Chapter 5

Additional Topic: 'Internal Standardisation'

It is helpful at this stage to introduce the process of 'internal standardisation' in our analytical approach and to consider its use for correction of data. An 'internal standard' [IS] is an independent material that can be added to the sample at a suitable stage in the sample preparation step, for measurement along with that of the analyte of interest. A known quantity of the IS is always added and it should match the analyte in terms of its required physical and chemical character.
Therefore an IS may be considered an 'element or compound that is not originally present in the sample at a significant concentration, that has the same required physical and chemical character and undergoes the same physical and chemical processes as the analyte'. It should be added in a known quantity to the sample or prepared sample which can then be measured along with the analyte - preferably at the same time. The same IS should be incorporated into each calibrant, at the same or a known quantity, where required and measured together with each calibrant's quantity of analyte.

Three areas can be identified (broadly) within the analytical approach, where this process is often critical to the quantitation step. These are:

i) to allow for differences between a calibrant's matrix and a sample's matrix during the measurement step; ii) to allow for changes in instrument performance during the measurement step and iii) to allow for changes in an analyte's quantity at various stages during the sample preparation step; e.g. extraction or transfer etc.

1. In order to compensate (and hence, correct) for certain physical differences between a prepared sample and the calibrants used, particularly where matrix matching may be difficult, it is preferable to incorporate an 'internal standard' (IS). This IS is measured and monitored alongside the analyte(s) throughout the analysis. In an ideal stable system, the instrumental response to this IS should always be the same. However, small differences between the calibrants and the prepared sample(s) can affect the quantity of analyte introduced to the instrument and its detector (matrix effects). The value from the IS can be used to correct for most, if not all of the above phenomena by ratio-ing the response from the analyte(s) to that of the IS, for every measurement made.
   An example to demonstrate this effect is "where the solution from an acid-digested fish-tissue sample, will have a high concentration of both acid and dissolved solid in it. The final solution measured will therefore have a very different viscosity and surface tension to that of the calibrant standards made up in only a simple 2% acid matrix. An internal standard helps correct for the different transport efficiency of the sample solution, from the nebuliser of a typical ICP instrument to its plasma."
2. Also, an instrument's performance, for various reasons, can vary with time due to internal and environmental conditions. This 'drift' effect may be considered a measure of an instrument's stability during the entire period of measurement. The value from the IS can be used to correct for most, if not all of the above phenomena by ratio-ing the response from the analye(s) to that of the IS, for every measurement made.
   An example to demonstrate this effect is "where you may observe over the timescale of the measurement step, the long term drift in the signal from the calibrants' analyte

and IS from an instrument, associated with a change in temperature within a laboratory".

3. Internal standardisation is also used as a 'procedural tool' during sample preparation in order to correct for possible transfer process effects (volume or mass-based transfer) and extraction efficiency effects. A known quantity of the matching IS is added to the sample at the beginning of the preparation step. Any process that the sample undergoes, such as extraction of analyte, dilution of extracted analyte, volume or mass transfer of the diluted analyte etc. will have a measurable quantity of IS within it. As one knows the original quantity of IS added and can measure the final quantity of IS in the prepared sample, this gives a 'scaling factor' which can be used to adjust the quantity of analyte measured in the processed sample back to the original sample. It is important to note that identical physical and chemical properties (for the series of processes undertaken) is expected between the IS and analyte of interest, for this 'scaling factor' to be valid. Various names are associated with this scaling factor, such as 'recovery factor' and 'extraction efficiency' etc. but these terms should be used with care.
   An example to demonstrate this effect is "where an internal standard will help correct (if only in part) for incomplete 'extraction' of an analyte from a solid sample, e.g. during a Soxhlet extraction. The known quantity of internal standard should be allowed to "soak in" to the known mass of solid sample and for a sufficient time to become equilibrated, and then dried prior to extraction". The above example also demonstrates the potential ambiguity associated with the terms "extraction efficiency" and "recovery". In the former, it refers to the assumption that the added IS has the same extraction efficiency factor as the analyte from the solid and in the latter, as having a measure of recovery of the IS from the solid.

   Of course, if the internal standard is added to the solution 'after' the extraction process, then this IS can be used as a "transfer efficiency" scaling factor. This factor would include the effect from all further processes performed on the extracted sample during the sample preparation step, right up to its measurement together with the analyte of interest.