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Application of NIR
(Near InfraRed spectroscopy)
in food and feed analysis

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Preface

Near Infrared spectroscopy, often abbreviated as NIR, NIRS, NIT (for transmission mode), has been in use for food analysis since the 1960-ies. NMKL recognized the need for guidance and standardisation in the use of NIR for food (and feed) analaysis. In connection with a seminar on non-destructive analytical methods arranged in Copenhagen in 2013, the project group elaborated the main content of this Technical Report. The project group after the Copenhagen meeting consisted of:

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This Technical Report is based on the ISO 12099:2010: Animal feeding stuffs, cereals and milled cereal products -- Guidelines for the application of near infrared spectrometry. For more and detailed information, we refer to this ISO standard [1].

Contents

1. General considerations

Near Infrared spectroscopy; NIR, NIRS and NIT (for transmission mode), is a fast and nondestructive technique and has been used in food and feed analysis since the 1960-ies when Karl Norris from the U.S. Department of Agriculture recognized the potential of this analytical technique and introduced “modern NIRS” into industrial practice [2].

The method accelerated in the early 1970-ies with “large scale” analysis of moisture and protein in cereals when filter NIR-instruments with multiple linear regression calibrations became commercially available. Since that time, NIR has become one of the standard choices in feed and food analysis.

It is important to remember that NIR, is considered to be a secondary method since the detected signal(s) must connect to the content of chemically defined constituents by a mathematical model. The success of NIR is thus largely the availability of inexpensive very powerful computers and chemometric software that has been developed during the last 50 years.

1.1 Purpose and scope

The purpose of this technical report is to give a simple introduction to the use of NIR in food and feed analysis, and some criteria that should to be fulfilled in order to do the analysis in a quality assured way.

Within the scope lays various combinations of instrument designs and chemometric methods for calibration used for analysis of nutritional components, such as moisture, protein, fat and carbohydrates (low-molecular as well as polysaccharides), all generally occurring in the analysed material at mass fractions above 0,001.

2. Principle

The NIR technique is a spectroscopic method using the wavelength range of 780-2500 nm. Diffuse monochromatic light is reflecting to a detector, due to reflection, refraction and diffraction on particles of the sample. The spectrum is a result of specific light absorption from C-H, O-H, N-H, C=O overtones and combination bands at different wavelengths [3]. The spectra then have to be combined with (wet) chemical data via a mathematical model within a process called calibration with the aim to predict constituents from the NIR spectra.

2.1 Apparatus

Most common today is NIR instruments designed for specific applications equipped with grating monochromator or filters, a light source as a traditional lamp, LED or lasers, in addition interferometric (Fourier Transform) instruments.

2.2 Sample preparation

Diffuse reflection is influenced by particle size and –distribution. This mean that sample preparation is crucial, i.e. that grinding of dry materials has to be standardised and considered, both during sample measurement and during calibration.

3 Calibration and validation

3.1 General

The procedure of calibration is a vital part of NIR analysis of food and feed. There are several mathematical methods for calibration. Most used are:

Multiple linear regression (MLR) using a few selected wavelengths,
Principal component regression (PCR) using full spectrum or parts of the spectrum,
Partial least squares regression (PLSR) using full spectrum or parts of the spectrum,
Neural networks (NN) a nonlinear multivariate method.

It is not possible to decide one and only method to be applied for all combinations of instruments, commodities and analytes, and thus the choice of calibration methods will not be covered by this NMKL-procedure. Among others, Naes et al [4], have published literature on calibration of NIR. Also, NIR instruments are normally accompanied by advanced software for calibration that is suitable for the respective instrument. These are necessary to get familiar with when working with the instruments.

Initial validation is an integer part of the calibration process. This could be performed by the use of an independent validation set and/or by cross-validation. This is a procedure where a fraction, for example 10 per cent of the calibration data set is held outside calibration and used for validation. The procedure is repeated sequentially until all samples have been included in the calibration set.

A good prediction model (calibration) covers all (NIR-) spectroscopic variation and all variation in the desired chemical constituents. It is thus necessary that the dataset (calibration set) contains all variation that is practically possible to collect. Obviously, this is valid for both spectroscopic (X) and chemical/reference data. Since NIR-spectra contains much information that is not directly coupled to the chemical constituents of interest (i.e. Y-data) it is important to pay attention to factors such as particle size, sample preparation, seasonal variation, growing conditions, processing, storage conditions, temperature (sample and instrument), difference between individual instruments, etc.

