

Traceable certified reference materials (CRMs) in far-ultraviolet spectrophotometry

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Ultraviolet/visible (UV/vis) spectrophotometry is still one of the most widely used instrumental techniques in the pharmaceutical industry: even in a post-millennium survey,¹ 43% of all respondents said they use it regularly. This article looks at the role of traceable calibration references in this technique, and how new materials were developed to extend its valid scope of application.

Why traceable CRMs?

Instrument calibration was standard practise in any good laboratory long before it was made obligatory by legislation to minimise the risk associated with "...numbers being produced by a black box". In the case of UV/vis spectrophotometry, laboratories and instrument vendors have used, and very often rejected, a variety of filters, solutions and other test materials to calibrate and check the performance of their instruments. The resultant procedures associated with the materials that have survived this rigorous examination still apply today, but one aspect that has changed significantly is the laboratory environment in which the test materials are employed. This change derives from the legislative control that is now enforced in many regulated laboratory environments.

It is well known that the Good Practice, GxP (where x = Laboratory, Manufacturing etc.) set of quality standards was born out of consumer protection. The powers of the Food and Drug Administration (FDA) in the USA to regulate drug production increased dramatically when the Thalidomide tragedy in Europe led to the New Drugs Amendments of 1962. This legislation required new drugs to

be subjected to increased safety and toxicological testing before their release. Unfortunately not all science is good science, and some laboratories performing this testing were subsequently shown to be reporting invalid results, largely because of inconsistent or incomplete record keeping. This could not be allowed to continue, so the FDA acted to regulate laboratory testing. Its GLP guidelines became law in 1976, and the GMP guidelines were published in the Federal Register on 21 July 1978. Through the auspices of the internationally recognised Organisation for Economic Co-Operation and Development (OECD) guidelines; these principles have been adopted on a global basis by the local regulatory bodies, and although there may be slight differences between its various manifestations, in every case the fundamental objective is the same: to ensure the validity of chemical measurements made in the interests of food and drug safety, health and environmental protection.

GxP, like any comprehensive quality scheme, rightly pays great attention to procedures, record keeping, audit trails and the like, in order to ensure the integrity, traceability and control of analytical data, and states that the equipment used shall be fit for purpose by stating that "adequate means shall be provided". Within this general statement is the implicit requirement that the equipment and instrumentation used to generate that data in the first place must be shown to have been calibrated against reliable reference materials, and be "under control". The old computer adage "garbage in, garbage out!" comes to mind. The objective of quantitative analysis is to

establish a concentration value, usually for comparison against a specification or another analysis made elsewhere, and such a comparison will be valid only if the uncertainty of the measurement is known. The overall uncertainty of the result will be the combination of the uncertainty of the measurement process itself *and* the uncertainties of any reference values used in the system, which obviously include any references used for instrument calibration. So how can the uncertainty level of the calibration references be established? It is now accepted that this can only be done if the calibration materials themselves can be compared with internationally agreed references. Those references may not be immediately accessible (there is only *one* International Kilogram, kept at the "Bureau International des Poids et Mesures" at Sèvres near Paris, France!) or if derived from physical constants, like atomic spectra, may be impractical to use in the average analytical laboratory. However, it is usually possible to establish working references that are "traceable" to those primary materials.

The International Organisation for Standardisation, ISO, defines traceability as:

"Traceability: Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties".²

In practical terms, this means that any reference materials used to calibrate our spectrophotometer must themselves have calibration values that can be traced to

internationally accepted references established by one of the National Metrology Institutes (NMIs). Examples of NMIs are the National Institute of Standards and Technology (NIST) in the USA or the National Physical Laboratory (NPL) in the United Kingdom. Although these organisations sell Standard Reference Materials (SRMs) on a commercial basis, or offer calibration services, themselves traceable to the primary standards, they are too expensive and too valuable to be used routinely. Fortunately, the uncertainty values quoted on these materials are sufficiently small to allow the introduction of another level of Certified Reference Materials (CRMs). By definition, these "secondary" materials will have a slightly greater uncertainty level brought about by the calibration against the NMI supplied "primary", but the traceability chain is maintained. Therefore, most laboratories

use these "secondary" CRMs for which the suppliers provide certification-proving traceability.

