Application note

Instrument Qualification: A Guide to IQ/OQ Procedures

UV-Visible spectroscopy is a highly versatile technique employed in a variety of different industries and workflows. The data acquired through UV-Visible spectrophotometers is often used for sample characterization or to quantify the concentration of a given analyte. It is imperative the instrument be installed correctly and tested against specific performance criteria in order to check that the instrument is operating appropriately. By performing these tests, the user can trust the instrument is correctly reporting data within the uncertainties established by the manufacturer. Herein, we aim to outline the processes and tests used to check the instrument, as well as define terms used in this process.

What is the difference between Validation and Qualification?

While both terms can appear similar, the tests required for each are different. A Qualification includes a set of methods used to verify the system components are working properly. This set of methods includes the IQ/OQ (discussed in the next section of this document) and the qualification process is often performed by the manufacturer or a trained representative. Because of the nature of these tests, qualification protocols are typically designed by the vendor or manufacturer.

A Validation includes procedures to confirm the complete system, as opposed to each individual part, works for the application or process required by the end user, including the qualification as discussed above. As this requires knowledge of the end user's exact needs, including the SOP's developed in their lab, validation is the responsibility of the user.

What is an IQ/OQ?

As previously discussed, the qualification of an instrument includes an IQ/OQ. This is comprised of two different qualification steps: an Installation Qualification (IQ) and an Operational Qualification (OQ). The IQ outlines procedures used to confirm the instrument and requisite software are installed correctly, while the OQ includes tests to check both the instrument and software are functioning as expected. In an OQ, this includes ensuring the specifications outlined for the instrument (e.g. photometric accuracy, stray light) are met.

A performance qualification (PQ) outlines a set of tests more specific to the end user's needs as well. Similar to the validation procedures, which encompass the entire system for the application, the PQ is specific to the requirements of the laboratory methods/SOPs in place. As such, it will not be discussed in detail in this document.

Instrument OQ Tests

Below are descriptions for the various tests used in the OQ for the UV-Visible instruments themselves. These tests check that the instrument specifications are met or that the instrument meets guidelines described in the United States and/or European Pharmacopoeia's (USP and EP, respectively) chapters on UV-Visible instrumentation.[1,2] For a full description of the various standards outlined by USP and EP, please see the Pharmaceutical Standards Guide for UV-Vis Instrumentation.[3] While these tests are used in OQ procedures, they can also be helpful when troubleshooting.

When choosing a standard for any test, it is important, especially for regulated environments, that the standard be considered "traceable". This indicates the reported value for the standard was measured on an instrument calibrated according to a national reference method and meeting appropriate standards, such as those outlined by the National Institute of Standards and Technology (NIST). These standards will include an uncertainty in the reported value that includes all sources of potential error, including uncertainty in the instrument used. For standards which require re-calibration, the manufacturer's guidance should be referred to regarding when re-calibration is needed. Many instrument manufacturers including Thermo Fisher Scientific, do recommend specific standards; Thermo Fisher Scientific also provides purchasing and renting options for these standards.

Multiple OQ tests for UV-Visible spectrophotometers outline describing either the accuracy of a parameter or the repeatability. In the context of these tests, repeatability can be considered synonymous with the precision of a particular measurement. Accuracy refers to how close a reported value is to the "true" value, while precision describes how close replicate measurements are to one another (see Figure 1 for a visual description). It is important to be able to demonstrate both accurate and precise measurements, as this implies the data obtained will be consistent between measurements and appropriately reflect the true characteristics of the sample analyzed.

Wavelength Accuracy and Repeatability

Wavelength accuracy tests check that the wavelength reported by the instrument is the true wavelength interacting with the sample. This is typically checked by measuring the spectrum of a standard with well-known absorption or emission peaks. A commonly used standard includes an elemental lamp source, like a low-pressure mercury (Hg) lamp. By its nature, atomic emission spectra are not dependent on preparation procedures and will not vary significantly over time, eliminating the need to re-calibrate. As these standards are dependent on fundamental properties of elements, they are considered primary standards and have a lower associated uncertainty than solution-phase samples such as holmium oxide. The instrument is considered passing if the measured value of the wavelength maximum reported is found to be within plus or minus a given limit. For example, for USP compliance, this limit is ± 1.0 nm in the UV spectral region and ± 2.0 nm in the visible region. It is important to note that the instrument is only considered qualified within the spectral range spanning the wavelengths measured in the experiment.

