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Animal feeding stuffs, cereals and milled cereal products — Guidelines for the application of near infrared spectrometry

Aliments des animaux, céréales et produits de mouture des céréales — Lignes directrices pour l'application de la spectrométrie dans le proche infrarouge

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 12099 was prepared by the European Committee for Standardization (CEN) Technical committee TC 327, *Animal feeding stuffs — Methods of sampling and analysis*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna agreement).

Introduction

This International Standard has been drafted using, as a basis, ISO 21543 | IDF 201[15], prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF).

Animal feeding stuffs, cereals and milled cereal products — Guidelines for the application of near infrared spectrometry

1 Scope

This International Standard gives guidelines for the determination by near infrared spectroscopy of constituents such as moisture, fat, protein, starch, and crude fibre as well as parameters such as digestibility in animal feeding stuffs, cereals and milled cereal products.

The determinations are based on spectrometric measurement in the near infrared spectral region.

2 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

2.1
near infrared instrument
NIR instrument
apparatus which, when used under specified conditions, predicts **constituent contents** (2.3) and **technological parameters** (2.4) in a matrix through relationships to absorptions in the near infrared range

NOTE In the context of this International Standard, the matrices are animal feeding stuffs, cereals and milled cereal products.

2.2
animal feeding stuff
any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals

EXAMPLES Raw materials, fodder, animal flour, mixed feed and other end products, and pet food.

2.3
constituent content
mass fraction of substances determined using the appropriate, standardized or validated chemical method

NOTE 1 The mass fraction is often expressed as a percentage.

NOTE 2 Examples of constituents determined include moisture, fat, protein, crude fibre, neutral detergent fibre, and acid detergent fibre. For appropriate methods, see, e.g., References [1] to [16].

2.4
technological parameter
property or functionality of a matrix that can be determined using the appropriate standardized or validated method(s)

EXAMPLE Digestibility.

NOTE 1 In the context of this International Standard, the matrices are animal feeding stuffs, cereals and milled cereal products.

NOTE 2 It is possible to develop and validate NIR methods for other parameters and matrices than listed, as long as the procedure from this International Standard is observed. The measuring units of the parameters determined have to follow the units used in the reference methods.

3 Principle

Spectral data in the near infrared (NIR) region are collected and transformed to constituent or parameter concentrations by calibration models developed on representative samples of the products concerned.

4 Apparatus

4.1 Near-infrared instruments, based on diffuse reflectance or transmittance measurement covering the NIR wavelength region, 770 nm to 2 500 nm (12 900 cm^{-1} to 4 000 cm^{-1}), or segments of this or at selected wavelengths or wavenumbers. The optical principle may be dispersive (e.g. grating monochromators), interferometric or non-thermal (e.g. light-emitting diodes, laser diodes, and lasers). The instrument should be provided with a diagnostic test system for testing photometric noise and reproducibility, wavelength or wavenumber accuracy and wavelength or wavenumber precision (for scanning spectrophotometers).

The instrument should measure a sufficiently large sample volume or surface to eliminate any significant influence of inhomogeneity derived from chemical composition or physical properties of the test sample. The sample pathlength (sample thickness) in transmittance measurements should be optimized according to the manufacturer's recommendation with respect to signal intensity for obtaining linearity and maximum signal/noise ratio. In reflectance measurements, a quartz window or other appropriate material to eliminate drying effects should preferably cover the interacting sample surface layer.

4.2 Appropriate milling or grinding device, for preparing the sample (if needed).

NOTE Changes in grinding or milling conditions can influence NIR measurements.

5 Calibration and initial validation

5.1 General

The instrument has to be calibrated before use. Because a number of different calibration systems can be applied with NIR instruments, no specific procedure can be given for calibration.

For an explanation of methods for calibration development see, for example, Reference [17] and appropriate manufacturers' manuals. For the validation, it is important to have a sufficient number of representative samples, covering variations such as:

a) combinations and composition ranges of major and minor sample components;

b) seasonal, geographic and genetic effects on forages, feed raw materials and cereals;

c) processing techniques and conditions;

d) storage conditions;

e) sample and instrument temperature;

f) instrument variations (differences between instruments).

