify only the use of accurate reference material standards certified in the spectral wavelength region of analytical interest. Extrapolations of measurement results from tests conducted in spectral regions removed from the analytical method wavelength will weaken the scientific defensibility of the validation.

Verification of the wavelength scale should be the first UV/VIS validation test performed. [1] The three most common wavelength reference material standards and their respective certified spectral ranges include: (i) holmium oxide solution, 241 nm - 641 nm; (ii) holmium oxide glass filter, 279 nm - 638 nm; and (iii) didymium oxide glass filter, 328 nm -880 nm. The certified spectral ranges of the three wavelength reference material standards are illustrated in Figure 1. For each wavelength standard, the number of certified wavelengths for a 1-nm spectral bandpass is shown in parenthesis, and the individual wavelengths for the certified absorption peaks are indicated by the symbols positioned along the horizontal wavelength scale.

The horizontal wavelength scale of Figure 1 is arbitrarily divided into 50-nm segmented regions, as indicated by the dashed vertical lines. By locating the segmented region in Figure 1 that includes the wavelength of the analytical method, the wavelength reference material standard of preference is the one which exhibits the most certified absorption peaks in the same spectral region of the analytical method. If no certified absorption peaks for any wavelength standard fall within the specified spectral region, scientific defensibility dictates the use of a wavelength reference material that exhibits a certified absorption peak closest to the analytical wavelength of interest.

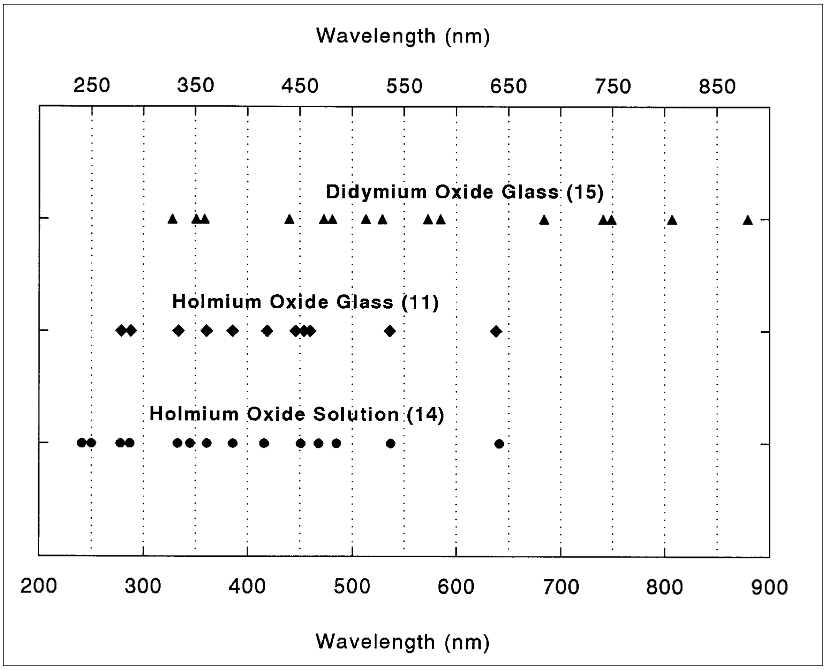

Figure 1. Certified spectral range coverage of wavelength reference material standards.

Holmium oxide solution is superior to the holmium oxide glass filter as a UV/VIS wavelength reference material because of its higher certification accuracy and better UV spectral range coverage. [2] State-of-the-art UV/VIS analysis and testing programs incorporate the use of the holmium oxide solution as the preferred reference material standard for the validation of the wavelength scale of a spectrophotometer in the ultraviolet and visible spectral regions. Having several certified absorption peaks in the 650 nm to 900 nm spectral range, the didymium oxide glass filter is preferred for validation of the wavelength scale in the near infrared spectral region.

