

पशु आहार सामग्री, अनाज और पिसे हुए
अनाज उत्पाद — नियर इन्फ्रारेड स्पेक्ट्रोमेट्री
अनुप्रयोग हेतु दिशानिर्देश

Animal Feeding Stuffs, Cereals and
Milled Cereal Products — Guidelines
for the Application of Near Infrared
Spectrometry

ICS 65.120

© BIS 2023



भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुर शाह ज़फर मार्ग, नई दिल्ली - 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI - 110002
www.bis.gov.in www.standardsbis.in

NATIONAL FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Animal Feeds and Nutrition Sectional Committee had been approved by the Food and Agriculture Division Council.

This Indian standard is identical with ISO 12099 : 2017 'Animal feeding stuffs, cereals and milled cereal products — Guidelines for the application of near infrared spectrometry' issued by the International Organization for Standardization (ISO).

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

Contents

Page

Introduction	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Apparatus	2
6 Calibration and initial validation	2
6.1 General.....	2
6.2 Reference methods.....	3
6.3 Outliers.....	3
6.4 Validation of calibration models.....	3
6.4.1 General.....	3
6.4.2 Bias correction.....	4
6.4.3 Slope adjustment.....	4
6.4.4 Expansion of calibration set.....	4
6.5 Changes in measuring and instrument conditions.....	4
7 Statistics for performance measurement	5
7.1 General.....	5
7.2 Plot the results.....	5
7.3 Bias.....	6
7.4 Root mean square error of prediction (S_{RMSEP}).....	8
7.5 Standard error of prediction (S_{SEP}).....	8
7.6 Slope.....	10
8 Sampling	12
9 Procedure	12
9.1 Preparation of test sample.....	12
9.2 Measurement.....	12
9.3 Evaluation of result.....	12
10 Checking instrument stability	13
10.1 Control sample.....	13
10.2 Instrument diagnostics.....	13
10.3 Instruments in a network.....	13
11 Running performance check of calibration	13
11.1 General.....	13
11.2 Control charts using the difference between reference and NIR results.....	14
12 Precision and accuracy	15
12.1 Repeatability.....	15
12.2 Reproducibility.....	15
12.3 Accuracy.....	15
12.4 Uncertainty.....	15
13 Test report	15
Annex A (informative) Guidelines for specific NIR standards	16
Annex B (informative) Examples of outliers and control charts	17
Annex C (informative) Supplementary terms and definitions	23
Bibliography	28

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).

Indian Standard

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 $\pm 3 s_{SEP}$.

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 ($y_{\text{ref}} = a + b \times y_{\text{NIRS}}$) 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 \(1\)](#):

$$e_i = y_i - \hat{y}_i \quad (1)$$

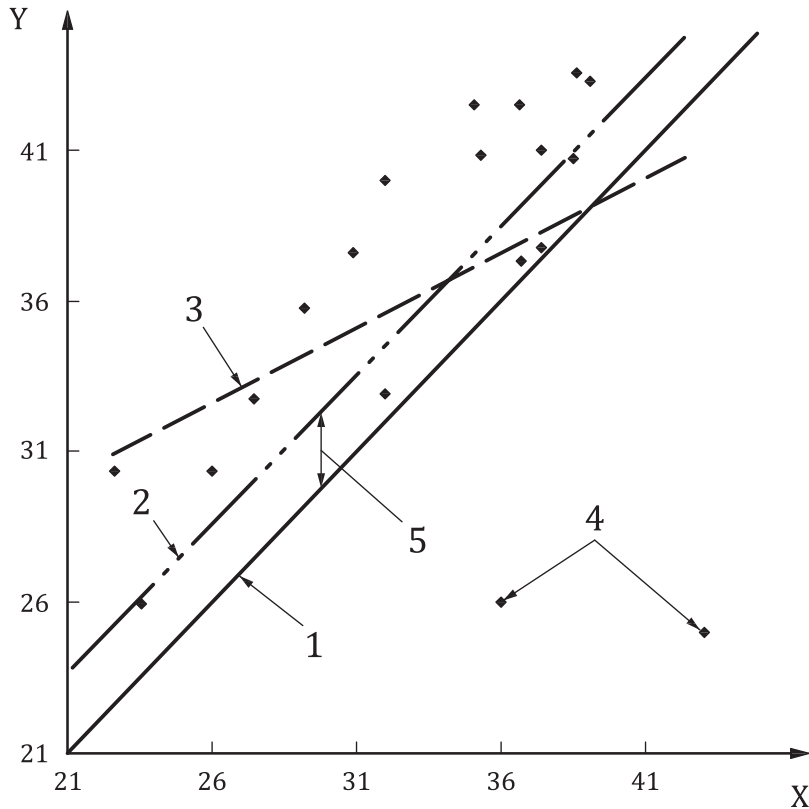
where

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 \(3\)](#):

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

where

- y_i is the i^{th} reference value;
- \hat{y}_i is the i^{th} predicted value;
- \bar{y} is the mean of the reference values;
- $\bar{\hat{y}}$ is the mean of the predicted 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 formula performance on the small set of samples chosen from the new population; see [Formula \(4\)](#):

$$T_b = \pm \left[t_{(1-\alpha/2)} s_{\text{SEP}} \right] / \sqrt{n} \quad (4)$$

where

- α is the probability of making a type I error;
- t is the appropriate student's t value for a two-tailed test with degrees of freedom associated with s_{SEP} and the selected probability of a type I error;
- n is the number of independent samples;
- s_{SEP} is the standard error of prediction (defined in [7.5](#)).

As an example, with $n = 20$, and $s_{\text{SEP}} = 1$, the BCLs are as in [Formula \(5\)](#):

$$T_b = \pm (2,09 \times 1) / \sqrt{20} = \pm 0,48 \quad (5)$$

This means that the bias tested with 20 samples shall be higher than 48 % of the standard error of prediction to be considered as different from zero.

Table 1 — Values of the t distribution with a probability $\alpha = 0,05$ (5 %)

n	t values	n	t values
10	2,23	75	1,99
15	2,13	100	1,98
20	2,09	200	1,97
30	2,04	500	1,96
40	2,02	1000	1,96
50	2,01	—	—

NOTE The Excel function "TINV" can be used.^a

^a Excel is the trade name of a product supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

7.4 Root mean square error of prediction (s_{RMSEP})

The s_{RMSEP} is defined by [Formula \(6\)](#):

$$s_{RMSEP} = \sqrt{\frac{\sum_{i=1}^n e_i^2}{n}} \quad (6)$$

where

- e_i is the residual of the i^{th} sample;
- n is the number of independent samples.

This value can be compared with s_{SEC} and s_{SECV} (see [Annex C](#)).

s_{RMSEP} includes the random error (s_{SEP}) and the systematic error (bias). It includes also the error of the reference methods (as do s_{SEC} and s_{SECV}); see [Formula \(7\)](#):

$$s_{RMSEP} = \sqrt{\frac{(n-1)}{n} s_{SEP}^2 + \bar{e}^2} \quad (7)$$

where

- n is the number of independent samples;
- s_{SEP} is the standard error of prediction (defined in [7.5](#));
- \bar{e} is the bias or systematic error.