3.2 Reference methods

Since NIR used in food and feed analysis in general is a secondary method it is vital that the reference (chemical) method is good and under proper statistical control. The NIR analysis can never be shown to be more accurate than the reference method. It has, however been showed that the precision in NIR analysis can be higher than for the reference method [5]. It should be noted that for empirically (method) defined analytes such as protein, fat, oil, fibre, starch etc. it is crucial to choose the reference method with care and consideration of scope of measurement. It is highly recommended to use internationally recognised methods such as ISO, EN or EU methods in all cases in order to be able to get results with validity in the respective area of the application.

3.3 Outliers

In many situations, statistical outliers are observed during calibration and validation. Outliers may be related to NIR data (x-outliers), or errors in reference data or samples with different relationship between reference data and NIR data (y-outliers).

For the purpose of validation; samples are not regarded as outliers if:

- they are within the working range of the constituents/parameters in the calibration(s), or
- they are within spectral variation of the calibration samples, e.g. as estimated by Mahalanobis distance, or
- the spectral residual is below a limit defined by the calibration process, or
- the prediction residual is below a limit defined by the calibration process.

If a sample appears as an outlier, it should be checked initially to see if it is an x-outlier. If it exceeds the x-outlier limits defined for the calibration, it should be removed. If it is not an x-outlier, both the reference value and the NIR predicted value should be checked. If these confirm the original values, the sample should not be deleted, and the validation statistic should include this sample. If the repeat values show that either the original reference values or the NIR predicted ones were erroneous, then the new values should be used.

3.4.1 General

Before use, calibration equations must be validated locally on an independent test set that is representative of the population to be analysed. For the determination of bias, at least 10 samples are needed and for the determination of standard error of prediction (SEP) at least 20 samples are needed. Validation should be carried out for each sample type, constituent or parameter, and temperature. The calibration is valid only for the variations, i.e. sample types, range and temperature, used in the validation.

Results obtained on the independent test set are plotted, reference against NIR, and residuals against reference results, to give a visual impression of the performance of the calibration. The SEP is calculated and the residual plot of data corrected for mean systematic error (bias) is examined for outliers, i.e. samples with a residual exceeding +/- 3s SEP

If the validation process shows that the model cannot produce acceptable statistics, then it should not be used.

NOTE. What is acceptable depends on such criteria as the performance of the reference method, the range covered, and the purpose of the analysis and is up to the parties involved to decide.

The next step is to fit NIR data, y NIR and reference data, y ref by linear regression (y ref =byNIR + a) to produce statistics that describe the validation results.

3.4.2 Bias correction

If the difference between means of the NIR predicted and the reference values is significantly different from zero then this indicates that the calibration is biased. A bias may be removed by adjusting the constant term.

3.4.3 Slope adjustment

If the slope, b, is significantly different from 1, the calibration is skewed.

Adjusting the slope or intercept of the calibration is generally not recommended unless the calibration is applied to new types of samples or instruments. If a reinvestigation of the calibration does not detect outliers, especially outliers with high leverage, it is preferable to expand the calibration set to include more samples. However, if the slope is adjusted, the calibration should then be tested on new independent test set.

3.4.4 Expansion of calibration set

If the accuracy of the calibration does not meet expectations, the calibration set should be expanded to include more samples or a new calibration is performed. In all cases, when a new calibration is developed on an expanded calibration set, the validation process should be repeated on a new validation set. If necessary, expansion of the calibration set should be repeated until acceptable results are obtained on a validation set.

4 Change in measuring and instrument conditions

Unless additional calibration is performed, a local validation of an NIR method stating the accuracy of the method can generally not be considered valid if the test conditions are changed.

For example, calibrations developed for certain population of samples may not be valid for samples outside this population, although the analyse concentration range is unchanged. This is due to the fact that NIR-spectra (X-data), contain a lot of information not connected to the Y-data, as discussed above (section 3.1). Thus, a calibration developed on grass silage from one area may not give the same accuracy on silages from *another* area if the genetic, growing and processing parameters are different. For animal products, a calibration made on fresh samples, may not have the same accuracy for frozen /thawed samples.

Changes in the sample presentation technique measuring conditions (e.g. temperature) not included in the calibration set may also influence the analytical results,

Calibrations developed on a certain instrument cannot always be transferred to an identical instrument operation under the same principle. If mat be necessary to perform bias, slope or intercept adjustments to calibration equations. In many cases it is necessary to standardize the two instruments against each other before calibration equations can be transferred [4]. Standardization procedures can be used to transfer calibrations between instruments of different types provided that samples are measured in the same way (reflectance, transmittance) and that the spectral region is common. Many commercial software packages bundled to instruments include proprietary calibration transfer procedures.

If the conditions are changed, a supplementary validation should be performed,

The calibration should be checked whenever any major part of the instrument (optical system, detector) has been changed or repaired.

5 References

1. ISO 12099:2010 Animal feeding stuffs, cereals and milled cereal products -- Guidelines for the application of near infrared spectrometry

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