Verification of the photometric scale and photodetector linearity should be performed only after the wavelength scale has been successfully validated. The four most common photometric reference material standards and their respective certified spectral ranges include: (i) potassium dichromate solutions, 235 nm- 350 nm; (ii) cobalt/nickel/nitrate solutions, 302 nm - 678 nm; (iii) neutral-density glass filters, 440 nm-635 nm; and (iv) neutral-density metal-on-quartz filters, 250 nm-635 nm.[3,4]

The certified spectral ranges of the four photometric reference material standards are illustrated in Figure 2. For each photometric material the number of standard wavelengths, at which the transmittance/absorbance certification is provided, is shown in parenthesis. The individual standard

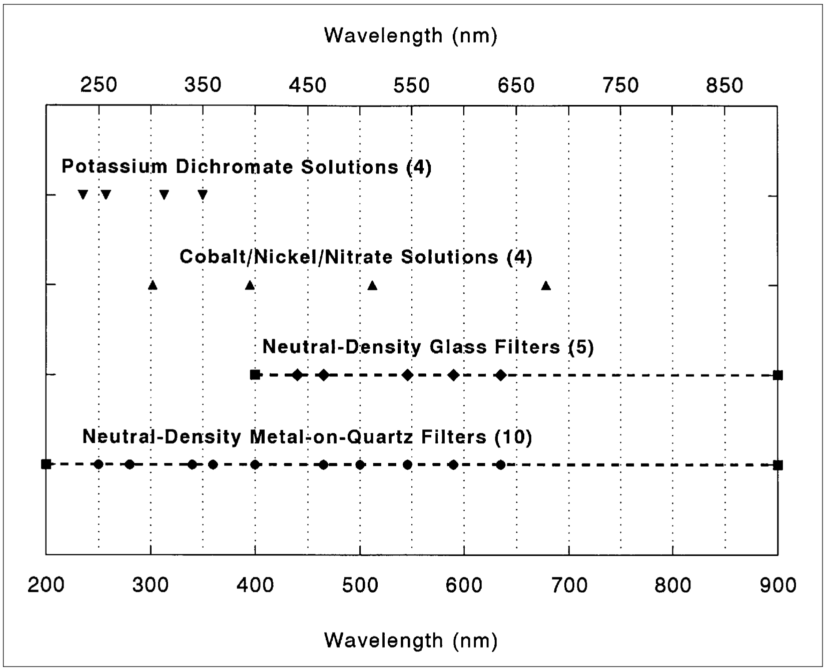

Figure 2. Certified spectral range coverage of photometric reference material standards.

wavelengths for the transmittance/absorbance certification are indicated by the symbols positioned along the horizontal wavelength scale.

The horizontal wavelength scale of Figure 2 is also arbitrarily divided into 50-nm segmented regions, as indicated by the dashed vertical lines. As specified for the wavelength validation tests, the spectral region that includes the wavelength of the analytical method must first be identified. By locating the segmented region in Figure 2 that includes the analytical method wavelength, the photometric reference material standard of preference is the one which exhibits the most certified transmittance/absorbance values at the standard wavelengths in the same spectral region of the analytical method.

A photometric reference materal is considered optically neutral if its transmittance/absorbance spectrum varies little with wavelength. Optical neutrality is a desirable spectral characteristic of a photometric reference material standard because it relaxes the restrictions on finite spectral bandpass specification and wavelength accuracy to validate transmittance/absorbance measurements. Solid photometric reference materials exhibit a higher degree of optical neutrality than photometric reference solutions. The optical neutrality attribute is one of several reasons why solid photometric standards are preferred over photometric reference solutions for most photometric validations.

The four standard wavelengths at which the transmittance/absorbance certification are provided for the potassium dichromate solutions and the cobalt/nickel/nitrate solutions correspond to absorption maxima and minima of their respective absorption spectra. Because of the lack of optical neutrality in their respective absorption spectra, the transmittance/absorbance certification for these two photometric reference solutions is restricted to their fixed standard wavelengths.

The high degree of optical neutrality of the solid photometric filters allows the certification of transmittance/absorbance measurements to be made at custom spectral wavelengths in addition to the standard spectral wavelengths. As illustrated in Figure 2, the horizontal dashed line for the neutral-density glass filters indicates that transmittance/absorbance certification is possible for this reference material at any custom wavelength within the approximate spectral range of 400 nm to 900 nm. The horizontal dashed line for the neutral-density metal-on-quartz filters indicates that transmittance/absorbance certification is possible for this reference material at any custom wavelength within the approximate spectral range of 200 nm to 900 nm.

