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Animal feeding stuffs — Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES

Aliments des animaux — Détermination des teneurs en calcium, sodium, phosphore, magnésium, potassium, fer, zinc, cuivre, manganèse, cobalt, molybdène, arsenic, plomb et cadmium par ICP-AES

Reference number
ISO 27085:2009(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 27085 was prepared by the European Committee for Standardization Technical Committee CEN/TC 327, *Animal feeding stuffs* (as EN 15510:2008), and was adopted under a special “fast-track procedure”, by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*, in parallel with its approval by the ISO member bodies.

Animal feeding stuffs — Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES

1 Scope

This International Standard specifies an inductively coupled plasma atomic emission spectroscopic (ICP-AES) method for the determination of:

a) the minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, and molybdenum in animal feeding stuffs;

b) the elements arsenic, lead and cadmium in minerals on their own, in premixtures or mixtures for use in animal feeding stuffs.

The method detection limit for each element is dependent on the sample matrix as well as on the instrument. The method is not applicable for determination of low concentrations of elements. The limit of quantification is 3 mg/kg or lower.

NOTE This method can also be used for the determination of minerals in products with high mineral content ($>$ 5 % mass fraction), yet for this purpose other more precise analytical techniques are available.

2 Normative references

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

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*[1)]

3 Terms and definitions

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

3.1
limit of detection
LOD
⟨animal feeding stuffs⟩ smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

NOTE The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations ($n \geqslant 10$, where n is the number of measures) performed under reproducibility conditions.

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1) To be published. (Revision of ISO 6498:1998)

3.2
limit of quantification
LOQ
⟨animal feeding stuffs⟩ lowest content of the analyte which can be measured with reasonable statistical certainty

NOTE If both trueness and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to 10 times the standard deviation of the mean of blank determinations ($n > 10$, where n is the number of measures) performed under reproducibility conditions.

3.3
feed additive
substance or preparation used in animal nutrition in order to: a) affect favourably the characteristics of feed materials or of compound feedingstuffs or of animal products; b) satisfy the nutritional needs of animals or improve animal production, in particular by affecting the gastro-intestinal flora or the digestibility of feedingstuffs; c) introduce nutrition elements conducive to attaining particular nutritional objectives or to meeting the specific nutritional needs of animals at a particular time; d) prevent or reduce the harmful effects caused by animal excretions or improve the animal environment

NOTE Adapted from Council Directive 96/51/EC[3].

3.4
feedingstuff
animal feeding stuff
product of vegetable or animal origin, in its natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, used singly or in mixtures, whether or not containing additives, for oral animal feeding

NOTE Adapted from Council Directive 2002/32/EC[4].

4 Principle

For the determination of the minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, and molybdenum, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic feeding stuffs) or wet digested with hydrochloric acid (in the case of mineral compounds).

For the determination of the elements arsenic, cadmium and lead, a test portion of the sample is wet digested with nitric acid.

The concentration of minerals and the elements iron, zinc, copper, manganese, cobalt, and molybdenum is determined by ICP-AES using external calibration or standard addition technique.

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate health and safety practices and determine the applicability of regulatory limitations prior to use.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade, and water conforming to ISO 3696, grade 2.

5.2 Nitric acid, concentrated, not less than 65 % (mass fraction), having a density of approximately $\rho(HNO_3)$ 1,42 g/ml.

5.3 Dilute nitric acid, 50 % (volume fraction), prepared by mixing 1 volume of nitric acid (5.2) with 1 volume of water.

5.4 Nitric acid solution, 5 % (volume fraction), prepared by pipetting 160 ml dilute nitric acid (5.3) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.5 Nitric acid solution, 2 % (volume fraction), prepared by pipetting 20 ml nitric acid (5.2) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.6 Hydrochloric acid, concentrated, not less than 30 % (mass fraction), having a density of approximately $\rho(\text{HCl})$ 1,15 g/ml.