There is no direct test for s_{RMSEP} . This is the reason to separate the systemic error (bias or \bar{e}) and the random error s_{SEP} .

7.5 Standard error of prediction (s_{SEP})

The standard error of prediction (s_{SEP}), i.e. standard deviation of the residuals, which expresses the accuracy of routine NIR results corrected for the mean difference (bias) between routine NIR and reference method, can be calculated by using [Formula \(8\)](#):

$$s_{SEP} = \sqrt{\frac{\sum_{i=1}^n (e_i - \bar{e})^2}{n-1}} \quad (8)$$

where

- n is the number of independent samples;
- e_i is the residual of the i^{th} sample;
- \bar{e} is the bias or systematic error.

The s_{SEP} should be related to the s_{SEC} (respectively, s_{SECV} ; see [Annex C](#)) to check the validity of the calibration model for the selected validation set.

The unexplained error confidence limits (UECLs), T_{UE} , are calculated from an F -test (ratio of two variances) (see Reference [18] and Table 2). See Formula (9):

$$T_{UE} = s_{SEC} \sqrt{F_{(\alpha, v, M)}} \quad (9)$$

where

- s_{SEC} is the standard error of calibration (see Annex C);
- α is the probability of making a Type I error;
- v is $n - 1$ numerator degrees of freedom associated with s_{SEP} of the test set;
- n is the number of samples in the validation process;
- M is $n_c - p - 1$ denominator degrees of freedom associated with s_{SEC} (standard error of calibration) [n_c is the number of calibration samples, p is the number of terms or PLS factors of the model or weights in the case of ANN (see Annex C). In ANN, weights are all unknown parameters in the model].

NOTE 1 s_{SEC} can be replaced by s_{SECV} , which is a better statistic than s_{SEC} ; very often, s_{SEC} is too optimistic; ($s_{SEP} > s_{SECV} > s_{SEC}$).

EXAMPLE Where $n = 20$, $\alpha = 0,05$, $M = 100$, and $s_{SEP} = 1$, gives the following value: $T_{UE} = 1,30$.

With 20 samples, a s_{SEP} that is up to 30 % larger than the s_{SEC} can be accepted.

NOTE 2 The Excel¹⁾ function “FINV” can be used.

The F -test cannot be used to compare two calibrations on the same validation set. It needs (as here) two independent sets to work. Another test shall be used to compare two or more models on the same data set.

1) Excel is the trade name of a product supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 2 — F values and squared root of the F values in function of the degrees of freedom of the numerator associated with s_{SEP} and of the denominator associated with s_{SEC}

		$F(\alpha, \nu, M)$							$\sqrt{F(\alpha, \nu, M)}$				
		df (s_{SEC})							df (s_{SEC})				
df (s_{SEP})		50	100	200	500	1 000	df (s_{SEP})		50	100	200	500	1 000
10		2,03	1,93	1,88	1,85	1,84	10		1,42	1,39	1,37	1,36	1,36
11		1,99	1,89	1,84	1,81	1,80	11		1,41	1,37	1,36	1,34	1,34
12		1,95	1,85	1,80	1,77	1,76	12		1,40	1,36	1,34	1,33	1,33
13		1,92	1,82	1,77	1,74	1,73	13		1,39	1,35	1,33	1,32	1,32
14		1,89	1,79	1,74	1,71	1,70	14		1,38	1,34	1,32	1,31	1,30
15		1,87	1,77	1,72	1,69	1,68	15		1,37	1,33	1,31	1,30	1,29
16		1,85	1,75	1,69	1,66	1,65	16		1,36	1,32	1,30	1,29	1,29
17		1,83	1,73	1,67	1,64	1,63	17		1,35	1,31	1,29	1,28	1,28
18		1,81	1,71	1,66	1,62	1,61	18		1,30	1,31	1,29	1,27	1,27
19		1,80	1,69	1,64	1,61	1,60	19		1,34	1,30	1,28	1,27	1,26
29		1,69	1,58	1,52	1,49	1,48	29		1,30	1,26	1,23	1,22	1,22
49		1,60	1,48	1,42	1,38	1,37	49		1,27	1,22	1,19	1,17	1,17
99		1,53	1,39	1,32	1,28	1,26	99		1,24	1,18	1,15	1,13	1,12
199		1,48	1,34	1,26	1,21	1,19	199		1,22	1,16	1,12	1,10	1,09
499		1,46	1,31	1,22	1,16	1,13	499		1,21	1,14	1,11	1,08	1,07
999		1,45	1,30	1,21	1,14	1,11	999		1,20	1,14	1,10	1,07	1,05

NOTE 1 See explanation to [Formula \(9\)](#).
NOTE 2 df is the degree of freedom; $n - 1$ for s_{SEP} ; and $n_c - p - 1$ for s_{SEC} .

7.6 Slope

The slope, b , of the simple regression $y = a + b \cdot \hat{y}$ is often reported in the NIR reports and publications.

Notice that the slope shall be calculated with the reference values as the dependent variable and the predicted NIR values as the independent variable, if the calculated slope is intended to be used for adjustment of NIR results (like in the case of the inverse multivariate regression used to build the prediction model).

From the least squares fitting, slope and intercept are calculated by [Formula \(10\)](#) and [Formula \(11\)](#), respectively:

$$b = \frac{s_{\hat{y}y}}{s_{\hat{y}}^2} \tag{10}$$

where

$s_{\hat{y}y}$ is the covariance between reference and predicted values;

$s_{\hat{y}}^2$ is the variance of the n predicted values.

$$a = \bar{y} - b\bar{\hat{y}} \quad (11)$$

where

\bar{y} is the mean of the reference values;

b is the slope;

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

As for the bias, a t test can be calculated to check the hypothesis that $b = 1$ as in [Formula \(12\)](#):

$$t_{\text{obs}} = |b - 1| \cdot \sqrt{\frac{s_{\hat{y}}^2 \cdot (n - 1)}{s_{\text{res}}^2}} \quad (12)$$

where

$s_{\hat{y}}^2$ is the variance of the n predicted values;

n is the number of independent samples;

s_{res} is the residual standard deviation.

The residual standard deviation, s_{res} , is defined in [Formula \(13\)](#):

$$s_{\text{res}} = \sqrt{\frac{\sum_{i=1}^n [y_i - (a + b\hat{y}_i)]^2}{n - 2}} \quad (13)$$

where

n is the number of independent samples;

a is the intercept (see [Formula \(11\)](#));

b is the slope (see [Formula \(10\)](#));

y_i is the i^{th} reference value;

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

NOTE s_{res} is like s_{SEP} when the predicted values are corrected for slope and intercept. Be aware to not confuse bias and intercept. See also [Figure 1](#).

The bias equals the intercept only when the slope is exactly one.

The slope, b , will be considered as different from 1 when [Formula \(14\)](#) applies:

$$t_{\text{obs}} \geq t_{(1-\alpha/2)} \quad (14)$$

where

t_{obs} is the observed t value, calculated according to [Formula \(12\)](#);

$t_{(1-\alpha/2)}$ is the t value obtained from [Table 1](#) for a probability of $\alpha = 0,05$ (5 %).

A too-narrow range or uneven distribution will lead to useless correction of the slope even when the s_{SEP} is correct. The slope can only be adjusted when the validation set covers a large part of the calibration range.