2. Use UV/VIS reference material standards that have high certification accuracy (low uncertainty).

The measurement validity and scientific defensibility of UV/VIS analytical procedures and protocols are maximized and long-term validation costs are decreased by only using UV/VIS reference material standards that have high intrinsic certification value (i.e., high accuracy, low uncertainty). UV/VIS reference standards are available as Standard Reference Materials® (SRMs) from the National Institute of Standards and Technology (NIST) and also as secondary NIST-traceable standards from suppliers in the private sector. Secondary standards from different suppliers vary in accuracy specifications and certification value despite their similar claims of NIST traceability.

Secondary UV/VIS reference materials uniquely patterned after the corresponding NIST standards offer the highest certification accuracy in the private sector. [5] For example, with its similar material design/production specifications and wavelengths at which the

Figure 3. The *SpectroStandards*™CRM 500 neutral-density metal-on-quartz filter set. CRM 500 consists of three individual filters in sparate metallic cuvette-style holders and one empty filter holder. The nominal percent transmittances of the three filters are 10%T, 30%T and 90%T. The filter holders are provided with removable sliding shutters that are intended to protect the filters when not in use. Each filter holder is engraved with a unique filter number (10, 30 or 90) and a set identification number. The filters are stored and transported in a cylindrical canister.

transmittance/absorbance certification is provided, the *SpectroStandards*™ CRM 500 shown in Figure 3 uniquely corresponds to the NIST SRM® 2031 [6], a neutral-density metal-on-quartz filter set used for UV/VIS photometric validation.

It is noteworthy that NIST traceability is a necessary but *not* sufficient condition for secondary UV/VIS reference material standards to have high accuracy. Accurate UV/VIS reference material standards from any supplier must exemplify the following characteristics: (i) exacting material design/production specifications, (ii) meaningful certification measurements, and (iii) for secondary standards, rigorous NIST intercalibration benchmarking and scientific traceability. Specifically, for neutral-density glass photometric filters, the candidate reference material standard must satisfy certain production design criteria including flatness, parallelism and optical transmittance uniformity, and the standard material must also pass stability requirements. [4,7]

Target specifications for certification accuracy are given in Table 1 for the holmium oxide solution wavelength standard and for neutral-density glass photometric standards comprised of filters that have transmittances within the range of 10%T to 50%T. Acquiring a holmium oxide wavelength standard or a neutral-density glass photometric standard (comprised of filters that have transmittances within the 10%T to 50%T range) from a supplier that can not provide these respective certification accuracies will also jeopardize the scientific defensibility of the UV/VIS validation. Such accuracy characteristics should not be trivialized because it is plausible that impending analytical quality systems such as the NIST Traceable Reference Material (NTRM) program may not formally recognize UV/VIS reference material standards which do not comply with certain production design specifications and certification accuracy requirements.

The purchase of low-accuracy reference material standards may appear to represent an attractive cost savings. The hidden costs of this strategy, however, will soon become evident from failed audits. When using reference material standards of low accuracy, the cost equation must also include the consequences of unproductive laboratory staff time and instrument downtime when trying to resolve measurement discrepancies that may exist between different spectrophotometers or when trying to interpret marginal or inconclusive UV/VIS validation data which border on the uncertainty or tolerance limits. The original cost savings is quickly negated and even turned into a financial loss when the laboratory realizes that it must upgrade its UV/VIS quality program by purchasing new reference material standards of higher accuracy and reputable certification.

It is important to recognize that the certification uncertainty of the UV/VIS reference material standard should be small (if possible) with respect to the relevant instrument specification and the accuracy requirement of the analytical test method. Accurate reference material standards will, therefore, facilitate the acquisition of validation data that have the highest interpretational value which will help evaluate marginal or borderline measurement data and instrument performance.

