

Operational Qualification and Performance Verification of UV-visible Spectrophotometers

Technical Note



In principle, operational qualification and performance verification (OQ/PV) is not difficult to perform, because spectrophotometers are relatively simple analytical instruments. However, in practice there are significant problems because of the need to conform closely to regulatory requirements and because of the non-availability of appropriate and easy-to-use standards.

This technical note reviews the regulatory requirements, the advantages and disadvantages of the standards currently available and describes a new approach which significantly reduces the time required to perform OQ/PV.

Introduction

In recent years quality requirements, as outlined by ISO 9000, GLP, GMP and NAMAS, have assumed increasing importance and as a consequence, in the pharmaceutical industry, the recommendations of pharmacopeias have also become more influential. Verification of the continued proper performance of UV-visible spectrophotometers is an important element of these quality requirements.



Definition The terms of

The terms operational qualification and performance verification are used interchangeably in this publication. Both terms are used to mean a series of tests designed to prove that an instrument is working within a set of required specifications.

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Regulatory requirements

There are many regulatory requirements that govern the use of UV-visible spectrophotometers. The following sections review the most important of these.

Good laboratory practice and good manufacturing practice

Good laboratory practice (GLP) and good manufacturing practice (GMP) requirements concerning the validation of instruments can be summarized as:

"Documented verification that the system or subsystem performs as intended throughout representative or anticipated operating ranges." ¹

In reference to spectroscopy, the following is suggested:

"Where appropriate, periodic performance checks should be carried out (for example, ... the resolution, alignment and wavelength accuracy of spectrophotometers, etc.)."² The statement *intended and anticipated operating ranges* is critical when considering an OQ/PV strategy. For example, if the intended purpose is to measure absorbance in the UV region (as is the case for most pharmaceutical analyses), it is not appropriate to verify photometric accuracy in the visible range—it must be done in the UV region and preferably at several wavelengths.

Similarly, it is not appropriate to verify wavelength accuracy at the 656.1 nm deuterium line because this is not a reliable indicator of wavelength accuracy in the UV region. Further, as the vast majority of samples to be measured in the pharmaceutical laboratory are solutions contained in cuvettes, it can be argued that the use of solid or emission standards is inappropriate, because these standards have different optical characteristics from the samples that will be analyzed. It is worthwhile noting that the American Standard Testing Methods (ASTM) and the National Institute of Standards and Technology (NIST) make similar statements. For example, the ASTM states that the wavelengths used for calibration should, if possible, bracket the analytical wavelength and NIST comments on the use of their various standards.

United States Pharmacopeia³

The regulatory requirements for UV-visible spectrophotometers are defined in the United States Pharmacopeia (USP) XIII, Section 851 on "Spectrophotometry and Light Scattering." It states:

"Check the instrument for accuracy of calibration. ... The wavelength scale may be calibrated also by means of suitable glass filters, which have useful absorption bands through the visible and ultraviolet regions. Standard glasses containing didymium (a mixture of praseodymium and neodymium) have been widely used. Glass containing holmium is considered superior.

For checking the photometric scale, a number of standard inorganic glass filters as well as standard solutions of known transmittance such as potassium chromate or potassium dichromate are available." The latter contains a cross-reference:

"For further details regarding checks on both wavelength and photometric scales of a spectrophotometer, reference may be made to the following publications of the National Institute of Science and Technology ..." The NIST⁴ provides a range of solid and liquid standards for determining wavelength accuracy, photometric accuracy and stray light. The most important are summarized in table 1.