When is a CRM not an appropriate reference?

Good spectroscopic practice recommends that where possible, both the wavelength and absorbance scales should be validated by appropriate references. CRMs for use in the visible region (370–680 nm) have been available for over 30 years, and include neutral density filters for absorbance and a variety of glass or silica filters incorporating rare earth oxides for wavelength. If measurements are to be performed in the UV region, it is inadvisable to use references certified in the visible, as instrumental characteristics may vary significantly between the two regions. Ideally the reference materials should "bracket"

the required wavelength or absorbance range, i.e. have certified values above and below the levels to be used for analysis. Indeed this philosophy should be applied to *any* calibration process if at all possible! In recent years, the availability of reference materials in solution, sealed in far-UV quartz cells, has extended the lower limit of the usable wavelength range down to approximately 240 nm. However, the pharmaceutical and bioscience industries routinely require analysis in the 200–280 nm region. By definition, these measurements cannot be validated using reference materials certified for higher wavelengths, creating a demand for new CRMs.

New CRMs—the certification process

If one looks at the general characteristics required of a certified reference mate-

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rial for spectrophotometry, they could be summarised in five statements.

They should:

- Be readily available, safe and easy to handle.
- Suit the optical properties of the instrument, e.g. spectral bandwidth.
- Be unaffected by environmental conditions, e.g. have a small temperature coefficient, good chemical stability etc.
- "A reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability".³
- Be calibrated in a quality environment, endorsed by an independent third party.

The first three points may influence the choice of material, but it is the last two that set quality CRMs apart. Clearly, only suppliers whose calibration procedures are themselves fully controlled and viable under intense screening, will be able to demonstrate traceability and certify parameter and uncertainty values reliably.

The internationally recognised standard covering the accreditation of calibration and test laboratories is ISO/IEC 17025. This standard evaluates the actual technical competence of the laboratory, unlike ISO 9001, which only relates to the laboratory's quality management system. In sourcing CRMs, it would therefore be a prudent risk management strategy to only obtain such materials from suppliers holding this type of accreditation. Accreditation to ISO/IEC 17025 is awarded by national accreditation bodies like the United Kingdom Accreditation Service (UKAS) in the United Kingdom or the American Association for Lab Accreditation (A2LA) and National Voluntary Laboratory Accreditation Program (NVLAP) in the USA. Most of these bodies are members of ILAC, the International Laboratory Accreditation Co-operation, formalised as a co-operation in 1996 when 44 national bodies signed a Memorandum of Understanding (MOU) in Amsterdam. This has facilitated



Figure 1. Sealed certified wavelength reference.

the setting up of Mutual Recognition Agreements, international agreements whereby one country recognises another's accredited laboratories as if they themselves had awarded the accreditation. This means that reference materials certified by a ISO/IEC 17025 accredited laboratory in one country will be acceptable as part of process validation in another.

New CRMs for the far-UV region (200–280 nm)

For the suppliers of these new CRMs,⁴ it was necessary to obtain an extension of their accreditation schedule from the national ISO/IEC 17025 accreditation body to cover the new materials, in their case the United Kingdom Accreditation Service (UKAS).

The first new CRM is a wavelength reference, produced by permanently sealing by heat fusion, an acidic rare earth solution into a far-UV grade quartz cell (Figure 1). The use of rare earth oxides solvated in acid is an established and well-recognised method for the validation of the wavelength scale of a spectrophotometer in the UV and visible regions. Holmium oxide is perhaps the best-known example. This new reference simply extends the usability of this elemental group into the required spectral region, having useful peaks at 201, 212, 223, 240 and 254 nm (nominal values), see Figure 2.

The second is a series of sealed cells containing solutions of nicotinic acid solvated in dilute hydrochloric acid. The use of nicotinic acid is a well-documented method⁵ for the validation of the absorbance scale and linearity of a spectrophotometer in the far-UV region. When

prepared in 0.1 M hydrochloric acid, nicotinic acid gives a spectral scan containing characteristic peaks at approximately 210 nm and 260 nm. Within the concentration range 5–25 mg L⁻¹ the measured absorbances of a series of concentrations will be a linear function of concentration (provided the spectrophotometer's absorbance scale is itself linear at the relevant values and the instrument has a spectral bandwidth less than 2 nm), see Figure 3.