EXAMPLE For $n = 20$ samples with a residual standard deviation [see [Formula \(13\)](#)] of 1, a standard deviation of the predicted values of $S_{\hat{y}} = 2$ and a calculated slope of $b = 1,2$, the observed t_{obs} value is 1,7 and then the slope is not significantly different from 1 as the t value (see [Table 1](#)) for $n = 20$ samples is 2,09. If the slope is 1,3, the t_{obs} value is 2,6 and then the slope is significantly different than 1.

8 Sampling

Sampling is not part of the method specified in this document.

NOTE Recommended sampling procedures are given in ISO 6497 and ISO 24333.

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage.

9 Procedure

9.1 Preparation of test sample

All laboratory samples should usually be kept under conditions that will not change the composition of the sample from the time of sampling to the time of commencing the procedure.

The preparation of samples for routine measurements needs to be made in the same way as the preparation of the validation samples. It is necessary to apply standard conditions.

Before the analysis, the sample should be taken in such a way as to obtain a sample representative of the material to be analysed.

For specific procedures, see specific NIR standards.

Guidelines for specific NIR standards are given in [Annex A](#).

9.2 Measurement

Follow the instructions of the instrument manufacturer/supplier.

The prepared sample should reach a temperature within the range included in the validation.

9.3 Evaluation of result

For the routine results to be valid, they shall be within the range of the calibration model used.

Results obtained on samples detected as spectral outliers cannot be regarded as reliable.

If multiple measurements are made on the same sample, calculate the arithmetic mean if the repeatability conditions (see [12.1](#)) are observed.

For the expression of results, refer to specific NIR standards.

10 Checking instrument stability

10.1 Control sample

At least one control sample should be measured at least once per day to check instrument hardware stability and to detect any malfunction. Knowledge of the true concentration of the analyte in the control sample is not necessary. The sample material should be stable and, as far as possible, resemble the samples to be analysed. The parameter(s) measured should be stable and, as far as possible, identical to or at least biochemically close to the sample analyte. A sample is prepared as described in 9.1 and stored in such a way as to maximize the storage life. These samples are normally stable for lengthy periods but the stability should be tested in the actual cases. Control samples should be overlapped to secure uninterrupted control.

The recorded day-to-day variation should be plotted in control charts and investigated for significant patterns or trends.

10.2 Instrument diagnostics

For scanning spectrophotometers the wavelength/wavenumber (see 5.1) accuracy and precision should be checked at least once a week, or more frequently if recommended by the instrument manufacturer, and the results should be compared to specifications and requirements (see 5.1).

A similar check of the instrument noise shall be carried out weekly or at intervals recommended by the manufacturer.

10.3 Instruments in a network

If several instruments are used in a network, special attention shall be given to standardization of the instruments according to the manufacturer's recommendations.

11 Running performance check of calibration

11.1 General

The suitability of the calibration for the measurement of individual samples should be checked. The outlier measures used in the calibration development and validation can be applied, e.g. Mahalanobis distance and spectral residuals. In most instruments, this is done automatically.

If the sample does not pass the test, i.e. the sample does not fit into the population of the samples used for calibration and/or validation, it cannot be determined by the prediction model, unless the model is changed. Thus, the outlier measures can be used to decide which samples should be selected for reference analysis and included in a calibration model update.

If the calibration model is found to be suitable for the measured sample, the spectrum is evaluated according to the validated calibration model.

NIR methods should be validated continuously against reference methods to secure steady optimal performance of calibrations and observance of accuracy. The frequency of checking the NIR method should be sufficient to ensure that the method is operating under steady control with respect to systematic and random deviations from the reference method. The frequency depends *inter alia* on the number of samples analysed per day and the rate of changes in sample population.

The running validation should be performed on samples selected randomly from the pool of analysed samples. It may be necessary to resort to some sampling strategy to ensure a balanced sample distribution over the entire calibration range, e.g. segmentation of concentration range and random selection of test samples within each segment or to ensure that samples with a commercially important range are covered.

The number of samples for the running validation should be sufficient for the statistics used to check the performance. For a solid validation, at least 20 samples are needed (to expect a normal distribution of variance). One can fill in the results of the independent validation set for starting the running validation. To continue with about 5 to 10 samples every week is sufficient to monitor the performance properly. Using fewer samples it is hard to take the right decision in case one of the results is outside the control limits.

11.2 Control charts using the difference between reference and NIR results

Results should be assessed by control charts, plotting running sample numbers on the abscissa and the difference between results obtained by reference and NIR methods on the ordinate; $\pm 2 s_{SEP}$ (95 % probability) and $\pm 3 s_{SEP}$ (99,8 % probability) may be used as warning and action limits where the s_{SEP} has been obtained on a test set collected independently of calibration samples.

If the calibration and the reference laboratories are performing as they should, then only 1 point in 20 points should plot outside the warning limits and 2 points in 1 000 points outside the action limits.

Control charts should be checked for systematic bias drifts from zero, systematic patterns and excessive variation of results. General rules applied for Shewart control charts may be used in the assessment. However, too many rules applied simultaneously may result in too many false alarms.

The following rules used in combination have proved to be useful in detection of problems:

- a) one point outside either action limit;
- b) two out of three points in a row outside a warning limit;
- c) nine points in a row on the same side of the zero line.

Additional control charts plotting other features of the running control (e.g. mean difference between NIR and reference results) and additional rules may be applied to strengthen decisions.

In the assessment of results, it should be remembered that s_{SEP} and measured differences between NIR and reference results also include the imprecision of reference results. This contribution can be reduced to a negligible part if the imprecision of reference results is reduced to less than one third of the s_{SEP} ^[17].

To reduce the risk of false alarms, the control samples should be analysed independently (in different series) by both NIR spectrometry and reference methods to avoid the influence of day-to-day systematic differences in, for example, reference analyses.

If the warning limits are often exceeded and the control chart only shows random fluctuations (as opposed to trends or systematic bias), the control limits may have been based on a too optimistic s_{SEP} value. An attempt to force the results within the limits by frequent adjustments of the calibration will not improve the situation in practice. The s_{SEP} should instead be re-evaluated using the latest results.

If the calibration equations after a period of stability begin to move out of control, the calibration should be updated. Before this is done, an evaluation should be made of whether the changes could be due to changes in reference analyses, unintended changes in measuring conditions (e.g. caused by a new operator), instrument drift or malfunction, etc. In some cases, a simple adjustment of the constant term in the calibration equation may be sufficient (an example is shown in [Figure B.6](#)). In other cases, it may be necessary to run a complete re-calibration procedure, where the complete or a part of the basic calibration set is expanded to include samples from the running validation, and perhaps additional samples selected for this purpose (an example is shown in [Figure B.7](#)).

Considering that the reference analyses are in statistical control and the measuring conditions and instrument performance are unchanged, significant biases or increased s_{SEP} values can be due to changes in the chemical, biological or physical properties of the samples compared to the underlying calibration set.

Other control charts, for example, using z-scores, may be used.

12 Precision and accuracy

12.1 Repeatability

The repeatability, i.e. the difference between two individual single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, which should not be exceeded in more than 5 % of cases, depends on the sample material, the analyte, sample and analyte variation ranges, method of sample presentation, instrument type and the calibration strategy used. The repeatability should be determined in each case.