5.7 Dilute hydrochloric acid, 50 % (volume fraction), prepared by mixing 1 volume of hydrochloric acid (5.6) with 1 volume of water.

5.8 Hydrochloric acid solution, 1 % (volume fraction), prepared by pipetting 60 ml dilute hydrochloric acid (5.7) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.9 Element stock solutions, Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo, Cd, Pb, As, ρ = 1 000 mg/l.

The user should choose a suitable stock solution. Both single-element stock solutions and multi-element stock solutions with adequate specification stating the acid used and the preparation technique are commercially available. It is advisable to use certified stock solutions.

Stock solutions shall be replaced after one year, if nothing else is mentioned.

Element stock solutions with concentrations different from 1 000 mg/l may also be used.

5.10 Standard solutions

5.10.1 General

Depending on the scope, different multi-element standard solutions may be necessary. In general, when combining multi-element standard solutions, their chemical compatibility and the possible hydrolysis of the components shall be taken into account. The examples given in 5.10.2 to 5.10.4 also take into account the measuring range of various ICP-AES instruments and the expected concentration of the element in animal feeding stuffs.

The multi-element standard solutions are considered to be stable for several months, if stored in the dark (observe the expiry date specified by the manufacturer).

Other combinations of elements at different concentrations can be used, provided that the element stock solutions (5.9) are diluted with the same acid at a similar concentration to the acid in the test solution to prepare a range of standards that covers the concentrations of the elements to be determined.

5.10.2 Multi-element standard solution — Minerals in HCl (5.8), ρ(Ca, Na, P, Mg, K) = 40 mg/l.

Pipette 40,0 ml of each element stock solution (Ca, Na, P, Mg, K) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml dilute hydrochloric acid (5.7). Make up to the mark with water and transfer to a suitable storage bottle.

5.10.3 Multi-element standard solution — Fe, Zn, Cu, Mn, Co, Mo in HCl (5.8), ρ(Fe, Zn, Cu, Mn, Co, Mo) = 50 mg/l.

Pipette 50,0 ml of each element stock solution (Fe, Zn, Cu, Mn, Co, Mo) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml dilute hydrochloric acid (5.7). Make up to the mark with water and transfer to a suitable storage bottle.

5.10.4 Multi-element standard solution — Cd, Pb, As in HNO_3 (5.4), ρ(Cd, Pb, As) = 100 mg/l.

Pipette 100,0 ml of each element stock solution (Cd, Pb, As) (5.9) into a 1 000 ml volumetric flask (6.7). Add 160 ml dilute nitric acid (5.3). Make up to the mark with water and transfer to a suitable storage bottle.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Laboratory grinders

6.1.1 Laboratory grinder capable of grinding to a particle size of less than or equal to 1 mm, e.g. a knife mill or equivalent.

6.1.2 Laboratory grinder capable of grinding to a particle size of less than or equal to 0,1 mm, e.g. a ball mill or equivalent.

6.1.3 Mortar with pestle, free of contamination.

6.2 Analytical balance, capable of weighing to an accuracy of 1 mg.

6.3 Electric hot plate, with temperature control.

6.4 Ashing crucibles, of platinum, quartz or porcelain.

6.5 Electric muffle-furnace, capable of being maintained at a temperature of 450 °C ± 20 °C.

The real temperature in the furnace has to be checked, because this temperature may be substantially different from the adjust temperature.

6.6 Beakers, of capacities 100 ml, 250 ml.

6.7 One-mark volumetric flasks, of capacities 100 ml, 500 ml, 1 000 ml.

6.8 ICP-AES instrument

The instrument shall be equipped with a radial plasma as a minimum requirement; an axial plasma is equally acceptable. Background correction shall also be performed when necessary. Settings of the working conditions (e.g. viewing height, gas flows, RF or plasma power, sample uptake rate, integration time, number of replicates) shall be optimized according to the manufacturer's instructions.

6.9 Freeze drying equipment, capable of freeze-drying liquid animal feeding stuffs.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497[2].