In both cases, permanently sealing the solution into a quartz cell provides a readily available, easy-to-use, safe material. These products are designed for use with commercial UV/vis spectrophotometers, and the accompanying certificates detail the spectral bandwidth etc. at which the measured values were obtained.

The certified values for the new materials are obtained using a high quality spectrophotometer, but in order to demonstrate an unbroken traceability chain, it is necessary to relate those measured results either to pre-existing CRMs or to fundamental physical references.

In the case of the wavelength standard, there were two sets of references against which the wavelength scale of the test instrument could be qualified. There was an overlap between part of the required wavelength range and certified values obtained using holmium oxide solution. In this case traceability links to the primary reference were established using NIST SRM 2034 (holmium oxide in perchloric acid).⁵ Where no overlap existed, mercury arc emission lines could be used. For the absorbance standard, having qualified the wavelength scale, physical attenuation using the "Double Aperture" technique⁶ was used to qualify the absorbance scale of the reference spectrophotometer. Once the instrument itself was thus comparable to traceable reference materials, it could be used to measure the values and uncertainties certified for the new standards.

Conclusions

Establishing reliable calibration references for analytical instruments that will function as required, and stand up to a rigorous quality audit is a complex procedure

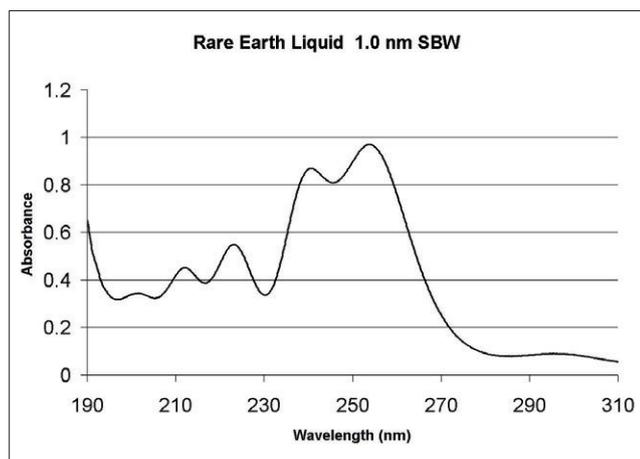


Figure 2. Wavelength scan of certified far-UV wavelength reference, recorded at 1 nm spectral bandwidth.

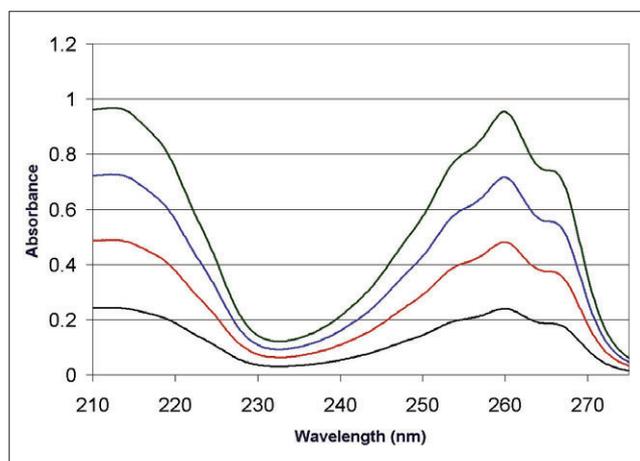


Figure 3. Wavelength scans of nicotinic acid absorbance references.

that may be impractical for laboratories to undertake themselves. In a situation where use of such an in-house reference has resulted in an OOS (Out of Specification) result, where does the problem reside—with the reference or the instrument, or both? By purchasing CRMs produced in an ISO/IEC 17025 environment by accredited suppliers, laboratories can largely eliminate the risks associated with this aspect of their quality management process and avoid the conundrum stated above.

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