Accuracy vs Repeatability (Precision)

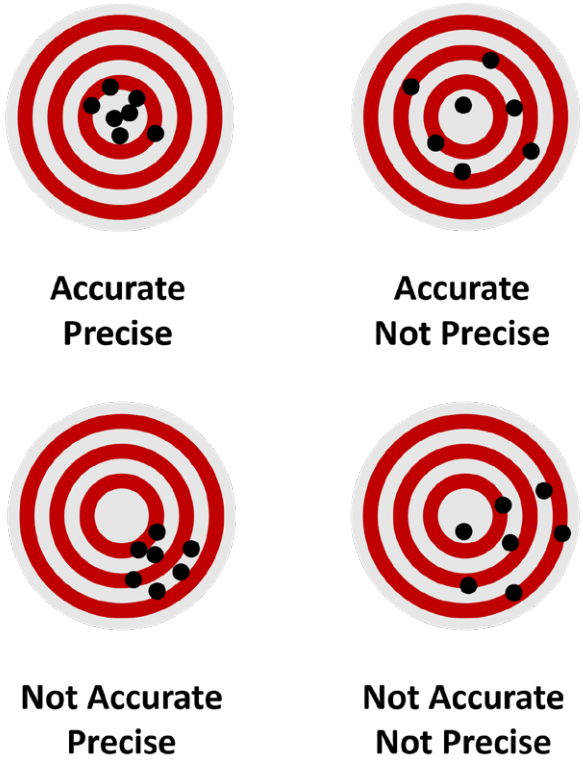

Figure 1. Accuracy vs precision depiction assuming the center of the target is the "true" value.

For example, if only a holmium oxide standard (Figure 2) is used, the shortest and longest certified wavelength maxima (~241 nm and ~640 nm) would define the range by which the instrument is considered qualified. Often, multiple standards are used to check the wavelength accuracy of an instrument to cover the full range required according to the intended analysis range. A description of the traditional standards used for wavelength accuracy measurements are expanded on in greater detail in the Pharmaceutical Standards Guide for UV-Vis Instrumentation.[3]

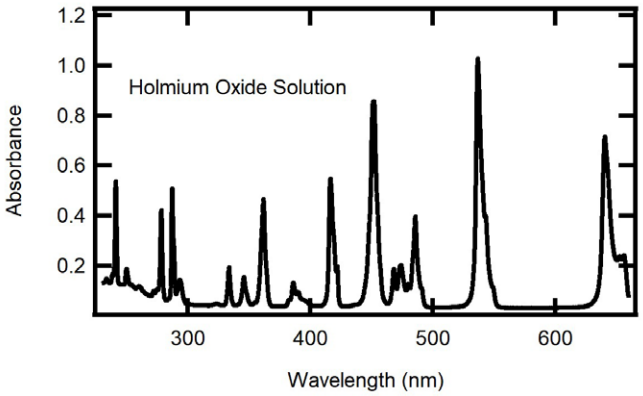

Figure 2. Spectrum of a holmium oxide standard in perchloric acid measured using a 0.05 s integration time, 1.0 nm bandwidth and 0.1 nm step size. This standard is NIST tracable and held in a 1.0 cm quartz cuvette.

For wavelength repeatability tests, the same measurement performed in wavelength accuracy tests is repeated multiple times. The standard deviation of the replicate measurements is then expected to fall below a given limit in order to pass. The combination of wavelength accuracy and repeatability tests help verify that the instrument is both accurate and consistent when reporting measured values. Note, while USP outlines a required repeatability of the measurement, this test is not required to qualify an instrument according to EP.

Photometric Accuracy and Repeatability

The photometric accuracy test compares the absorbance of a given standard (e.g., potassium dichromate) against the accepted absorbance of the material. This test checks that the reported absorbance collected for a given sample is accurate and falls within acceptable limits. As stated previously, standards should be traceable and include a certification for the "true" absorbance of the material and associated uncertainty in the measurement. As with wavelength accuracy measurements, the photometric accuracy of the instrument is only qualified in the range covered by the standards used.