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

8 Preparation of the test sample

8.1 General

Prepare the test sample in accordance with ISO 6498:

- the grinding shall be done in conditions such that the substance is not appreciably heated;
- the operation is to be repeated as many times as is necessary and it shall be effected as quickly as possible in order to prevent any gain or loss of constituents (water);
- the whole ground product is placed in a flask made of e.g. polypropylene, which can be stoppered and stored in such way to prevent any change in composition;
- before any weighing is carried out for the analysis, the whole test sample shall be thoroughly mixed for reasons of homogeneity.

8.2 Animal feeding stuffs which can be ground as such

Grind the laboratory sample (usually 500 g), using a grinder (6.1.1) or mortar, until a particle size of 1 mm or less has been reached.

8.3 Liquid animal feeding stuffs

Liquid feeding stuffs shall be pre-dried according to the procedure described in 8.3.1 or freeze-dried according to the procedure described in 8.3.2.

8.3.1 Pre-drying

Pre-dry the laboratory sample at a temperature of 70 °C ± 5 °C during at least 16 h to reduce the moisture content. The mass of the sample before and after the pre-drying is to be determined using an analytical balance (6.2). Grind the pre-dried sample in accordance with 8.2.

8.3.2 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (6.9). The mass of the sample before and after the freeze-drying is to be determined using an analytical balance (6.2). Grind the freeze-dried sample in accordance with 8.2.

8.4 Mineral animal feeding stuffs

Mineral compounds, except mineral products containing crystalline water, e.g. $MgCl_2 \cdot 6H_2O$, shall be ground using a grinder (6.1.2) or mortar until a particle size of 0,1 mm or less has been reached. Mineral products containing crystalline water should not be ground.

9 Procedure

9.1 Digestion

9.1.1 Selection of the procedure

9.1.1.1 Determination of Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo

If the test sample concerns a mineral compound or a product potentially containing phosphates, proceed in accordance with 9.1.2.

If the test sample contains organic substances and if it is free from phosphates rendering insoluble products on ashing, proceed in accordance with 9.1.3.

If the test sample contains organic substances and phosphates, proceed in accordance with 9.1.2.

9.1.1.2 Determination of Cd, Pb, As in minerals

For the determination of Cd, Pb and As in minerals, proceed in accordance with 9.1.4.

9.1.2 Extract with HCl (5.8)

Weigh about 1 g of the prepared test sample to the nearest 1 mg into a 250 ml beaker (6.6).

Add 30 ml dilute hydrochloric acid (5.7). Add about 100 ml water.

Cover the beaker (6.6) with a watch-glass and boil for 30 min on a hot plate (6.3).

Allow to cool. Transfer the liquid into a 500 ml volumetric flask (6.7), rinsing the beaker and the watch-glass several times with water.

Leave to cool, make up to the mark with water.

After homogenizing, filter through a dry folded filter paper into a dry Erlenmeyer flask. Use the first portion of the filtrate to rinse the glassware and discard that part. If the determination is not carried out immediately, the container with the filtrate shall be stoppered.

Carry out a blank test at the same time as the extraction with only the reagents, and follow the same procedure as for the samples.

Proceed in accordance with 9.2.

When the expected concentration of the element is lower than 100 mg/kg, proceed as described in this subclause, but use 6 ml dilute hydrochloric acid (5.7) and about 70 ml water, and transfer the liquid into a 100 ml volumetric flask (6.7).

9.1.3 Dry ashing — HCl (5.8)

Weigh about 5 g of the prepared test sample to the nearest 1 mg in an ashing crucible (6.4).

Ash in the furnace (6.5), set at a temperature of 450 °C, until white or grey ash is obtained (a small quantity of carbon does not interfere).

Transfer the ash to a 250 ml beaker (6.6) with 30 ml dilute hydrochloric acid (5.7). Add about 100 ml water.

Cover the beaker (6.6) with a watch-glass and boil for 30 min on a hot plate (6.3).