12.2 Reproducibility

The reproducibility, i.e. the difference between two individual single test results, obtained on identical test material by different laboratories and by different operators at different times, which should not be exceeded in more than 5 % of cases, depends on the sample material, the analyte, sample and analyte variation ranges, method of sample presentation, instrument type and the calibration strategy used. The reproducibility should be determined in each case.

12.3 Accuracy

The accuracy, which includes uncertainty from systematic deviation from the true value on the individual sample (trueness) and uncertainty from random variation (precision), depends *inter alia* on the sample material, the analyte, sample and analyte variation ranges, method of sample presentation, instrument type and the calibration strategy used. The accuracy should be determined in each case. The reported s_{SEP} and s_{RMSEP} values also include uncertainty of reference results which may vary from case to case.

12.4 Uncertainty

Uncertainty, U_e , is a parameter characterizing the dispersion of values that can reasonably be attributed to the result. For NIR predicted results, the uncertainty is usually expressed by [Formula \(15\)](#):

$$U_e = \pm 2s_{RMSEP} \quad (15)$$

If a multiplier of 2 is used, this can be understood to indicate limits corresponding to 95 % probability of the true value lying within the range $\pm U_e$.

The s_{RMSEP} shall be determined locally.

13 Test report

The test report shall specify:

- a) all information necessary for complete identification of the sample;
- b) the test method used, with reference to the relevant International Standard;
- c) all operating conditions not specified in this document, or regarded as optional;
- d) any circumstances which may have influenced the results;
- e) the test result(s) obtained;
- f) the current s_{SEP} and bias (if statistically significant), estimated from running a performance test on at least 20 test samples (see [Clause 11](#)).

Annex A (informative)

Guidelines for specific NIR standards

Specific NIR standards may be developed for specific calibrations for the determination of specific constituents and parameters in animal feeding stuff, cereals and milled cereal products by NIR spectrometry.

A specific standard should

- respect the recommendation of this document for the process of validation,
- indicate particular specifications to be met as range of concentration, accuracy and precision,
- fix the sources of variation that shall be taken into account in the development of the model, and
- specify the procedure, the calculation and the expression of results.

A specific standard is not made for a particular apparatus and calibration.

These standards should follow the ISO format and provide information regarding

- the type of samples and constituents/parameters determined followed by “near infrared spectrometry” and the calibration model(s) used in the title and the scope,
- the reference methods used for the validation under “Normative references”,
- the spectroscopic principle (e.g. NIR, NIT) and calibration principle (e.g. PLS, ANN),
- the procedure(s) including preparation of the test sample(s), measurement and quality control, and
- specifications to be followed in terms of accuracy, precision and range; for example, as in [Table A.1](#) for a validation set.

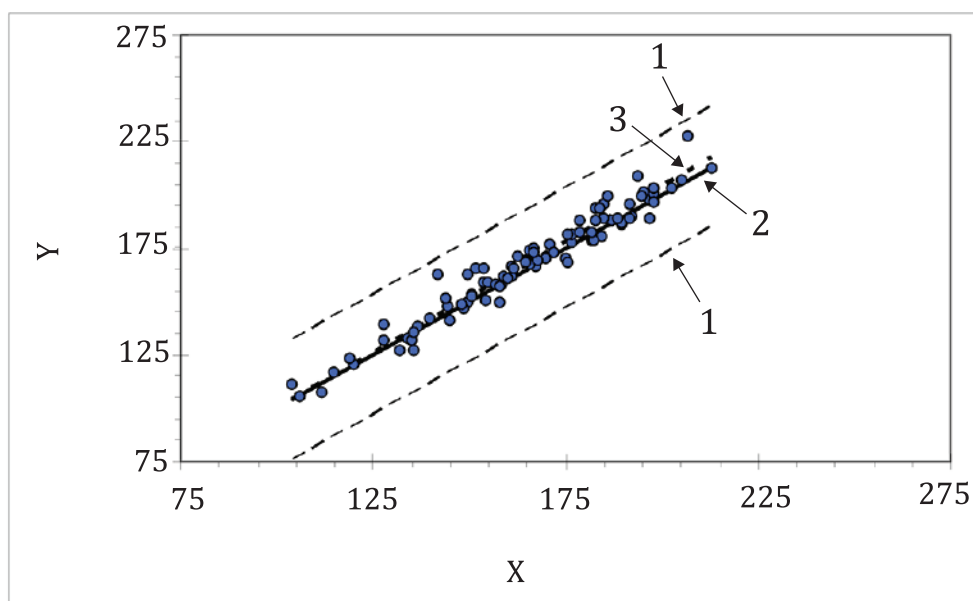
Table A.1 — Example for specifications given for a validation set

Component	Model	N	Precision (s_{SEP})	Min %	Max %	RSQ
Fat	ANN	183	0,50	2,8	12,9	0,94
Moisture	ANN	183	0,47	9,2	12,3	0,83
Protein	ANN	179	0,72	11,0	29,1	0,96
Fibre	ANN	123	1,11	0,5	18,0	0,90
Starch	PLS	113	1,80	7,8	50,2	0,92

Annex B (informative)

Examples of outliers and control charts

[Figure B.1](#) shows the determination of crude protein in forages, which is an example with no outliers. The results were obtained on an independent test set of 95 samples using the developed calibration equation: $s_{SEP} = 4,02$; $s_{RMSEP} = 6,05$; Slope, $b = 1,04$.

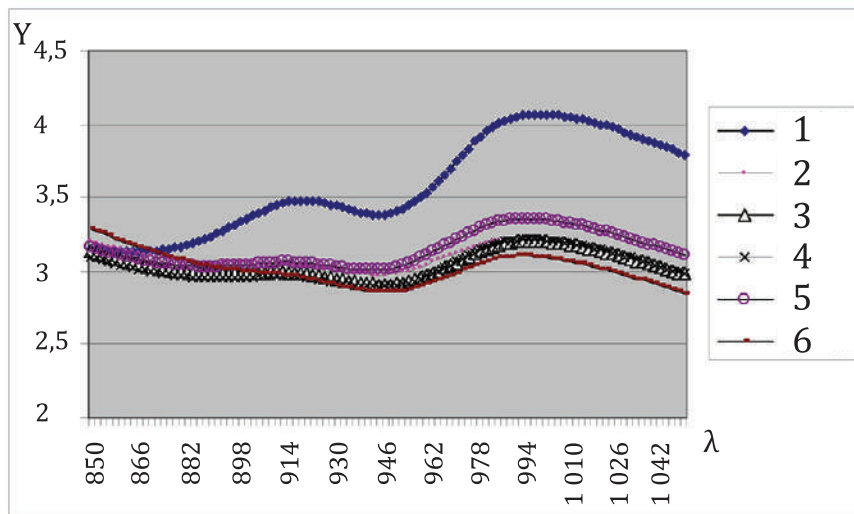


Key

- | | | | |
|---|---|---|-----------|
| 1 | $\pm 3 s_{SEP}$ limits, | X | NIR |
| 2 | 45 degree line (ideal line with slope = 1 and bias = 0) | Y | reference |
| 3 | regression line | | |

Figure B.1 — No outliers

Figure B.2 shows an absorbance spectra with X-outlier. The series 1 (upper) spectrum indicates a spectral outlier.

