INTERNATIONAL STANDARD

ISO 12099

Second edition
2017-08

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

Reference number
ISO 12099:2017(E)

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition cancels and replaces the first edition (ISO 12099:2010), which has been technically revised.

Introduction

This document has been drafted using, as a basis, ISO 21543 | IDF 201, which was 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 document gives guidelines for the determination by near infrared spectroscopy of constituents such as moisture, fat, protein, starch and crude fibre and 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 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
near infrared instrument
NIR instrument
apparatus which, when used under the conditions defined in this document, predicts *constituent contents* (3.3) and *technological parameters* (3.4) in *animal feeding stuffs* (3.2), cereals and milled cereal products through relationships to absorptions in the near infrared range

3.2
animal feeding stuffs
substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals

EXAMPLE Raw materials, fodder, meat and bone meal, mixed feed and other end products, pet food, etc.

3.3
constituent content
mass fraction of substances determined using the appropriate, standardized or validated chemical method

Note 1 to entry: The mass fraction is often expressed as a percentage.

Note 2 to entry: For examples of appropriate methods, see References [1] to [12].

EXAMPLE Moisture, fat, protein, crude fibre, neutral detergent fibre and acid detergent fibre.

3.4
technological parameter
property or functionality of *animal feeding stuffs* (3.2), cereals and milled cereal products that can be determined using the appropriate, standardized or validated method(s)

Note 1 to entry: It is possible to develop and validate NIR methods for other parameters and sample types than listed above, as long as the procedure from this document is observed. The measuring units of the parameters determined follow the units used in the reference methods.

EXAMPLE Digestibility.

4 Principle

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

5 Apparatus

5.1 Near infrared instruments.

Instruments based on diffuse reflectance or transmittance measurement covering the near infrared wavelength region of 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/wavenumber accuracy and wavelength/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 path length (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.

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

NOTE Changes in grinding or milling conditions can influence NIR measurements due, for example, to heating which can drive off volatile components such as water.

6 Calibration and initial validation

6.1 General

The instrument shall be calibrated before use. Calibration involves the comparison with a reference and adjustment processes to the instrument. 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 Reference [16] and the respective manufacturer's manual. For the validation, it is important to have a sufficient number of representative samples, covering variations such as the following:

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

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

c) processing techniques and conditions;

d) storage conditions;

e) sample and instrument temperature;

f) instrument variations (i.e. differences between instruments).

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

6.2 Reference methods

Internationally accepted reference methods for determination of moisture, fat, protein and other constituents and parameters should be used. See References [1] to [12] for examples.

The reference method used for calibration should be in statistical control. It is essential to know the precision of the reference method.

Where possible, references that provide measurement traceability to the SI (International system of units), such as certified reference materials, should be used.

6.3 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 examples.

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

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

b) if they are within the spectral variation of the calibration samples, as, for example, estimated by Mahalanobis distance;

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

d) if 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, e.g. by repeated measurements. 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.

6.4 Validation of calibration models

6.4.1 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, slope and for the determination of standard error of prediction (SEP, see 7.5), at least 20 samples are needed. Validation shall be carried out for each sample type, constituent/parameter, temperature and other factors known to affect or expected to have an effect the measurement. The calibration is valid only for the variations, i.e. sample types, range and temperature, used in the validation.

NOTE 1 Calibration models can only be used in the range they have been validated.

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 7.5) and the residual plot of data corrected for mean systematic error (bias) is examined for outliers, i.e. samples with a residual exceeding ±3 s_{SEP}.

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

NOTE 2 What will be acceptable will depend, for example, on the performance of the reference method, the covered range, the purpose of the analysis, etc., and is up to the parties involved to decide.

Where available and suitable, reference materials or certified reference materials can be used as part of validation of calibration models.

The next step is to fit NIR, y_{NIRS}, and reference data, y_{ref}, by linear regression (y_{ref} = a + b × y_{NIRS}) to produce statistics that describe the validation results.

