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Reagents, Reference Materials & Laboratory Chemicals

# Compliance with the latest USP and EP chapters on UV-visible spectrophotometry

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New editions of United States General Pharmacopeia Chapter <857> and European Pharmacopoeia Chapter 2.2.25, giving guidance on instrument qualification for ultraviolet and visible spectrophotometry, have recently been introduced. The former became mandatory on 1st December 2019 and the latter on 1st January 2020. Both introduce new approaches to qualification and suggest a variety of new reference materials for qualification measurements. While there are several areas of conformity between the two new standards, there are also some important differences.

## What do they have in common?

Earlier versions of both standards contained a fairly limited set of tests to control an instrument's performance for wavelength, absorbance, stray light and resolution (spectral bandwidth). For some parameters, measurement of a single reference at one wavelength was sufficient and provided an instrument passed these tests it could be claimed that it was 'pharmacopoeia compliant'. The limitation of this approach is that a qualification carried out under one set of operating conditions might not be valid under another set. A simple example would be a qualification carried out in the UV region using one light source when the actual analysis was to be carried out in the visible region using a different source. In the new regime, the same parameters must still be qualified, but the requirement is now to demonstrate 'fitness for purpose', namely that the instrument has the performance to undertake the actual analysis to the required accuracy and precision. The user must therefore determine, for these four parameters, the range over which the system will be used in the actual analysis and prove compliance over that range. Both standards now recommend, directly or by implication, that the values of the references used for qualification should "bracket" the values to be used in the proposed analysis, so more than one reference may be needed for each parameter to be tested. There is also a specific new requirement, in both standards, to qualify absorbance linearity: this will almost certainly mean the use of three or more absorbance references. To help meet these requirements, new reference materials are cited in both standards. While users can prepare their own reference solutions, this can be risky and prone to error but fortunately both standards still allow - indeed the USP strongly advocates - the use of commercially available Certified Reference Materials.

## How do they differ?

The major difference between the two new standards is their scope. Whereas USP <857> is limited to UV-visible spectrophotometers as described in USP <1857>, the new EP standard is extended to encompass HPLC UV detectors and PAT (process analytical technology) applications. This is a considerable divergence – USP <857> specifically excludes HPLC detectors from its scope whereas EP 2.2.25 will be mandatory for HPLC detectors. The following notes relate to spectrophotometers.

Regarding the test materials to be used for qualification, the USP states: 'Whenever possible, certified reference materials (CRMs) are to be used in preference to laboratory-prepared solutions' and to make it easier to establish 'fitness for purpose' over a range of operating parameters now lists a greater variety of commercially available CRMs. The EP, conversely, gives details for the laboratory preparation of relatively few test solutions, one of which (caffeine) is not available commercially as a CRM. Fortunately, however, the use of Certified Reference Materials is still allowed, so compliance can still be achieved without the need to prepare solutions in the laboratory.

## Will I need new reference materials?

Most laboratories working in a regulated environment will already have a selection of references for instrument qualification. To determine if any additional references are needed to meet the new regulations the wavelength and absorbance values expected in the proposed analyses should be checked to see if they are encompassed by the available references. If not, additional references will be required. For example, holmium oxide solution is the most widely used wavelength reference, with 14 peaks covering wavelengths from 240 nm to 650 nm. Provided the wavelengths to be used for analysis lie within these limits, no additional wavelength references should be required. For wavelengths below 240 nm, however, both standards now recommend cerium oxide solution, covering 200 nm to 270 nm. For even lower wavelengths, a 'Deep UV' CRM is available from Starna Scientific (Hainault UK), with certified wavelength and absorbance values down to 191 nm. For analyses carried out above 650 nm, didymium oxide has peaks up to 870 nm and for even higher wavelengths a 'Wide Range Wavelength Reference' certified to 1945 nm is available from Starna Scientific.

For absorbance qualification, the new linearity specifications mean that more than one reference will certainly be required: the USP recommends that linearity is controlled at a minimum of three absorbance levels over the expected range. Potassium dichromate solution has been used for many years and covers wavelengths from 235 to 350 nm. For lower wavelengths, nicotinic acid is now recommended, covering 210-270 nm. Indeed, nicotinic acid is the only UV absorbance reference now cited specifically by the EP for spectrophotometer qualification. Potassium dichromate, while recommended by the USP, is no longer recommended by the EP is because it is cited in the REACH regulations. At the concentrations and quantities used for instrument qualification, however, any risk to operators is vanishingly small and is non-existent when using commercially supplied CRMs in permanently sealed cells - the form in which most laboratories already hold this reference. It therefore remains a perfectly acceptable absorbance reference but if users still wish to avoid potassium dichromate, Starna Green solution is a very stable, non-toxic and REACH compliant reference with peaks at 258, 416 and 630 nm that can be certified at absorbances up to 5 A. All three materials are commercially available as CRMs with a wide range of absorbance values and can be purchased in convenient 'linearity sets'. For the visible region, the USP now lists metal-on-quartz filters; these filters can be used over a wide wavelength range (250 to 850 nm) but are not compatible with all instruments – users should check with their instrument supplier. For the visible region, well-established neutral density (grey glass) filters are available. These cover wavelengths from 440 nm to 635 nm and absorbance values up to 3.5A