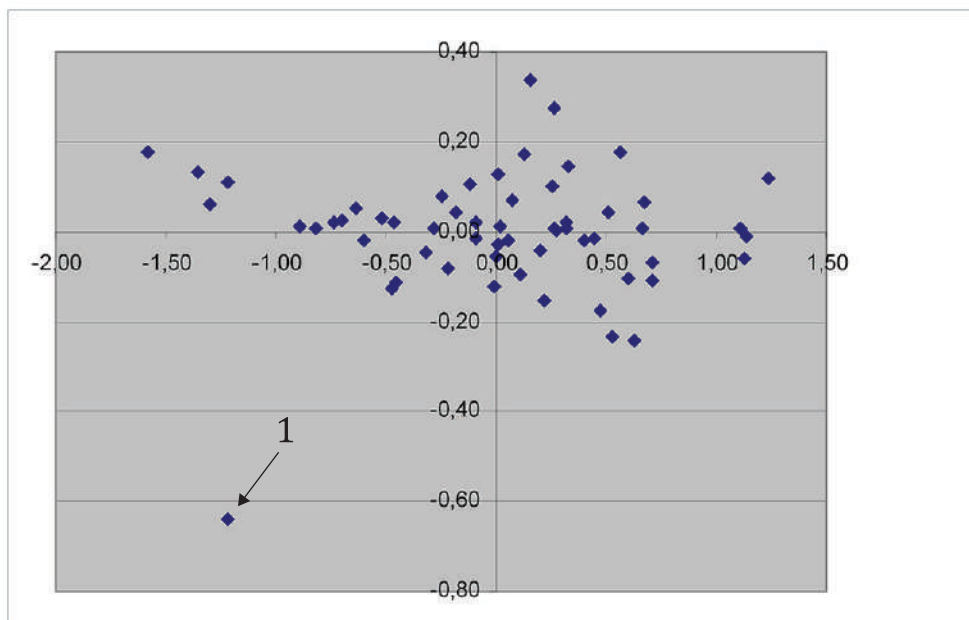


Key

- | | | | |
|---|----------|---|-----------------|
| 1 | series 1 | 5 | series 5 |
| 2 | series 2 | 6 | series 6 |
| 3 | series 3 | λ | wavelength (nm) |
| 4 | series 4 | Y | absorbance |

Figure B.2 — Absorbance spectra with X-outlier

Figure B.3 shows a PCA score plot with X-outlier (1).

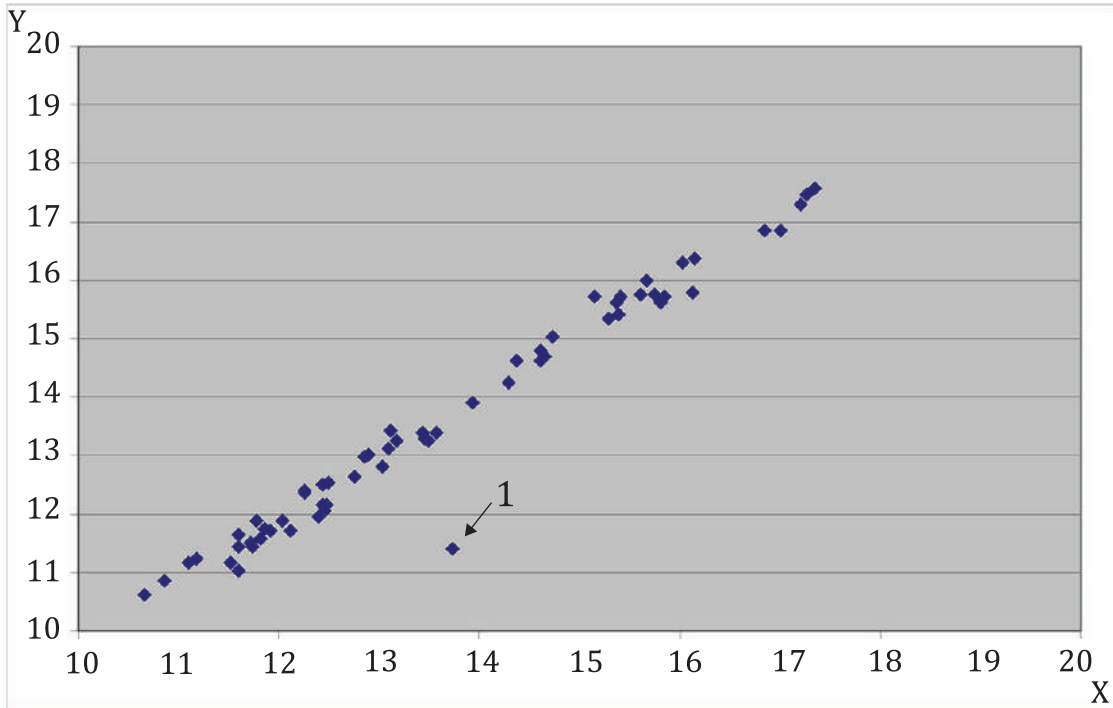


Key

- 1 outlier

Figure B.3 — PCA score plot with X-outlier (1)

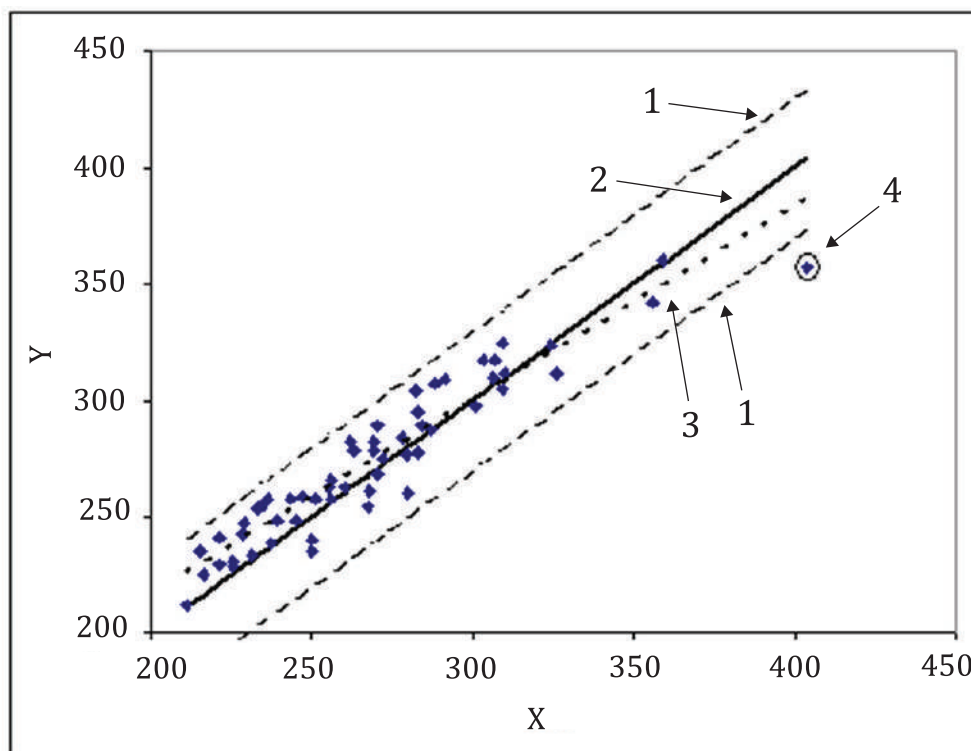
Figure B.4 shows a scatter plot with Y-outlier (1). The plot of reference vs. predicted values (or vice versa) shows one sample (1) that strongly deviates from the other samples. If the reason for this deviation is not related to NIR data (X-outlier), this sample will be a Y-outlier due to erroneous reference data or a different relationship between reference data and spectral data.