6.4.2 Bias correction

The data are also examined for a 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 7.3) in the calibration equation.

6.4.3 Slope adjustment

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

Adjusting the slope/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.

6.4.4 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 should be made. 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.

6.5 Changes in measuring and instrument conditions

Unless additional calibration is performed, a local validation of a 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 or slope / intercept adjustments to calibration equations. In many cases, it will be necessary to standardize the two instruments against each other before calibration equations can be transferred[16]. 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.

7 Statistics for performance measurement

7.1 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 will be new batches; in agriculture, it will be a new crop or a new experiment location.

This set of samples shall be carefully analysed following the reference methods. The care to analyse validation samples shall be emphasized 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.

The NIR protocol used for the determination of the performances of the prediction model shall be the same as that used in routine (one measurement or two measurements).

7.2 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 by Formula (1):

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

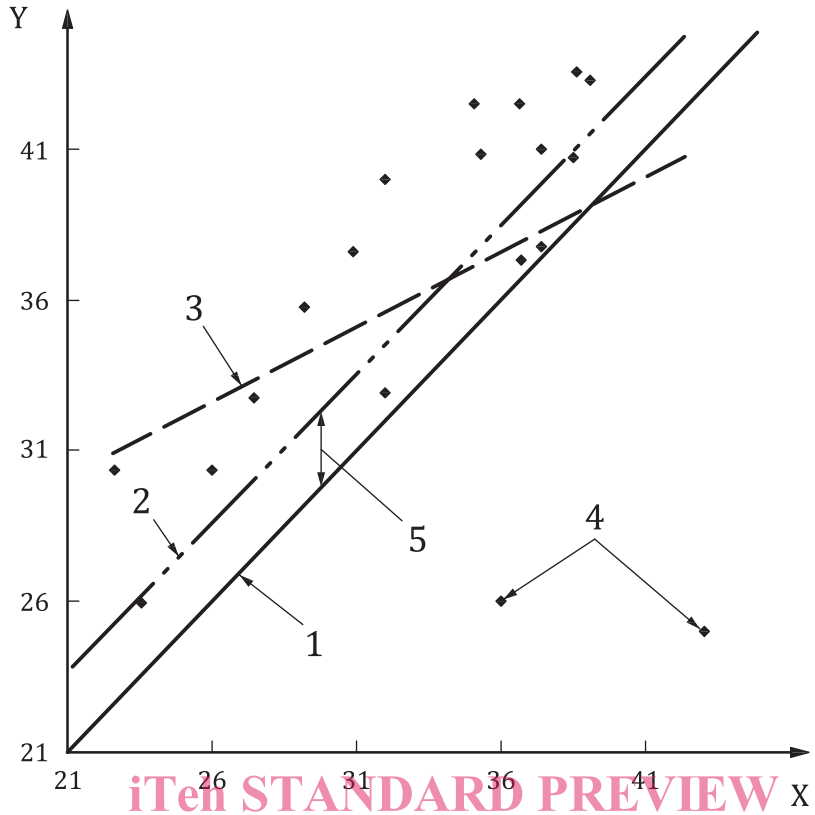
where

y_i is the i^{th} reference value (y_{ref});

$\hat{y}_i$ is the i^{th} predicted value (y_{NIRS}) obtained when applying the multivariate NIR model.

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

A plot of the data immediately gives an 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 = 0 and slope = 1)	X	Y_{NIRS}
2	(45° – bias) line	Y	Y_{ref}
3	linear regression line		
4	outliers		
5	bias		

NOTE The outliers (key 4) 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, $y_{ref} = f(a + b \times y_{NIRS})$

7.3 Bias

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

With n, the number of independent samples, the bias (or offset) is the mean difference and can be defined by Formula (2):

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

where e_i is the residual as defined by Formula (1) resulting in Formula (3):

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