| SRM# | Туре | NIST Description |
|------|--|---|
| 930 | Neutral density glass filters | This SRM is for the verification and calibration of the transmittance and absorbance scales of visible absorption spectrometers. |
| 931 | Cobalt and nickel solution in nitric/perchloric acid mixture | This SRM is for the verification and calibration of the absorbance scales of ultraviolet and visible absorption spectrometers having narrow band passes. |
| 935 | Potassium dichromate solid for preparation of test solution | This SRM is for the verification and calibration of the transmittance and absorbance scales of ultraviolet absorption spectrometers. |
| 2031 | Metal-on-quartz | This SRM is for the verification and calibration of the transmittance and absorbance scales of ultraviolet and visible absorption spectrometers. |
| 2034 | Holmium oxide solution in perchloric acid | This SRM is for use in the verification and calibration of the wavelength scale of ultraviolet and visible absorption spectrometers having nominal spectral bandwidths not exceeding 3 nm. |
| 2032 | Potassium iodide solid for preparation of test solution | This SRM is for use in the assessment of heterochromic stray radiant energy (stray light) in ultraviolet absorption spectrophotometers. |

Table 1 NIST standards

European Pharmacopoeia⁵

The European Pharmacopoeia (EP) is based on the requirements of national pharmacopoeias such as the British Pharmacopoeia (BP) and the Deutsche Arzneimittelbuch (DAB) in Germany, and is very specific in its requirements for UV-visible spectrophotometers. The EP requirements are described in the following sections.

Note that the British Pharmacopoeia states that the ratio should "... *be not less than* 1.5 unless otherwise specified in the monograph" and this is often taken as a de facto minimum performance specification for pharmaceutical applications. **Control of wavelengths**—Verify the wavelength scale using the absorption maxima of holmium perchlorate solution R, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapor arc shown below. The permitted tolerance is ± 1 nm for the UV range and ± 3 nm for the visible range.

| 241.15 nm 253.7 nm 287.15 nm 302.25 nm | n (Ho) (Hg) n (Ho) n (Hg) | 404.66 nm (Hg) 435.83 nm (Hg) 486.0 nm (Db) 486.1 nm (Hb) 526 2 nm (Hz) |
|---|------------------------------------|---|
| 313.16 nm 334.15 nm 361.5 nm 365.48 nm | n (Hg) n (Hg) (Ho) n (Hg) | 536.3 nm (Ho) 546.07 nm (Hg) 576.96 nm (Hg) 579.07 nm (Hg) |
| | | |

Control of absorbance—Check the absorbance using a solution of potassium dichromate R at the wavelengths indicated in the following table, which gives for each wavelength the exact values of the specific absorbance and the permitted limits. The tolerance for the absorbance is ± 0.001 .

| Wavelength (nm) | A (1 percent, 1 cm) | Maximum Tolerance |
|-----------------|---------------------|-------------------|
| 235 | 124.5 | 122.9 to 125.2 |
| 257 | 144.0 | 142.4 to 145.7 |
| 313 | 48.6 | 47.0 to 50.3 |
| 350 | 106.6 | 104.9 to 108.2 |

Limit of stray light—Stray light may be detected at a given wavelength with suitable filters or solutions. For example the absorbance of a 1.2 percent m/V solution of potassium chloride Rin a 1-cm cell should be greater than two at 200 nm when compared with water R as compensation liquid.

Resolution power—When prescribed in a monograph, measure the resolution of the apparatus as follows: record the spectrum of a 0.02 % V/V solution of toluene R in hexane R. The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monograph.

Recommended OQ/PV test parameters and standards

When the above regulatory requirements are considered together there are many inconsistencies and, in some cases, disagreements. However, it is possible to define a strategy of tests and standards that essentially satisfies all requirements. This is illustrated in table 2 where the implied USP methods (based on NIST standards) and the EP methods are summarized and then the GLP *filter* of suitability for purpose (liquid standards calibrated in the UV range) is laid over the matrix.

In addition, although not specified by the USP or EP, it is generally accepted that to characterize properly the performance of a spectrophotometer the parameters noise, baseline flatness and stability should also be tested.