- Key**
- 1 outlier
 - X NIR
 - Y reference

Figure B.4 — Scatter plot with Y-outlier (1)

Figure B.5 shows the determination of ADF in forages with Y-outlier (1).



Key

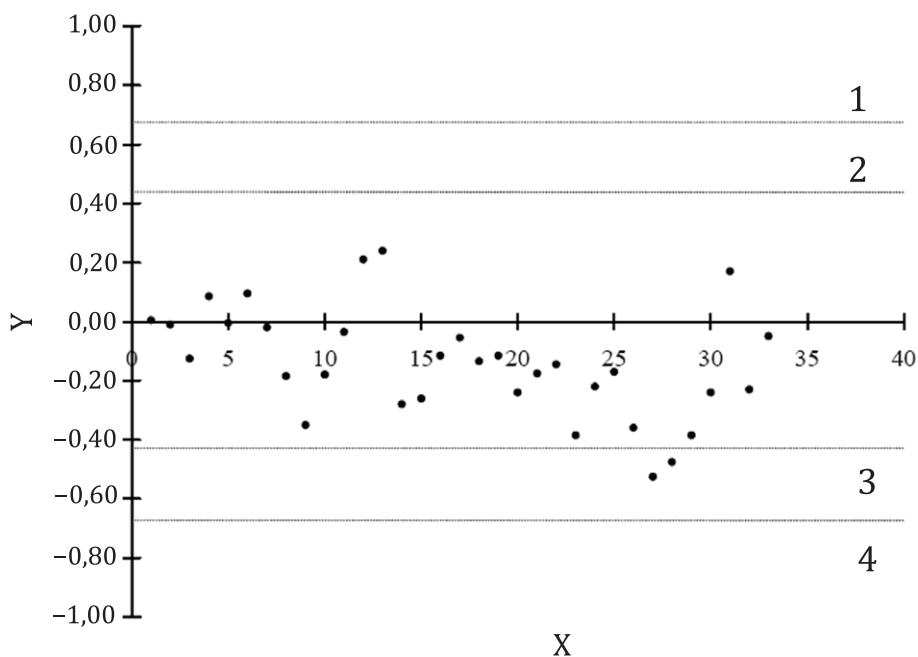
- | | | | |
|---|------------------------|---|-----------|
| 1 | $\pm 3 s_{SEP}$ limits | X | NIR |
| 2 | 45 degree line | Y | reference |
| 3 | regression line | | |
| 4 | outlier | | |

Figure B.5 — Determination of ADF in forages with Y-outlier (1)

Figure B.6 shows a control chart for determination of percent fat in cereals. No points are outside the upper action limit (UAL) or the lower action limit (LAL). However, 9 points in row (e.g. 14 to 22) are on the same side of the zero line. That indicates a bias problem. Two points (27 and 28) out of 3 points are outside the lower warning limit (LWL) but none are outside the upper warning limit (UWL). This also indicates a bias problem. No increase in random variation is observed. The spread is still less than 3 s_{SEP} .

In conclusion, the calibration should be bias adjusted.

(Difference Reference - NIR is plotted. UAL/LAL = 3 s_{SEP} and UWL/LWL = 2 s_{SEP})



Key

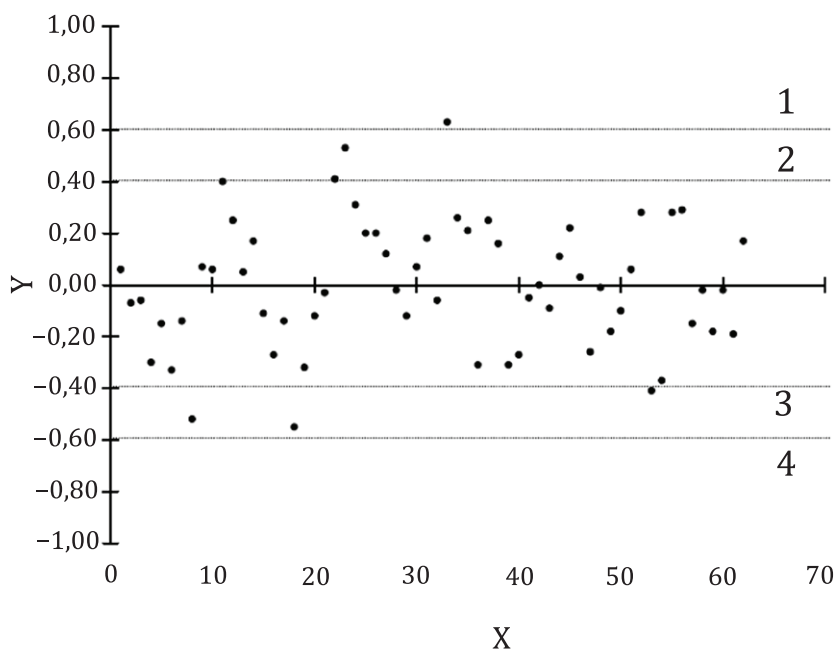
- | | |
|-----------------------------|-------------------|
| 1 upper action limit (UAL) | X run number |
| 2 upper warning limit (UWL) | Y reference - NIR |
| 3 lower warning limit (LWL) | |
| 4 lower action limit (LAL) | |

Figure B.6 — Control chart for determination of percent fat in cereals

Figure B.7 shows a control chart for determination of XXXX in YYY in the range 44 % to 57 %, where recalibration was performed at point 35.

Viewing the first 34 points, one point is outside the UAL. This indicates a serious problem. Two points (22 and 23) out of 3 points are outside the UWL. Two separate points are also outside the LWL. The spread is uniform around the zero line (the 9-points rule is obeyed) but 5 point out of 34 points are outside the 95 % confidence limits (UWL, LWL) and 1 point out of 34 points is outside the 99,9 % confidence limits (UAL, LAL). This is much more than expected.

One reason for this picture could be that the s_{SEP} value behind calculation of the limits is too optimistic. This means the limits should be widened. Another reason could be that the actual samples are somewhat different from the calibration samples. To test this possibility, the calibration set was extended to include the control samples and a new calibration was developed. The performance of this calibration was clearly better, as shown by the control samples number 35 to 62.