Accurate reference material

Reference Material Standard	Optical Property	Certification Uncertainty
Holmium Oxide Solution	Wavelength	± 0.1 nm
Neutral-Density Glass Filter	Photometric	± 0.003 AU

Table 1. Target specifications for UV/VIS certification accuracy.

Certification Accuracy of Reference Material Standard	Spectrophotometer / Method Accuracy		
	High	Medium	Low
HIGH ACCURACY	YES	YES	YES
LOW ACCURACY	NO	NO	YES

Table 2. Qualitative rating matrix for UV/VIS reference material standards.

standards are cost-effective because of their flexibility. They can be used for quality control, validation and troubleshooting of all laboratory spectrophotometers and/or UV/VIS analytical methods. Reference standards of lower certification accuracy are restricted in the scope of their application. A qualitative rating matrix is provided in Table 2 that summarizes the appropriateness and scientific defensibility of using UV/VIS reference material standards of both high and low accuracy specifications for quality control, validation and troubleshooting.

The summary is provided for three arbitrary scenarios of relative spectrophotometer specifications and method accuracy requirements: high, medium and low. It is evident from Table 2 that reference material standards of high accuracy are advantageous because a single array of the standards can be used for every spectrophotometer or UV/VIS method in the analytical laboratory. On the other hand, the use of reference material standards of low certification accuracy are not appropriate or defensible in the quality control and validation scenarios of medium to high measurement accuracy.

3. Use the appropriate UV/VIS reference material standard for the relevant spectrophotometer specification or parameter.

UV/VIS reference material standards are certified for a specific optical property such as a wavelength or photometric value. Wavelength and photometric reference material standards differ significantly in their spectral characteristics. A series of isolated absorption bands of narrow bandwidth is desirable for a wavelength standard whereas an optically-neutral spectrum is a desirable characteristic of a photometric standard. A single reference material standard can not be used for both wavelength and photometric validations because of the opposing intrinsic spectral characteristics of wavelength and photometric standards.

Using an accurate reference material standard is appropriate and defensible only if it is used for quality control or validation of the spectrophotometer or method parameter for which the standard is intended. For example, using a holmium oxide solution to validate the wavelength scale is appropriate and defensible whereas using a potassium dichromate solution for the same wavelength validation is not appropriate or defensible. On the other hand, using a potassium dichromate solution to validate photometric accuracy and photodetector linearity is appropriate and defensible whereas using a holmium oxide solution for the same photometric accuracy/linearity validation is not appropriate or defensible. Either case applies equally to the use of a NIST standard or a secondary reference material standard which has claims of NIST traceability.

The intended quality control and validation purposes of certified UV/VIS reference material standards are summarized in Table 3. Using a reference material standard to validate an instrument parameter for which it is not intended will also weaken the scientific defensibility of the validation.

Reference Material Standard	Spectrophotometer Parameter		
	Wavelength	Photometric/Linearity	Stray Light
Wavelength	YES	NO	NO
Photometric	NO	YES	NO
Stray Light	NO	NO	YES

Table 3. Relevance of reference material standards for instrument parameters.

4. Use an appropriate photometric reference material standard to verify photodetector linearity over the transmittance/absorbance range of the analytical method.

Photometric reference materials are available in different transmittance/absorbance levels. A defensible UV/VIS validation procedure will specify the use of an appropriate photometric standard comprised of filters having at least 3 different transmittance/absorbance levels to verify photodetector linearity over the transmittance/absorbance range of the analytical method. For example, by using the familiar NIST series of neutral-density glass filters (SRM® 930 and SRM® 1930), a 6-point stepwise verification of the transmittance scale in the visible spectrum is achievable over the range