By measuring multiple standards, the photometric linearity over the intended analysis range can be assessed as well. According to Beer's law (eq.1), where A is the measured absorbance, c is the concentration of the sample measured, l is the path length the light passes through and ε is the extinction coefficient of the material, absorbance is linear with concentration.

Equation 1. $$A = cl\varepsilon$$

In an ideal circumstance, this linearity would hold for any measured absorbance; however, instrument limitations cause deviations from linearity (Figure 3). This is primarily an issue for highly absorbing samples as minimal light is able to pass through the material. The detector is limited in how little light can be observed, leading to deviations and greater error for highly absorbing samples.

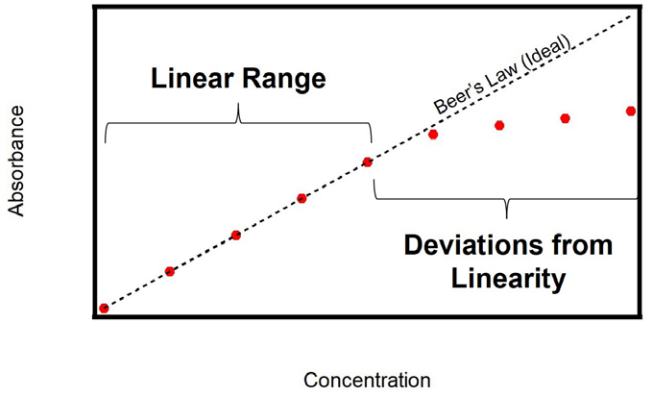

Figure 3. Absorbance as a function of concentration. The dashed line describes Beer's law as an ideal case while the red dots simulate what a real data set may look like. Deviations from linearity can be observed for high-concentration samples.

In general, the best practice is to avoid quantitative measurements above 1.0 A. However, ensuring the instrument meets linearity requirements will still qualify the instrument for absorbance beyond 1.0 A but only up to the absorbance of the most concentrated/highest absorbing standard. EP requirements indicate the coefficient of determination (R^2) for the line fit to the measured absorbance of each photometric accuracy standard should be between 1.000 and 0.999, whereas USP considers the linearity requirement satisfied given each standard measured passes the photometric accuracy test.

The photometric repeatability of the instrument is tested by measuring each given photometric accuracy standard multiple times and comparing the standard deviation against the required specification or acceptance limit. Like with wavelength accuracy and repeatability measurements, these tests help make sure the reported results are accurate and consistent for the qualified instrument, as well as define a working absorbance range for future experiments.

Stray Light

For UV-Visible instrumentation, light which “leaks” in from other places (e.g., room light) or scatters off optical components within the instrument in non-ideal ways has the possibility to reach the detector. This stray light can lead to over-estimations of the light intensity observed by the detector, resulting in inaccuracies in the reported absorbance. Instrument manufacturers have taken precautions to be certain a minimum stray light is observed. However, as the presence of stray light has direct implications on the measured absorbance, it is important to check that minimal stray light is detected within the instrument.

As introduction of stray light is often due to scattering of light, and the property of scattering is more prominent for shorter wavelengths, this test is performed using UV light. A standard is chosen which is expected to absorb highly at the chosen analysis wavelength, allowing virtually no light to pass through. For example, a 12 g/L aqueous solution of potassium chloride is expected to absorb almost all light at 198 nm. The absorbance or percent transmission (%T) is collected and compared against a given limit. If the sample absorbance is above a given limit, or conversely if the light transmittance is below a given limit, the instrument is considered passing.

Resolution

In UV-Visible measurements, resolution can best be described as the ability to distinguish between two peaks in a spectrum. For samples with absorption maxima far away from one another, poor resolution may not significantly affect the measured spectrum. However, spectra which contain peaks with substantial overlap or in which the absorption maxima are a few nanometers away from one another, the ability to resolve these features can be valuable.