Linearity is also often considered to be an important factor for performance verification. However, the primary instrumental reason for non-linearity is tested for. Other reasons for non-linearity are strongly sample or sampling dependent and for this reason it is the authors opinion that this parameter is best measured during a system suitability test as described below.

| Type of Test | USP | EP | Comment |
|---|-----|----|--|
| Wavelength accuracy Deuterium discharge lamp | | 4 | Only two peaks |
| Mercury discharge lamp Holmium oxide glass | 4 | 4 | Position of peaks may vary from batch to batch and must be calibrated. |
| Holmium perchlorate solution | 4 | 4 | Peaks throughout UV and visible ranges. |
| Photometric accuracy | _ | | |
| Neutral density glass filters | 4 | | Blocks in UV range so only useable for visible range |
| Metal on quartz filters | 4 | | Often problems with interreflection errors and very temperature sensitive |
| Potassium dichromate solution | 4 | 4 | Has a tendency to decompose |
| Stray light | | | |
| Sodium nitrite solution | | | Not essential but gives a good data point in the mid-UV range at 340 nm |
| Potassium iodide solution | 4 | | Use for 220 nm |
| Potassium chloride solution | | 4 | Use for 200 nm |
| Resolution | | | |
| Toluene in Hexane | | 4 | |

Table 2 Regulatory test requirements

The major problem in doing performance verification is the fact that all of the above are wavelength dependent and, in the case of stray light, standard dependent as well. It is not practicable to perform all tests at all wavelengths so in practice a few wavelengths, representative of the intended purpose, should be used. The results of these test should be compared to the instrument performance specifications and/or to the instrumental performance requirements for the methods in use. Performance verification then effectively demonstrates whether

the performance characteristics have changed in a way which could affect the quality of the analytical results.

It should be noted that compliance with the above criteria determined with an appropriate set of reference standards does not necessarily guarantee that a particular analysis can be performed with a required accuracy and linearity. Since many parameters are sample dependent this can only be demonstrated with an appropriate system suitability test that uses the sample itself.

Practical aspects

Whilst liquid standards have the advantage that an appropriate set can fulfill the regulatory requirements as outlined above they do have disadvantages when compared to solid standards. Some attempts have been made to reduce these disadvantages by sealing liquid standards into cuvettes but the results are not entirely satisfactory. The relative advantages and disadvantages of the different types of standards are compared in table 5.

| | Solid Standards | Liquid Standards | Comment |
|-------------|--|---|---|
| Handling | Straightforward, but user must take care to avoid dust or scratching. | Normally prepared by user using pure chemicals, calibrated balance and volumetric flasks. User requires some skill to eliminate cross contamina- tion. Using liquid standards is to some extent a test of proper sample handling. | Straightforward, but user must take care to avoid dust or scratching. |
| Calibration | User must calibrate each standard individually. | If prepared accurately, these are absolute standards that do not require calibration. | Because the cuvette con- taining the sample is also a factor, each standard must be calibrated individually. |
| Stability | Good, but require recalib- ration at 1–2 years. | If not properly stored, some standards show significant instability. Standards are best pre- pared fresh for each test. | Generally good, but some (potassium dichromate) are less stable than solid standards. User may need to recalibrate at 6-monthly intervals. |
| Cost | High, because each stan- dard must be individually calibrated and then recali- brated at intervals. | Can be low, because chemical costs are minimal and no calibra- tion is required. Can also be prepared in bulk. | High, because each standard must be individually calibra- ted and then recalibrated at intervals. |

Table 5

Comparison of practical aspects of different standard types

A practical solution: liquid standards in ampules

The problems associated with the preparation and use of liquid standards have been virtually eliminated by making them available as prepared solutions in sealed ampules. To use them it is only necessary to break open the ampule, pour in the curette and measure. The contents of the standards kit and the purpose of each standard are summarized in table 6.

| Standard | Blank | Test |
|--|----------------------|-----------------------|
| 40 g/l holmium oxide in 10 % perchloric acid | 10 % perchloric acid | Wavelength accuracy |
| 60 mg/l potassium dichromate in 0.01 N sulfuric acid | 0.01 N sulfuric acid | Photometric accuracy |
| 50 g/l sodium nitrite in water | Water | Stray light at 340 nm |
| 10 g/l sodium iodide in water | Water | Stray light at 220 nm |
| 12 g/l potassium chloride in water | Water | Stray light at 200 nm |
| 0.02 % v/v toluene in hexane | Hexane | Resolution |

