Spectrophotometry: How to ensure essential measurements can be made precisely and accurately

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In 2001, the Starna laboratory in Hainault (UK) became the first to be accredited to ISO/IEC standard 17025 for the calibration of Certified Reference Materials (CRMs) for UV-visible spectrophotometry. For the first time, users of commercially produced reference materials could be confident that their measurements were not only accurate but would be universally accepted as such.

The requirement for instrument qualification had its origins in the 1970s, when the FDA discovered serious procedural failures at two major testing laboratories in the USA, ranging from poor record keeping to poor staff training and even deliberate falsification of test results. A major scandal erupted, and the episode was described as "the most massive scientific scandal in the history of this country and perhaps the world." in January 1976, Senator Edward Kennedy told a United States Senate hearing that "Accurate science is the best protection the American people have from an unsafe and ineffective drug supply. Inaccurate science, sloppy science, fraudulent science - these are the greatest threats to the health and safety of the American people." Prosecutions and imprisonments followed, but the most long-lasting legacy of the affair was the introduction of Good Laboratory Practice (GLP) guidelines covering such topics as control of test substances, facilities and equipment, reporting of results, storage and retrieval of data, and compliance. Official GLP regulations were released in December 1978 and compliance with them became US law in 1979. They were also adopted in Organization for Economic Corporation and Development (OECD) guidelines in 1981. Initially followed mainly in pharmaceutical and life sciences laboratories, GLP has expanded into most areas of regulated laboratory analysis: chemicals, biocides and pesticides, food and animal feeds, cosmetics, detergents and the environmental sector.

There was now a legal requirement for laboratories to prove that their equipment was working properly and capable of producing results of the required quality, and a need for that proof to be accepted internationally. Until the 1970s, most laboratories had used home-made test solutions or relied on the manufacturer to calibrate their instruments as part of routine maintenance. The advent of GLP led to a huge increase in the demand for reference materials for routine instrument qualification. At the time the only available references with internationally recognised and certified calibration values were those from National Measurement Institutes (NMIs) such as the National Institute of Standards and Technology (NIST) in the United States, whose products were trademarked as Standard Reference Materials (SRMs), but these were expensive and production capacity was limited. Since its inception, Starna Ltd had been producing spectrophotometry reference materials with calibration values traceable to primary standards or SRMs. NIST invited Starna to be a partner on the NTRM (NIST Traceable Reference Material) programme, with a view to harnessing the skills of commercial producers to the production of internationally recognised reference materials to satisfy the demand.

To achieve international recognition, the laboratories calibrating the references would need to be accredited to an international standard. ISO Guide 25 was first released in 1978 and covered "General requirements for the competence of calibration and testing laboratories". Later, the need to achieve compatibility with ISO 9001 led to a revision of the standard to ISO/IEC 17025, issued in 1999. Incidentally, while ISO 9001 and ISO 17025 are compatible, they are not interchangeable. While laboratories accredited to ISO/IEC 17025 will also satisfy the requirements of ISO 9001, the reverse is not true: conformity to ISO 9001 does not demonstrate the technical competence of the laboratory to produce valid calibrations. Accreditation to ISO/IEC 17025 is a complex and detailed process: Starna achieved this in 2001 and in so doing became the first commercial supplier accredited to calibrate liquid and glass reference materials for UV-visible spectrophotometry. Materials calibrated in an ISO/IEC 17025 accredited laboratory and meeting other requirements for stability and homogeneity can be described as "Certified Reference Materials" or CRMs, and measurements using instruments qualified with these materials will be universally accepted. A CRM is defined by ISO as a "Reference Material, characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability." All Starna CRMs are supplied with a certificate showing the traceability of the certified value to primary references or SRMs and a statement of the measurement uncertainty, as required by the ISO definition of a CRM.

Traceability is a vital aspect of the calibration process and is defined in ISO Guide 99:2007 as the "property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty". This means that the instrument used to generate the calibration values should itself be qualified using NIST SRMs. The Starna calibration laboratory uses a number of reference spectrophotometers, all qualified against NIST SRMs or primary physical references.

At the beginning of the NTRM programme there were only a few SRMs available for spectrophotometry. For wavelength, for example, only two: holmium oxide solution for the near UV and visible and a rare-earth doped glass for the near infra-red. One of the objectives of the programme was to enable commercial producers to develop CRMs to extend this range and fill the gaps. Over the years, the range of Starna wavelength CRMs has extended into the deep UV and the infra-red:

Reference material	Usable range (nm)
Starna Deep UV (DUV)	190 – 230
Cerium oxide	200 - 270
Samarium perchlorate	230 - 500
Holmium oxide	240 - 650
Combined Holmium/Didymium oxides	240 - 795
Didymium oxide	290 - 870
Starna Near IR reference	930 -2550
Polystyrene	3.2 µm - 18.5 µm

Indeed, availability of an increasing range of commercially produced CRMs has allowed NIST to discontinue production of some of their materials. The other instrument parameters requiring qualification are absorbance accuracy, resolution (bandwidth) and stray light. Starna now offers the world's most comprehensive range of CRMs to cover these parameters.

Calibration is an essential step in the production of CRMs, however it is not the complete process. Consequently, in 1999, ISO Guide 34 'General requirements for the competence of Reference Material Producers' was published. This covers the wider aspects of production such as management systems for quality, administrative and technical operations. In 2004, the International Laboratory Accreditation Cooperation (ILAC) resolved that reference material producers should be accredited to ISO Guide 34 in combination with ISO/IEC 17025. In 2006, Starna became the first, and only, supplier of spectrophotometry references accredited to both. ISO Guide 34 was revised to become ISO 17034:2016 in 2016, with Starna's accreditation being updated to the new standard.

A particularly important aspect of these accreditations is their Scope, which defines the range of materials that can be produced as CRMs and the measurements that the calibration laboratory is entitled to perform. A laboratory could claim to be "accredited to ISO/IEC 17025" on the strength of just one measurement, so users should satisfy themselves that a proposed supplier's Schedule of Accreditation includes the references they intend to purchase. Starna Scientific's accreditations have the widest scope of any producer of spectrophotometry CRMs.

As mentioned above, the pharmaceutical industry was one of the first to adopt GLP, and national and international pharmacopoeias now publish detailed requirements for the qualification of instruments used for analysis. Originally these contained a few generic tests, based on which an instrument could be claimed to be 'pharmacopoeia compliant'. It is now recommended that instrument qualification measurements represent more closely those to be made in the actual analysis. A laboratory performing a wide range of analyses may therefore require several different CRMs to cover the range of the parameters used. Starna Scientific offers the world's widest range of spectrophotometry CRMs, as well as for spectrofluorometry. The pharmacopoeias do contain instructions for the preparation of reference solutions, but these may involve solvation or liquid transfer operations, with their attendant risks. Starna liquid references are permanently flame-sealed into their cells, eliminating many such risks, and allowing Starna to offer a lifetime guarantee on all their CRMs, subject only to periodic recertification. USP Chapter <857> states: "Wherever possible… certified reference materials (CRMs) are to be used in preference to laboratory-prepared solutions."

Starna Scientific has been a key player in the development and propagation of reliable reference materials for spectrophotometry and 20 years ago was the first supplier to be accredited to the coveted ISO/IEC 17025 standard. The company was also the first, and is still currently the only supplier of these materials accredited to both ISO 17034 and ISO/IEC 17025 Starna is also committed to the process of continuous improvement demanded by those standards to ensure essential measurements can be made precisely and accurately by users.