It is important to note that the resolution is highly dependent on the chosen instrument parameters, and the acceptable limits are different as a function of the given parameters. One key parameter is spectral bandwidth, which describes the range of wavelengths that are allowed to pass through a slit in the instrument after the light is dispersed by a monochromator. In the context of resolution, increasing the bandwidth will essentially average the signal across the wavelengths measured in a single measurement, leading to a loss of resolution.

As shown in Figure 4, by increasing the spectral bandwidth for the measurement from 1.0 nm to 2.0 nm, fine details in the spectrum are lost and some drastic changes in the reported absorbance of the sample can be observed. For samples with very close and narrow absorption bands, the ability to resolve these features is very important. However, in a majority of cases the absorptive features of a substance tend to be fairly broad, and changes to the bandwidth do not significantly change the measured spectra.

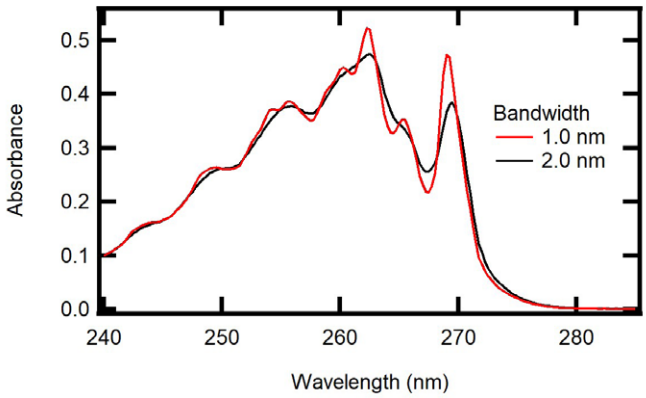

Figure 4. Spectra of 0.02% toluene in hexane collected using a (red) 1.0 nm and (black) 2.0 nm spectral bandwidth. Both spectra were collected using a 1.0 cm NIST traceable standard with an integration time of 0.25 nm step size and 0.5 s integration time. Hexane was used to establish the blank (NIST traceable).

To test this function when required, the absorption of a 0.02% v/v toluene in hexane solution is typically measured at 269 nm and 266 nm. The ratio of these values is then compared to a given acceptance limit (eq. 2), a value that depends on the bandwidth used in the measurement.

$$\frac{A_{269}}{A_{266}} > limit$$

Equation 2.

In USP compliant environments, this check is required and should result in a ratio greater than 1.3 when using a 2.0 nm spectral bandwidth. EP however only specifies checking the resolution if the monograph calls for this. In environments outside of pharmacopoeia, this test is still helpful to perform to confirm narrow bands can be readily resolved.

Extra OQ Tests

The following performance tests are not strictly included in either USP <857> or EP Ch. 2.2.25 as required tests, however these checks can be very helpful to verify the instrument is operating appropriately. Current IQ/OQ procedures for the Evolution instruments include the following tests.

Photometric Noise

Given that instruments do not operate under ideal conditions, there will inherently be noise detected across the spectrum. This noise can come from a variety of different sources (e.g., flicker noise, shot noise, dark noise, etc.), some of which can be limited through careful experimental techniques, while some cannot be removed (e.g., shot noise). The instrumental noise is expected to be worse at the limits of the detector, specifically when analyzing highly absorbing substances where minimal light is able to reach the detector.

It can be helpful to check that the observed noise meets the specifications of the instrument, especially in the absorbance range at which the instrument will be operating. Keep in mind, the measured noise for the instrument is highly dependent on the parameters chosen for the measurement. For example, using a short integration time will result in a noisier spectrum than if a long integration time is chosen, as depicted in Figure 5.

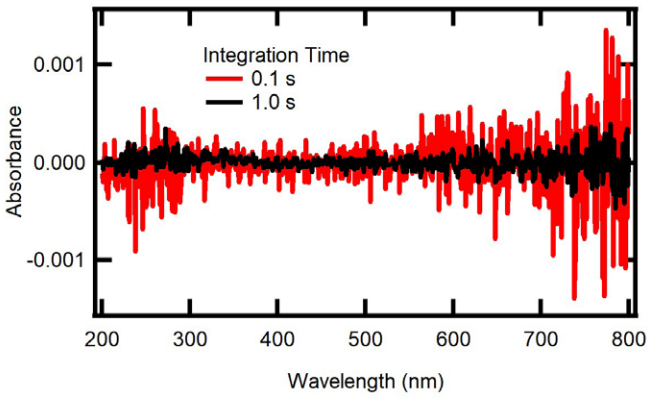

Figure 5. Baseline absorption measurement collected against air using a (black) 1.0 s and (red) 0.1 s integration time. Measurements were colleced using a 1.0 nm spectral bandwidth and 1.0 nm data interval. Air was used a blank measurement.