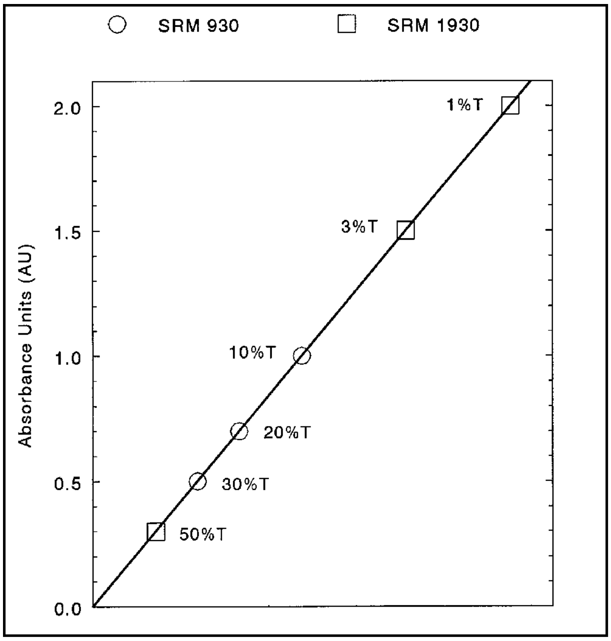

Figure 4. Linearity verification using the NIST series of neutral-density glass filters.

of 1%T to 50%T [3,7]. This corresponds to a linearity verification over the range of 0.3 to 2 absorbance units, which is illustrated in Figure 4.

Comprehensive validation of the spectrophotometer at periodic intervals should include a rigorous verification of photodetector linearity using the full transmittance/absorbance range of the photometric reference material standards. Daily quality control checks are also recommended, and these measurements may be conveniently achieved by using a single photometric standard of the appropriate transmittance/absorbance for the analytical method.

5. Partner with a reputable UV/VIS measurement science organization.

The ultimate laboratory objective of UV/VIS reference material standardization is to help ensure the integrity and reliability of the analytical measurement data in UV/VIS chemical analysis and testing. This objective is best fulfilled by purchasing accurate UV/VIS reference material standards for the appropriate validation test from a reputable supplier specializing in UV/VIS measurement science and standardization. By partnering with a reputable standards supplier that is solely focused on the production and certification of UV/VIS reference materials, and one which can also provide authoritative technical support, the analytical laboratory is assured the highest level of quality, integrity and scientific defensibility in the reference material standards that will withstand the test of time.

Because of the intrinsic nature of UV/VIS reference materials, recertification at periodic intervals is recommended to ensure that the certified values assigned to the reference material standard are meaningful and accurate. A reputable measurement science and standards supplier will also be able to provide an affordable and efficient recertification service of high quality. The supplier's commitment to a timely recertification turnaround is required to minimize any interruption in the laboratory's quality control, calibration and validation/qualification services while it is without the use of a certified reference standard. For a secondary standards supplier, the recertification service must be conducted with an uncompromising rigor of NIST benchmark inter-calibration and defensible traceability to ensure that the customer receives the highest measurement accuracy and recertification quality value. [5]

Summary

The successful development, validation, transfer and application of UV/VIS laboratory methods will be compromised if existing systematic errors are not controlled. Reference material standardization, the underlying concept of analytical measurement science, is the practical laboratory means to investigate potential instrument-related systematic errors and, if present, to evaluate their impact on the quality of the data. The scientific defensibility of any UV/VIS validation, whether conducted in-house or out-sourced to an independent validation firm, can be no better than the accuracy of the reference material standards and the relevance of the test procedure or protocol used for the validation.

By integrating reference material standardization into its standard operating procedures and validation/qualification protocols, the analytical laboratory will have documented evidence of achieving the highest level of scientific defensibility in the validation and daily quality control of their UV/VIS instrumentation. Reference material standardization, therefore, facilitates the acceptance and use of the analytical results for their intended purpose with greater confidence. Partnering with a reputable UV/VIS measurement science and standards organization reinforces or enhances the laboratory's quality consciousness, image and reputation which will further support its commitment to analytical excellence in the competitive global marketplace.

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Acknowledgement

One of the authors (JDM) gratefully acknowledges Dr. Radu Mavrodineanu, internationally renowned analytical spectroscopist and the "founding father" of NIST's contemporary spectrophotometric SRM® program, for his treasured mentorship and invaluable insight into high-accuracy spectrophotometry and UV/VIS reference material standardization which uniquely provide the scientific cornerstone to the SpectroStandards™ UV/VIS measurement science program.

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