Technical Note -

Repeatability and standardisation explained

Have you got samples you know are outside your usual sample set? Do you have two NIR machines that are giving you results with some variation between them? Do you want to know how you can adjust your calibration to include everything and carry on working? Keep reading…

Applying a repeatability file

A good database for NIR modelling must contain data obtained from all the varying conditions that you would expect to encounter during routine analysis. But this is not always possible for a number of reasons including time restrictions, lack of space for sample storage or geographical limitations. Other sources of variation come from carrying out analyses on different NIR machines and the differences in the temperature of both the sample and the instrument at the time the analysis is carried out. A way to reduce the impact of this lack of sample information is to use a repeatability file.

A repeatability (rep) file contains spectra of one or more samples scanned using different instruments and in different conditions. The goal of including a rep file in a calibration is to develop an equation that gives the same predicted value across all conditions represented in the scans; the equation is developed so that it is not sensitive to the rep file's spectral variation. A rep file is an ingenious way of introducing variability to the calibrations without actually having any reference values for the new samples. Also, there is no need to worry about having enough samples to influence a large calibration set.

How is a rep file used in the calibration process?

In normal regression, the predicted value for the average spectrum is the average reference value. With a rep file, we want the average spectrum plus and minus the differences in the rep file spectra (i.e. standard deviation in the rep file) to still predict close to the average reference value.

This is done by centring and scaling each rep file (i.e. a series of spectra associated to a single sample scanned under different conditions) separately and assigning 0 as their reference values. The next step is to add these to the centred calibration

dataset followed by the usual regression. This process ensures that the regression coefficients calculated will minimise the prediction errors under different conditions.

Calibrations developed using rep files are usually slightly less accurate but offer much more reliable results when there are differences between instruments and assay conditions.

The standardisation process

The reason for standardizing an instrument is to make its spectra match those of a master instrument. This is a particularly useful tool when a calibration built with data from a grating instrument is transferred to other similar instruments.

This transformation is very important for getting the best performance in the secondary instrument, but it has some limitations. The standardisation process is only good at the time it was carried out but not after any changes have been made to the instrument, for example lamp repairs. In this scenario, another standardisation process must be carried out.

For calibrations that are designed to function across a number of NIR instruments and conditions, the most robust and reliable method to ensure accurate predictions is to employ rep files and standardisation.

References:

Westerhaus, M.O. "Improving repeatability of NIR calibrations across instruments." *Proceedings of the Third International Conference on Near Infrared Spectroscopy,* 1990.

Westerhaus, M.O. "Eastern Analytical Symposium Award for outstanding achievements in Near Infrared Spectroscopy: my contributions to Near Infrared Spectroscopy." (2014). NIR News, Vol 25 No 8. pg 16-20.