As the noise is expected to be smallest for the baseline, where the absorbance is 0 A, it is good practice to confirm the noise in this photometric region is minimized. The photometric noise at 0 A also defines the limit of detection (LOD) and limit of quantification (LOQ) for the instrument. In general, the instrument LOD and LOQ can be estimated as the points at which the signal is three times larger and ten times larger, respectively, than the noise level of the instrument.

When the data collected will be used for quantification, the LOQ is a more appropriate limit. The LOD and LOQ of the instrument can be reported in terms of absorbance units as well as converted to concentration through Beer's law (eq. 1).

For example, if the photometric noise of the instrument at 0 A is ±0.01 A, the LOD and LOQ are approximately 0.03 A and 0.10 A, respectively. Keep in mind, there are more rigorous methods by which the LOD and LOQ can be estimated, including methods which involve the construction of a standard curve. However, the method described here can serve as a general baseline when assessing if the analyte can be reliably detected by the instrument, or if the resulting absorbance can be used for further quantification..

Photometric Drift

Often, from when the blank/baseline measurement is collected to when the last sample is measured, a large amount of time can pass. This is particularly true for kinetics experiments, where the instrument may be measuring a single or multiple samples for anywhere from a few minutes to hours. As UV-Visible measurements are based on the ratio of the light intensity of the blank and the light intensity of the sample, changes to the lamp intensity over time can cause slight errors in the absorbance reported. While modern spectrophotometers are designed to minimize these issues, it can be very helpful to monitor this behavior through photometric drift tests.

A photometric drift test involves measuring the absorbance at one wavelength as a function of time and is typically performed using an empty sample holder. In this method, the change in absorbance overtime is calculated from the data set and must fall within a set range of values to be considered passing.

It is important to realize that while photometric drift tests can assist in confirming that changes in the baseline are not originating from the instrument, this particular test is incapable of determining whether the solvent/buffer is undergoing any changes during the course of the experiment. Changes to the solution used to establish the background can lead to changes in the perceived absorbance of the sample. To mitigate issues resulting from the blank material, it can be helpful to place a matched reference cuvette with the solvent/buffer blank in the reference cell position. This will aid in accounting for variations in the blank absorbance overtime; this method is most commonly used for kinetics measurement where other components may also change as the experiment progresses. Alternatively, re-taking a blank measurement can also aid in correcting for changes in the blank spectrum. If this method is used, it can be helpful to measure the blank solution as a sample to check that the baseline was properly collected.

Baseline Flatness

If a sample with no absorption features is measured, it is expected the spectrum should appear flat with an average absorbance of 0 A. This measurement would represent the “baseline” absorbance spectrum for the instrument and is typically collected by measuring the absorbance of an empty sample holder.

What about IQ/OQ for Software?

While it is very important to check the instrument is operating according to specifications, it is equally important to make sure the software is also working properly. IQ/OQ procedures for the instrument control software cover basic operation and initialization of the software. For regulated environments, such as US FDA 21 CFR Part 11 complaint laboratories, additional security software is required. This software allows users to record logs of all the actions performed either during an experiment or on an existing data set as well as implement user restrictions on specific software functions. In addition to the general testing of the instrument control software, tests specific to the security functions must also be qualified through IQ/OQ procedures. For software-specific OQ procedures, there are no standards required to complete the tests, unlike the instrument OQ. However, it is helpful to have an IT representative available when performing the software portion of the IQ/OQ. As Insight Pro™ and related security software rely on the Windows™ platform to enforce security measures, an IT representative can aid in troubleshooting network and computer issues interfering with the function of the software.

References

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