Key

- | | | | |
|---|---------------------------|---|-----------------|
| 1 | upper action limit (UAL) | X | run number |
| 2 | upper warning limit (UWL) | Y | reference - NIR |
| 3 | lower warning limit (LWL) | | |
| 4 | lower action limit (LAL) | | |

Figure B.7 — Control chart for determination of XXXX in YYY (range 44 % to 57 %)

Annex C (informative)

Supplementary terms and definitions

General	
Reference method	ISO or otherwise internationally recognized and validated method, which gives the “true” or “assigned” value of the parameter to be measured, including the measurement uncertainty.
Indirect method	Method that measures properties that are functionally related to the parameter(s) to be determined. The obtained signal is related to the “true” value(s) as determined by the reference method(s).
Near infrared spectroscopy (NIRS)	NIR spectroscopy is the measurement of the intensity of the absorption of near infrared light by a sample within the range 700 nm to 2 500 nm (14 300 to 4 000 cm ⁻¹). NIR instruments use either part of, the whole, or ranges that include this region (e.g. 400 nm to 2 500 nm). Multivariate calibration techniques are then used to relate a combination of absorbance values either to composition or to some property of the samples.
Near infrared reflectance (NIR)	NIR spectroscopy where the basic measurements are the absorption of near infrared light diffusely reflected back from the surface of a sample to be collected by a detector in front of the sample.
Near Infrared transmittance (NIT)	NIR spectroscopy where the basic measurements are the absorption of near infrared light that has travelled through a sample and is then collected by a detector behind the sample.
NIR network(s)	A number of near infrared instruments operated using the same calibration models. Instruments in a network are usually standardized so that the differences in predicted values for a set of standard samples are minimized.
Standardization (of instruments)	A process whereby a group of near infrared instruments are adjusted so that they predict similar values when operating the same calibration model. A number of techniques can be used but these can be broadly defined as either pre-prediction methods where the spectra of samples are adjusted to minimize the differences between the response of a “master” instrument and each instrument in the group and “post-prediction” methods where linear regression is used to adjust the predicted values produced by each instrument to make them as similar as possible to those from a “master” instrument.
Z-Score	Performance criterion calculated by dividing the difference between the NIR predicted result and the reference value by a target value for the standard deviation, e.g. the RMSEP.

Calibration techniques	
PCA	<p>Principle component (PC) analysis is a form of data compression. For a set of samples, it works solely with the X (spectral) data and finds principal components (factors) according to a rule that says that each PC expresses the maximum variation in the data at any time and is uncorrelated with any other PC. The first PC expresses as much as possible of the variability in the original data. Its effect is then subtracted from the X data and a new PC derived again expressing as much as possible of the variability in the remaining data. You can derive as many PCs as there are either data points in the spectrum or samples in the data set but the major effects in spectra can be shown to be concentrated in the first few PCs and therefore the amount of data that needs to be considered is dramatically reduced.</p> <p>PCA produces two new sets of variables at each stage: PC Scores represent the response of each sample on each PC; PC Loadings represent the relative importance of each data point in the original spectra to the PC.</p> <p>PCA has many uses, for example, in spectral interpretation, but is most widely used in the identification of spectral outliers.</p>
PCR	<p>Principle component regression uses the scores on each PC as regressors in a MLR against Y values representing composition of samples. As each PC is orthogonal to every other PC, the scores form an uncorrelated data set with better properties than the original spectra. While it is possible to select a combination of PCs for regression based on how well each PC correlates to the constituent of interest, most commercial software forces the regression to use all PCs up to the highest PC selected for the model (“the top down approach”).</p> <p>When used in NIRS, the regression coefficients in PC space are usually converted back to a prediction model using all the data points in wavelength space.</p>
PLS	<p>Partial least square regression is an analytical technique which, like PCA, is a form of data compression. With PLS, the rule used to derive the factors is that each factor in turn maximizes the covariance between the Y data and all possible linear combinations of the X data. PLS is therefore a balance between variance and correlation with each factor being influenced by both effects. PLS factors are therefore more directly related to variability in Y values than are principal components. PLS produces three new variables, loading weights (which are not orthogonal to each other), loadings, and scores which are both orthogonal.</p> <p>PLS models are produced by regressing PLS scores against Y values. As with PCR, when used in NIRS, the regression coefficients in PLS space are usually converted back to a prediction model using all the data points in wavelength space.</p>
MLR	<p>Multiple linear regression uses a combination of several X variables to predict a single Y variable. In NIRS, the X values are either absorbance values at selected wavelengths in the near infrared or derived variables such as PCA or PLS scores.</p>
ANN	<p>Artificial neural networks describes a nonlinear modelling technique loosely based on the architecture of biological neural systems. The network is initially “trained” by supplying a data set with several X (spectral or derived variables such as PCA scores) values and reference Y values. During the training process, the architecture of the network may be modified and the neurons assigned weighting coefficients for both inputs and outputs to produce the best possible predictions of the parameter values.</p> <p>Neural networks require a lot of data in training. Some optimization schemes for ANN require a stop set to end the optimization of the model (e.g. weights).</p>
Multivariate model	<p>Any model where a number of X values are used to predict one or more Y variables.</p>

Calibration techniques	
Outliers	Outliers are points in any data set that can be shown statistically to have values that lie well outside the expected distribution. For NIRS data, outliers are normally classified as either X (spectral) outliers or Y (reference data) outliers.
X-outliers	Outlier related to the NIR spectrum. An X-outlier can be a spectrum with instrumental faults or from a sample type that is radically different from the other samples or in prediction, a sample type not included in the original calibration set.
Y-outliers	Outliers related to errors in reference data, e.g. a transcription error or in the value obtained by the reference laboratory.
Leverage	A measure of how far a sample lies from the centre of the population space defined by a model. Samples with high leverage have high influence on the model. Leverage is calculated by measuring the distance between a projected point and the centre of the model.
Mahalanobis distance	The distance in PC space between a data point and the centre of the PC space (see h-value below). This is a linear measurement. In PC space, a set of samples usually form a curve shaped distribution. The ellipsoid that best represents the set's probability distribution can be estimated by building the covariance matrix of the samples. The Mahalanobis distance is simply the distance of the test point from the centre of mass divided by the width of the ellipsoid in the direction of the test point.
h-value	In some software, the Mahalanobis distance is referred to as the "Global h-value" and outlier detection depends upon how many standard deviations of h a sample is from the centre. A second measure "Neighbourhood h" is the distance in PC space between a data point and its "n" nearest neighbours and indicates whether a sample is isolated or in a well-populated part of the distribution.
Residual(s)	The difference between a reference value and the value predicted by a regression model. Used in the calculation of regression statistics.
Spectral residual	Residual after chemometric treatment (e.g. PCA, PLS, etc.) of a spectrum. Spectral variation not described by the model.
Test set	When testing a regression model, any set of samples that excludes those used to develop the calibration.
Independent test set	A test set that consists of samples that are from a different geographical region, a new plant (in industrial terms), or have been collected at a later time (e.g. from a different harvest) than those used to create and validate a regression model. These samples form a "true" test of a prediction model.
Validation set	Samples used to validate or "prove" a calibration. Usually samples having the same characteristics as those selected for calibration. Often alternate or "n th " samples (ranked in order of the constituent of interest) are allocated to the calibration and validation data sets from the same pool of samples.
Monitoring set	Set of samples that is used for the routine control of calibration models.

