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Soil quality — Determination of carbon and nitrogen by near-infrared spectrometry (NIRS)

Qualité du sol — Dosage du carbone et de l'azote par spectrométrie proche infrarouge (SPIR)

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Soil quality — Determination of carbon and nitrogen by near-infrared spectrometry (NIRS)

1 Scope

This International Standard specifies a method for the determination of carbon and nitrogen in soils by direct measurement of sample spectra in the near-infrared spectral region. The spectra are evaluated by a suitable calibration model derived from the results obtained by reference methods.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11464, *Soil quality — Pretreatment of samples for physico-chemical analysis*

3 Principle

Soil samples are measured by reflectance near-infrared (NIR) spectroscopy. Diffuse reflectance NIR spectroscopy offers a non-destructive means for measurement of soil properties based on reflectance spectra of illuminated soils. Spectral data are evaluated by a suitable calibrating model derived from the measurement of a sufficient number of representative soil samples with known content of carbon and/or nitrogen determined by reference methods. Calibration equations reflect the relationship between the constituents of the sample and NIR spectral information. The soil samples and the set of calibrating samples for the NIR measurement are prepared the same way.

NOTE 1 NIR spectrometry is a very fast non-destructive and environmentally friendly analytical technique when compared to the standard chemical methods used as reference methods.

NOTE 2 Soils generally have similar reflectance spectra in the 1 100 nm to 2 500 nm range. The absorption peaks for soils in the near-infrared region are difficult to assign to specific chemical components.

4 Apparatus

4.1 Near-infrared instrument, based on measurement of reflectance spectra in the near-infrared region (wavelength range from 900 nm to 2 500 nm is usually applied). The instrument should be equipped with a suitable measurement cell for pulverized solid samples. The instrument should measure sufficiently large sample volume to eliminate any significant influence of inhomogeneity of the sample. The software shall allow instrument tests, calibration, sample measurement and data evaluation.

Resolution of the instrument should be equal to 8 nm or better.

NOTE Wavelengths of spectra recorded in higher resolution may be averaged to reduce spectra noise and there is a risk of over fitting of the calibration model. Instruments with lower resolution may be used if their performance is verified for intended purposes.

5 Procedure

5.1 Preparation of samples

Soil samples shall be prepared the same way as soils used for the instrument calibration. Any difference in sample preparation may influence the measurement. Sample preparation shall ensure a good homogenization of the sample.

Sample preparation according to ISO 11464, particle size < 2 mm, is generally used. Air-dried or oven-dried samples can be used for analysis. The method is not suitable for samples with water content higher than 10 %. Oven drying is recommended for samples with high specific surface area that are susceptible to changes in water content due to fluctuations in air humidity.

5.2 Instrument calibration

5.2.1 General

A suitable set of uniformly prepared soil samples is measured by NIR spectroscopy. The spectra and the results of the content of carbon and/or nitrogen determined by a reference method are used for calculation of the calibration model. Calibration should include enough samples to cover most of the possible spectral variability encountered during routine analysis and to predict the composition of unknown samples accurately. The calibration sample set shall be selected to gain an evenly distributed coverage of the property range.

The NIR spectra represent cumulative information about the chemical and physical properties of a sample. Influence of physical properties of a sample (e.g. particle size), is reduced by mathematical corrections as derivatives, standard normal variate (SNV), multiplicative scatter corrections (MSC), etc. There are several possible ways for development of calibration equations and no specific procedure can be given. The choice shall aim at minimising the calibration error. The methods most frequently applied in the development of calibration equations are: PCR (principal component regression), PLS (partial least square regression), LWR (locally weighted regression), SMLR (stepwise multiple linear regression) and ANN (artificial neural network regression). Among these methods, only ANN methods can give calibration for the whole concentration range for carbon and nitrogen in soils but ANN methods only apply with more than 500 calibration samples. For other statistical methods, splitting of the concentration range into two calibrations was found to be the best solution. Removal of outliers from the calibration set usually reduces the robustness of the calibration and should be avoided.

For samples from different locations and soil types, a minimum of 60 to 100 calibration samples is required. A smaller number of calibration samples can be used for sample sets with lower variability such as samples from a defined location.

NOTE 1 Transformations of the reference measurements or the spectra using e.g. log or square root transformed reference measurements may help to reduce the calibration error.

NOTE 2 It is possible that calibrations developed on a certain instrument may not always be transferred directly to an identical instrument. It may be necessary to perform bias and slope adjustments to calibration equations. In many cases it is necessary to standardize the two instruments against each other before calibration equations can be transferred. Standardization procedures can be used to transfer calibrations between instruments of different types provided that samples are measured the same way and that the spectral region is identical.