Table 6

Contents of liquid standards kit from Agilent Technologies

The only additional requirement is water as blank for the non-critical stray-light tests, all other reference solvents are included. The advantages of these standards are that they:

- save time because no preparation is required
- eliminate false OQ/PV failures due to errors in preparation
- are inexpensive compared to other calibration standards
- include a certificate of analysis for traceability, and
- have been shown to have excellent stability.

Errors due to poor sample handling can still occur but, if desired, these can be significantly reduced by using a flow cell instead of a standard cuvette. The test solutions are drawn into the cell using a syringe and the potential for cross contamination is greatly reduced. A further advantage of this procedure is that it saves considerable time compared to using a standard cuvette which must be rinsed carefully several times between standards. Test have shown that a full OQ/PV including noise and baseline flatness tests can be performed in about 30 minutes on the Agilent 8453 UV-visible spectrophotometer and its performance verification software.

Because diode-array spectrophotometers such as the Agilent 8453 have excellent stability and reliability it is only necessary to repeat the OQ/PV tests at 6–12-month intervals.

Additional test recommendations

To ensure continued proper operation between OQ/PV testing, and to ensure suitability of the system for the specific analyses being performed, the following additional tests are recommended.

Instrument selftest

The Agilent ChemStation software for UV-visible spectroscopy includes a set of self-test routines that check proper electronic and optical operation of the spectrophotometer as well as wavelength accuracy checks with the two lines at 486.1 and 656.0 nm from the deuterium lamp.

System suitability

System suitability should not be confused with method validation. System suitability is designed to evaluate the components of the analytical system to show that the performance of the system meets the standards required by the method. While method validation is performed once at the end of method development, system suitability tests are performed on a given system periodically to determine its adequacy or effectiveness. System suitability requirements for chromatography systems have been well defined but no similar definition exists yet for UV-visible spectroscopy systems.

In practice users have developed their own strategies for performing system suitability. For example:

- Measure and calibrate using one standard with a concentration equal to 100 percent of the expected component concentration. Then measure and quantify the standard and the standard diluted by a factor of 2. The results of both samples should be within a specified percentage of the known concentration. Remeasuring the standard demonstrates the quality of the initial measurement.
- Measure the standard and then measure a series of dilutions of the standard and calculate the extinction coefficient (absorbance/concentration) for each concentration. The values of the extinction coefficients should not vary by more than a specified percentage.

Summary

Performing OQ/PV that exactly meets international regulatory requirements and GLP has, until now, been very time consuming and prone to errors. Using liquid standards in ampules virtually eliminates these problems.

References

1

Alford, J.S., F.L. Cline, F.L., "PMA's Computer System Validation Committee, Computer System Validation—Staying Current,: Installation Qualification," *Pharmaceutical Technology*, **1990**, *September*, 88–104

$\mathbf{2}$

"EURACHEM Guidance Document No. 1/WELAC Guidance Document No. WGD 2: Accreditation for chemical laboratories: Guidance on the interpretation of the EN 45000 series of standards and ISO/IEC Guide 25," **1993**

3

United States Pharmacopeia XXIII/National Formulary XVIII, "Spectrophotometry and lightscattering," General Chapter 851, page 1830, **1996**, The United States Pharmacopoeia Convention, Inc., Rockville, MD, USA

4

National Institute of Standards and Technology, Standard Reference Materials Catalog, Section 204, Optical Properties, pages 104–106, **1995**, Gaithersburg, MD, USA

5

European Pharmacopoeia, 3rd Edition, Section 2.2.25, "Absorption Spectrophotometry, Ultraviolet and Visible," pages 28–29, **1996**, Strasbourg, France

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