NOTE For a solid validation at least 20 samples are needed.

5.2 Reference methods

Internationally accepted reference methods for determination of moisture, fat, protein, and other constituents and parameters should be used. See References [1] to [16] for examples.

The reference method used for calibration should be in statistical control, i.e. for any sample, the variability should consist of random variations of a reproducible system. It is essential to know the precision of the reference method.

5.3 Outliers

In many situations, statistical outliers are observed during calibration and validation. Outliers may be related to NIR data (spectral outliers, hereafter referred to as x-outliers) or errors in reference data or samples with a different relationship between reference data and NIR data (hereafter referred to as y-outliers) (see Figures B.1 to B.5).

For the purpose of validation, samples are not to be regarded as outliers if:

a) they are within the working range of the constituents/parameters in the calibration(s);

b) they are within the spectral variation of the calibration samples, e.g. as estimated by Mahalanobis distance;

c) the spectral residual is below a limit defined by the calibration process;

d) the prediction residual is below a limit defined by the calibration process.

If a sample appears as an outlier then 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, then both the reference value and the NIR predicted value should be checked. If these confirm the original values then the sample should not be deleted and the validation statistics should include this sample. If the repeat values show that either the original reference values or the NIR predicted ones were in error then the new values should be used.

5.4 Validation of calibration models

5.4.1 General

Before use, calibration equations shall be validated locally on an independent test set that is representative of the sample population to be analysed. For the determination of bias, at least 10 samples are needed; for the determination of standard error of prediction (SEP, see 6.5) at least 20 samples are needed. Validation shall 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 (see 6.5) and the residual plot of data corrected for mean systematic error (bias) is examined for outliers, i.e. samples with a residual exceeding $\pm 3s_{\text{SEP}}$.

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 ($y_{\text{ref}} = by_{\text{NIR}} + a$) to produce statistics that describe the validation results.

5.4.2 Bias correction

The data are also examined for bias between the methods. If the difference between means of the NIR predicted and 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 (see 6.3) in the calibration equation.

5.4.3 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 a new independent test set.

5.4.4 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 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.

5.5 Changes 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 a certain population of samples may not be valid for samples outside this population, although the analyte concentration range is unchanged. A calibration developed on grass silages from one area may not give the same accuracy on silages from another area if the genetic, growing and processing parameters are different.

Changes in the sample presentation technique or the 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 directly to an identical instrument operating under the same principle. It may 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 (Reference [17]). 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.

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

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

6 Statistics for performance measurement

6.1 General

The performances of a prediction model shall be determined by a set of validation samples. This set consists of samples which are independent of the calibration set. In a plant, it is new batches; in agriculture, it is a new crop or a new experiment location.

This set of samples shall be carefully analysed following the reference methods. Care is essential in analysing validation samples and the precision of these results is more important for the validation set than for the samples used at the calibration phase.

The number of validation samples shall be at least 20 to compute the statistics with some confidence.

6.2 Plot the results

It is important to visualize the results in plots, i.e. reference vs predicted values or residuals vs predicted values.

The residuals are defined as:

$$e_i = y_i - \hat{y}_i \tag{1}$$

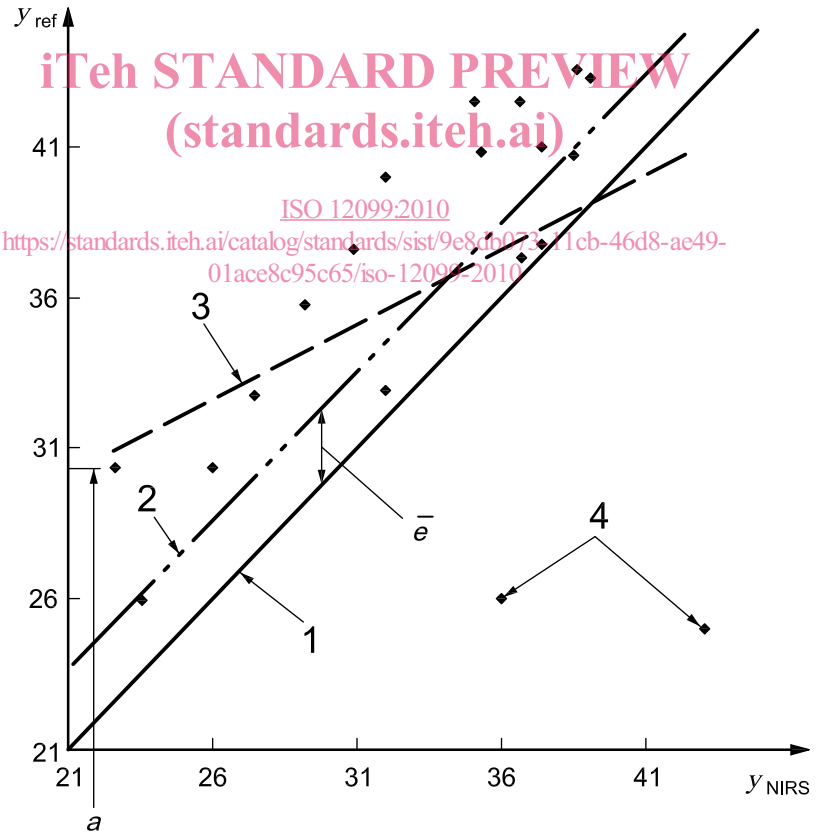
where

y_i is the ith reference value;

$\hat{y}_i$ is the ith predicted value obtained when applying the multivariate NIR model.

The way the differences are calculated gives a positive bias when the predictions are too high and a negative one when the predictions are too low compared to the reference values.

A plot of the data gives an immediate overview of the correlation, the bias, the slope, and the presence of obvious outliers (see Figure 1).



Key

1	45° line (ideal line with bias, $\bar{e}$ = 0 and slope, b = 1)	a	intercept
2	45° line displaced by bias, $\bar{e}$	$\bar{e}$	bias
3	linear regression line with y_{ref}-intercept, a	y_{NIRS}	near infrared spectroscopy predicted values
4	outliers	y_{ref}	reference value

NOTE The outliers have a strong influence on the calculation of the slope and should be removed if the results are to be used for adjustments.

Figure 1 — Scatter plot for a validation set, $y_{ref} = f(a + by_{NIRS})$

6.3 The bias

Most of the time, a bias or systematic error is what is observed with NIR models. Bias can occur due to: new samples of a type not previously seen by the model, drift of the instrument, drift in wet chemistry, changes in the process, and changes in the sample preparation.

With the number of independent samples, n, the bias (or offset) is the mean difference, $\bar{e}$, and can be defined as:

$$\bar{e} = \frac{1}{n}\sum_{i=1}^{n} e_i \tag{2}$$

where e_i is the residual as defined in Equation (1), or

$$\bar{e} = \frac{1}{n}\left(\sum_{i=1}^{n} y_i - \sum_{i=1}^{n} \hat{y}_i\right) = \bar{y} - \bar{\hat{y}} \tag{3}$$

where

y_i is the ith reference value;

$\hat{y}_i$ is the ith predicted value obtained when applying the multivariate NIR model;

and

$\bar{\hat{y}}$ is the mean of the predicted values;

$\bar{y}$ is the mean of the reference values.

The significance of the bias is checked by a t-test. The calculation of the bias confidence limits (BCLs), T_b, determines the limits for accepting or rejecting equation performance on the small set of samples chosen from the new population.

$$T_b = \pm \frac{t_{(1-\alpha/2)}\, s_{SEP}}{\sqrt{n}} \tag{4}$$

where

α is the probability of making a type I error;

t is the appropriate Student t-value for a two-tailed test with degrees of freedom associated with SEP and the selected probability of a type I error (see Table 1);

n is the number of independent samples;

s_{SEP} is the standard error of prediction (see 6.5).

EXAMPLE With $n = 20$, and $s_{SEP} = 1$, the BCLs are

$$T_b = \pm \frac{2,09 \times 1}{\sqrt{20}} = \pm 0,48 \tag{5}$$