NOTE 3 If the reference method is unbiased and a good linear calibration model is achieved, increasing number of calibration samples averages out errors in the reference method. Therefore, the lack of repeatability in the reference method can be compensated for by using high number of calibrating samples.

5.2.2 Validation of the calibration model

5.2.2.1 General

There are two main methods for validation of the calibration model:

- cross (internal) validation (see 5.2.2.2), and
- external validation (see 5.2.2.3).

Cross validation (see 5.2.2.2) is used to determine the number of factors used for PLS by determining a minimum RMSECV (root mean square error of cross validation), and when not enough samples are available for external validation. The number of factors shall be as small as possible to avoid over fitting of the calibration model. An external validation (see 5.2.2.3) shall be used to determine the calibration error since cross validations tend to underestimate the calibration error.

In all cases, if a new calibration is developed on an expanded calibration set, the validation process should be repeated. The calibrations should be checked whenever any major part of the instrument (optical system, detector) has been changed or repaired.

Next to the initial validation, NIR calibrations should be validated on a regular basis against reference methods to ensure optimal performance of calibrations. The frequency of checkings depends mainly on the number of changes in the sample population. The number of samples for the continuous checking should be sufficient for the statistics applied. The validation exercise is valid only for the range and for the sample types used in the validation.

The prediction ability of the calibration model is given by the correlation coefficient (R) and the root mean squared error of prediction (RMSEP) which is also called root mean squared error of cross validation (RMSECV) when using cross validation. These characteristics should be reported with the results. If the difference between two parallel measurements is higher than RMSECV or RMSEP, the results may not be valid and should be investigated further.

5.2.2.2 Cross validation

The set of calibration data is divided into K groups, each group has n/K individual samples. One group of the K groups is retained for validation of the model and the remaining $K - 1$ groups are used as training (calibration) data. This step is repeated for all K groups. When cross validation is completed, each sample is measured once for validation and the set of measured predicted values is used for calculation of RMSECV.

NOTE 1 For $K = n$ the method is called leave-one-out cross validation (LOOCV). A single observation is used as the validation data, and the remaining observations are used as training (calibration) data.

NOTE 2 $K = 10$ is commonly used.

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(y_i^* - y_{i,ref}^*)^2}{n}} \quad (1)$$

where

$y_{i,ref}^*$ is the prediction concentration;

y_i^* is the concentration of calibration sample;

n is the number of calibration samples.

5.2.2.3 External validation

Two independent and representative sets of samples are required for external validation: one set is used for calibration while the other set is used for validation. The differences between predictions and the known reference values are used to calculate root mean squared error of prediction (RMSEP).

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i^{\,*} - y^{*}_{\,i,ref}\right)^2}{n_t}} \qquad (2)$$

where

$y^{*}_{i,ref}$ is the prediction concentration;

y^{*}_{i} is the concentration of calibration sample;

n_t is the number of validation samples.

5.2.2.4 Correlation coefficient

The correlation coefficient (R) expresses the correlation between laboratory reference values and values predicted from the NIR measurement.

$$R = \frac{\sum_{i=1}^{N_K}(x_i - x_{av})(y_{i,NIR} - y_{NIR,av})}{\sqrt{\sum_{i=1}^{N_K}(x_i - x_{av})^2 \sum_{i=1}^{N_K}(y_{i,NIR} - y_{NIR,av})^2}} \qquad (3)$$

where

$y_{i,NIR}$ is the predicted concentration;

x_i is the reference concentration of the calibration sample;

$y_{NIR,av}$ is the average concentration of Y_{NIR};

x_{av} is the average concentration of x_i;

N_K is the number of calibration samples.

5.3 Sample measurement

A sub-sample is transferred to the sample cup and the surface is levelled. The spectrum of the sample is scanned following the instrument manual instructions. The number of scans or the dwell time on each wavelength should be large enough to reduce the noise of the measurement to a negligible level and should be part of the calibration definition. The windows are cleaned between the individual measurements (using a soft brush).

It is recommended to carry out additional measurements of the individual sample after moving the sample cell between the individual measurements to minimize the influence of sample inhomogeneity.

The calibration model for an individual sample should be tested for the stability of the model after the spectra measurement. This is necessary because calibrations developed for a certain population of samples may not be valid for samples outside this population, although the analyte concentration range is unchanged. Principal component analysis (PCA) or cluster analysis are usually used for this purpose.