Calibration techniques	
Cross validation	<p>A method of generating prediction statistics where, repeatedly, a subset of samples are removed from a calibration population, a model being calculated on the remaining samples and residuals calculated on the validation subset. When this process has been run a number of times, prediction statistics are calculated on all the residuals. Full cross validation omits one sample at a time and is run n times (where there are n calibration samples). Where a larger subset is removed, the cross validation cycle is usually run at least eight times before the statistics are calculated. Finally, a model is calculated using all the calibration samples.</p> <p>Cross validation should be used with caution. Firstly, cross validation statistics tend to be optimistic when compared with those for an independent test set. Secondly, care should be taken if there is any duplication in the calibration data (e.g. the same sample scanned on several instruments or at different times) to always assign all copies of the same sample to the same cross validation segment; otherwise, very optimistic statistics are produced.</p>
Overfitting	The addition of too many regression terms in a multiple linear regression. As a result, when samples not in the calibration set are predicted, statistics such as s_{RMSEP} or s_{SEP} are much poorer than expected.
PLS factors	See PLS above.
Scores/ score plots	Plots where the scores on one PC or PLS factor are plotted against those of another PC or PLS factor. Most useful if sample ID or concentration values are used to identify each point in the plot. Patterns in the data can then be seen which are not obvious from the raw data.

Statistical expressions	(see also Clause 7)
Bias, \bar{e}	The difference between the mean reference value \bar{y} and the mean value predicted by the NIR model $\bar{\hat{y}}$.
BCL, T_b	The bias confidence limit is the value greater than which a bias is significantly different from zero at the confidence level specified (see 7.3).
SEC, s_{SEC}	For any calibration model, the standard error of calibration (SEC) is an expression of the average difference between predicted and reference values for samples used to derive the model. In this and subsequent statistics, this expression of the average difference refers to the square root of the sum of squared residual values divided by the number of values corrected for degrees of freedom, where 68 % of the errors are below this value.
SECV, s_{SECV}	For a calibration model, the standard error of cross validation (SECV) is an expression of the bias corrected average difference between predicted and reference values for the subset of samples selected as prediction samples during the cross validation process (see “cross validation” above).
SEP, s_{SEP}	<p>The standard error of prediction (SEP) is an expression of the bias corrected average difference between predicted and reference values predicted by a regression model when applied to a set of samples not included in the derivation of the model.</p> <p>The SEP covers a confidence interval of 68 % (multiplied with 1,96 an interval of 95 %).</p>
RMSEP, s_{RMSEP}	<p>The root mean square error of prediction is an expression of the average difference between reference values and those predicted by a regression model when applied to a set of samples not included in the derivation of the model.</p> <p>NOTE RMSEP includes any bias in the predictions.</p>

Statistical expressions	(see also Clause 7)
RMSECV, s_{RMSECV}	The root mean square error of cross validation (RMSECV) is an expression of the average difference between predicted and reference values for the subset of samples selected as prediction samples during the cross validation process (see “cross validation” above). NOTE s_{RMSECV} includes any bias in the predictions.
UECL, T_{UE}	Unexplained error confidence limit, i.e. the limit which a validation s_{SEP} exceeds in order to be significantly different from the s_{SEC} at the confidence level specified.
RSQ (R^2)	The square of the multiple correlation coefficient between predicted and reference values. When expressed as a percentage, it represents the proportion of the variance explained by the regression model.
Slope, b	For a regression line, it represents the amount Y increases for unit increase in X.
Intercept	For a regression line, the value of Y at X = zero.
Residual standard deviation, s_{res}	The residual standard deviation is an expression of the average size of the difference between reference and fitted values after a slope and intercept correction has been performed.
Covariance, $S_{\hat{y}y}$	Covariance is a measure of how much two random variables vary together. If, for a population of samples, an increase in X is matched by an increase in Y, then the covariance between the two variables will be positive. If an increase in X is matched by a decrease in Y, then the covariance will be negative. When values are uncorrelated, then the covariance is zero.
Ue	Measurement uncertainty, characterizing the dispersion of values that can reasonably be attributed to the result.

Bibliography

- [1] ISO 712, *Cereals and cereal products — Determination of moisture content — Reference method*
- [2] ISO 5983-2, *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 2: Block digestion and steam distillation method*
- [3] ISO 6492, *Animal feeding stuffs — Determination of fat content*
- [4] ISO 6496, *Animal feeding stuffs — Determination of moisture and other volatile matter content*
- [5] ISO 6865, *Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration*
- [6] ISO 9622 | IDF 141, *Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry*
- [7] ISO 11085, *Cereals, cereals-based products and animal feeding stuffs — Determination of crude fat and total fat content by the Randall extraction method*
- [8] ISO 13906, *Animal feeding stuffs — Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents*
- [9] ISO 16472, *Animal feeding stuffs — Determination of amylase-treated neutral detergent fibre content (aNDF)*
- [10] ISO 16634-1, *Food products - Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content — Part 1: Oilseeds and animal feeding stuffs*
- [11] ISO 16634-2, *Food products — Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content — Part 2: Cereals, pulses and milled cereal products*
- [12] ISO 20483, *Cereals and pulses — Determination of the nitrogen content and calculation of the crude protein content — Kjeldahl method*
- [13] ISO 6497, *Animal feeding stuffs — Sampling*
- [14] ISO 24333, *Cereals and cereal products — Sampling*
- [15] ISO 21543 | IDF 201, *Milk products — Guidelines for the application of near infrared spectrometry*
- [16] NÆS T., ISAKSSON T., FEARN T., DAVIES T. *A user-friendly guide to multivariate calibration and classification*. NIR Publications, Chichester, 2002
- [17] SHENK J.S., WESTERHAUS M.O., ABRAMS S.M. Protocol for NIRS calibration: monitoring analysis results and recalibration. In: *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of forage quality*. USDA ARS Handbook 643, (MARTEN G.C., SHENK J.S., BARTON F.E. eds.). U.S. Gov. Print. Office, Washington, DC, 1989, pp. 104–110.
- [18] SØRENSEN L.K. Use of routine analytical methods for controlling compliance of milk and milk products with compositional requirements. *IDF Bulletin* **390**, 2004

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act, 2016* to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Head (Publication & Sales), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the website- www.bis.gov.in or www.standardsbis.

This Indian Standard has been developed from Doc No.: FAD 05 (20374).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones: 2323 0131, 2323 3375, 2323 9402

Website: www.bis.gov.in

Regional Offices:

	Telephones
Central : 601/A, Konnectus Tower -1, 6 th Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002	{ 2323 7617
Eastern : 8 th Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091	{ 2367 0012 2320 9474
Northern : Plot No. 4-A, Sector 27-B, Madhya Marg, Chandigarh 160019	{ 265 9930
Southern : C.I.T. Campus, IV Cross Road, Taramani, Chennai 600113	{ 2254 1442 2254 1216
Western : Plot No. E-9, Road No.-8, MIDC, Andheri (East), Mumbai 400093	{ 2821 8093

Branches : AHMEDABAD. BENGALURU. BHOPAL. BHUBANESHWAR. CHANDIGARH. CHENNAI. COIMBATORE. DEHRADUN. DELHI. FARIDABAD. GHAZIABAD. GUWAHATI. HIMACHAL PRADESH. HUBLI. HYDERABAD. JAIPUR. JAMMU & KASHMIR. JAMSHEDPUR. KOCHI. KOLKATA. LUCKNOW. MADURAI. MUMBAI. NAGPUR. NOIDA. PANIPAT. PATNA. PUNE. RAIPUR. RAJKOT. SURAT. VISAKHAPATNAM.