Stray light qualification is performed using cut-off filters that absorb all light at a certain wavelength. Any light then reaching the detector at that wavelength must be stray light. Different solutions cut off a different wavelengths: whereas EP 2.2.25 formerly named just one stray light reference, potassium chloride solution, both standards now list several references covering wavelengths from 190 nm to 400 nm; the measured absorbance at the recommended wavelength must exceed the stated value. Note that USP <857> now permits the use of the traditional "specified wavelength" method as well as the 'filter ratio' or Mielenz method cited in the 2015 edition. The references recommended by both standards are shown in the table below:

| COMPOUND | CONCEN -TRATION | USP | | EP | |
|---|---|---|---|---|---|
| | | Recommended wavelength | Absorb-ance | Recommended wavelength | Absorb-ance |
| Potassium Chloride | 12 g/l | 198 | ≥ 2.0 | 198 | ≥ 2.0 |
| Sodium Iodide | 10g/l | 220 | ≥ 2.0 | 220 | ≥ 3.0 |
| Potassium Iodide | 10g/l | 220 | ≥ 2.0 | 250 | ≥ 3.0 |
| Sodium Nitrite | 50g/l | 340 | ≥ 2.0 | 340 and 370 | ≥ 3.0 |
| Acetone | Spectroscopy grade | 300 | ≥ 2.0 | Not listed | |

For resolution (spectral bandwidth) qualification both standards recommend the well-established toluene-in-hexane solution.

Other than the references named in the standards, a wide range of CRMs for spectrophotometry is available from commercial suppliers:

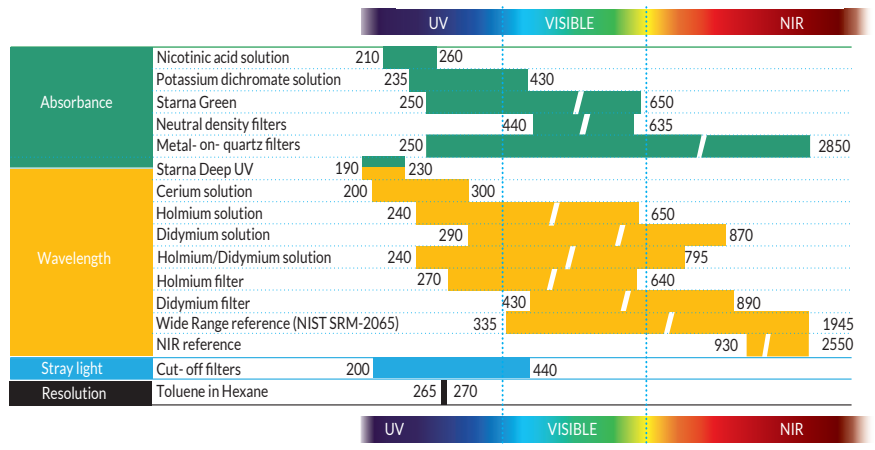


*Figure 1. Wavelength ranges of Certified Reference Materials*

## Conclusions

The new editions of USP <857> and EP 2.2.25 take a more holistic approach to the qualification of UV-visible spectrophotometers, which must now be performed under conditions that approximate as closely as possible to those to be used for analysis: the judicious selection of Certified Reference Materials will allow instrument users to comply with the new regulations.

Find out more at: **www.starna.com/uv-visible-spectroscopy**

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## Range of Reference Standards Expanded to Include FFPE and Liquid Biopsy

**Horizon Discovery Group plc** has announced it has added two new sample formats to its cell-based OncoSpan reference standards for use in the development and validation of workflows for cancer diagnostic assays.

Horizon's OncoSpan range now includes formalin-fixed, paraffin-embedded (FFPE) and cell-free DNA (cfDNA) formats for mimicking solid tumours and liquid biopsy samples, respectively, in addition to its well-established genomic DNA (gDNA) format. Its cfDNA format offers one of the largest number of variants and genes, over 380 and 152 respectively, of any characterised reference standard to help standardise liquid biopsy testing.

These cell-line derived reference standards closely mimic patient samples and offer an unlimited and reproducible resource to help ensure consistency during the establishment and validation of diagnostic assays. All three formats are delivered with batch-specific, next-generation sequencing (NGS) data, orthogonally validated by ddPCR, to allow researchers to further validate their analysis pipeline.

Paul Brooks, Head of Business Operations, Horizon Discovery said: "Horizon offers a range of premium reference standards for various molecular platforms including NGS and digital PCR. By extending our OncoSpan portfolio to include these additional sample formats, we are now able to provide more researchers working at the forefront of molecular diagnostics, with the reagents needed to suit their specific workflows. These cell-line derived standards offer significant benefits over less-commutable synthetic standards, meaning researchers can rely on our reference standards to deliver consistent results and have confidence in the performance of their diagnostic assays."

More information online: ilmt.co/PL/5a53

**For More Info, email:** 52298pr@reply-direct.com

## Expanded Production of Novel COVID-19 Reference Materials Announced


With the rapid global spread of the SARS-CoV-2 virus and the urgent need for reliable diagnostic solutions, **LGC**'s SeraCare Life Sciences is expanding production of their novel COVID-19 reference materials to include high titre stocks that serve as a valuable tool for diagnostic manufacturers.

Michael Sweatt, Executive Vice President and General Manager, LGC Diagnostics BU, stated: "As a leading provider to the in vitro diagnostic industry, we are keenly aware of the important role that reliable reference materials play in the development of accurate diagnostic tests. Offering our AccuPlex COVID-19 solution in a high titre format enables diagnostic manufacturers to accelerate development efforts through use of concentrated, lot-specific reference material to complete their assay validation activities. Our dedicated team of scientists are expediting production of these stocks, and we anticipate availability in the coming week."

AccuPlex reference materials, while mimicking wild-type pathogenic viruses, are safe, non-infectious, and replication-deficient. They serve as true, full-process, quality control solutions that challenge the entire diagnostic workflow, making them a valuable alternative to infectious materials.

More information online: ilmt.co/PL/xQ65

**For More Info, email:** 51853pr@reply-direct.com