If the sample does not pass the test (that means that the sample does not fit into the population of the samples used for calibration and/or validation) it cannot be determined by the NIR method unless the calibration model is changed. (These samples are usually used for improvement of the calibration model).

If the calibration model is found to be suitable for the measured sample, the spectrum is evaluated according to the validated calibration model. At least one control sample should be measured before and after an uninterrupted series of sample analyses to check instrument hardware stability and to detect any malfunction. The recorded day-to-day variation should be plotted in control charts and investigated for significant patterns or trends. The wavelength or wavenumber accuracy, precision, photometric response and instrument noise should be checked according to the recommendations of the manufacturer.

6 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) identification of the reference method used for calibration;

c) complete identification of the sample;

d) the results of the determination;

e) root mean square error of cross validation (RMSECV) or root mean square error of prediction (RMSEP) and regression coefficient (R) of the calibration equation;

f) any details not specified in this International Standard or which are optional, as well as any factor which may have affected the results.

Annex A
(informative)

Precision data

An interlaboratory trial was organized by Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic in 2011 and 2012. Ten independent data sets from nine participating laboratories from five countries were reported. The data were measured on five different types of instruments.

Validation samples: six soil samples were selected for the validation study. Mineral soils with lower content of organic matter (Sample 1 to Sample 3) and soils with higher content of organic matter (Sample 4 to Sample 6) were measured separately. All soil samples were air-dried and sieved (<2 mm fraction).

Calibration data sets: soil samples for calibration with their results obtained by reference methods were provided to avoid any influence of variability of the reference method. The samples were prepared for direct measurement. There were 60 calibration samples for each concentration range.

The statistical data of results evaluated according to ISO 5725-2[1] are presented in Tables A.1, A.2 and A.3.

Conclusion: repeatability of the method was very good for all parameters in both concentration ranges. Reproducibility of the method was fairly good for higher concentrations of the measurand and acceptable also for low concentrations. The number of samples in the calibration set (60 samples in each range) was found to be adequate for a screening method. Nevertheless the results could be improved if more samples are used for calibration of the instruments.

Table A.1 — Precision data for Cox

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
l	10	8	10	10	10	10
N_{Lab}	10	10	10	10	10	10
n	40	32	40	40	40	40
$w(\mathrm{X})$	5,3	3,9	2	31,7	22,4	36,9
s_R	0,6	1,3	0,7	2	2,6	2,4
s_r	0,3	0,1	0,3	0,9	1,6	0,9
$C_{V,R}$	11,48	34,09	35,49	6,40	11,54	6,46
$C_{V,r}$	6,07	2,63	15,94	2,98	7,31	2,47
R	1,7	3,7	1,9	5,6	7,2	6,6
r	0,9	0,3	0,9	2,6	4,5	2,5
R rel.	31,80	94,43	98,31	17,73	31,98	17,89
r rel.	16,81	7,27	44,17	8,24	20,24	6,84

l	is the number of laboratories after elimination of outliers;
N_{Lab}	is the total number of laboratories;
n	is the number of results;
$w(\mathrm{X})$	is the element mean value in percent (%);
s_R	is the reproducibility in percent (%);
s_r	is the repeatability in percent (%);
$C_{V,R}$	is the coefficient of variation of reproducibility in percent (%);
$C_{V,r}$	is the coefficient of variation of repeatability in percent (%);
R	is the reproducibility limit, R (2,77 × s_R);
r	is the repeatability limit, r (2,77 × s_r);
R rel.	is the reproducibility limit;
r rel.	is the repeatability limit.

Table A.2 — Precision data for Ctot

Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
l	9	9	9	10	10	10
N_{Lab}	10	10	10	10	10	10
n	36	36	36	40	40	40
$w(\mathrm{X})$	5,7	4,2	2,2	32,8	23,5	38,1
s_R	0,8	1,4	0,5	2,3	3	2,6
s_r	0,2	0,1	0,1	1,3	1,6	0,9
$C_{V,R}$	13,94	34,32	24,70	7,17	12,74	6,75
$C_{V,r}$	3,64	3,39	5,84	3,94	6,68	2,34
R	2,2	4	1,5	6,5	8,3	7,1
r	0,6	0,4	0,4	3,6	4,3	2,5
R rel.	38,62	95,08	68,43	19,86	35,30	18,70
r rel.	10,08	9,38	16,19	10,92	18,50	6,47

For explanation of the symbols see Table A.1.