Allow to cool. Transfer the liquid into a 500 ml volumetric flask (6.7), rinsing the beaker and the watch-glass several times with water.

Leave to cool, make up to the mark with water.

After homogenizing, filter through a dry folded filter paper into a dry Erlenmeyer flask. Use the first portion of the filtrate to rinse the glassware and discard that part. If the determination is not carried out immediately, the container with the filtrate shall be stoppered.

Carry out a blank test at the same time as the extraction with only the reagents, and follow the same procedure as for the samples.

Proceed in accordance with 9.2.

When the expected concentration of the element is lower than 100 mg/kg, proceed as described in this subclause, but use 6 ml dilute hydrochloric acid (5.7) and about 70 ml water, and transfer the liquid into a 100 ml volumetric flask (6.7).

9.1.4 Extract with HNO_3 (5.4)

Weigh about 2 g of the prepared test sample to the nearest 1 mg into a 250 ml beaker (6.6).

Add 16 ml dilute nitric acid (5.3). Add about 70 ml water.

Cover the beaker (6.6) with a watch-glass and boil for 30 min on a hot plate (6.3).

Allow to cool. Transfer the liquid into a 100 ml volumetric flask (6.7), rinsing the beaker and the watch-glass several times with water.

Leave to cool, make up to the mark with water.

After homogenizing, filter through a dry folded filter paper into a dry Erlenmeyer flask. Use the first portion of the filtrate to rinse the glassware and discard that part. If the determination is not carried out immediately, the container with the filtrate shall be stoppered.

Carry out a blank test at the same time as the extraction with only the reagents, and follow the same procedure as for the samples.

Proceed in accordance with 9.2.

9.2 Calibration

9.2.1 General

Calibration shall be performed by means of external calibration or standard addition technique or internal standard. It is important that the measurements be made in the linear range of the instrument. Appropriate matrix matching of the calibration solutions shall be performed if an (external) calibration method is used (see Annex B).

9.2.2 External calibration

The calibration is performed with at least two calibration solutions of which one is a blank calibration solution. In all cases linearity should be checked on a regular basis. If linearity is guaranteed, calibrate with at least two calibration solutions; if linearity is not guaranteed, calibrate with three calibration solutions (B.3.2).

Proceed as described in this subclause using an internal standard such as beryllium by use of an additional tube on the peristaltic pump and a Y connector. Follow the instrument manufacturer's instructions for internal standard calibration.

9.2.3 Standard addition technique

The standard addition curve should consist of at least two points of which one should be an addition (B.3.4). For those elements whose concentration is near the limit of quantification, the standard addition curve should consist of at least four points of which three should be additions. If three additions are used, the concentration of the highest standard should be three times to five times the concentration in the sample solution.

9.2.4 Example of calibration with one addition after dry ashing — HCl (5.8)

EXAMPLE Determination of copper in a mixed feed with expected concentration 200 mg/kg Cu.

9.2.4.1 Preparation of the test solution

Pipette 50,0 ml of the filtrate of the test portion (9.1.3) into a 100 ml volumetric flask (6.7) and make up to the mark with dilute hydrochloric acid (5.8).

9.2.4.2 Preparation of the blank solution

Pipette 50,0 ml of the filtrate of the blank test (9.1.3) into a 100 ml volumetric flask (6.7) and make up to the mark with hydrochloric acid solution (5.8).

9.2.4.3 Preparation of the addition

Pipette 50,0 ml of the filtrate of the test portion (9.1.3) into a 100 ml volumetric flask (6.7), add 2,0 ml of the multi-element standard solution — Fe, Zn, Cu, Mn, Co, Mo in HCl (5.8) (see 5.10.3) — and make up to the mark with hydrochloric acid solution (5.8).

9.2.5 Example of calibration with one addition after wet digestion — HCl (5.8)

EXAMPLE Determination of calcium in a mineral compound with expected concentration 2 000 mg/kg Ca.

9.2.5.1 Preparation of the test solution

Pipette 50,0 ml of the filtrate of the test portion (9.1.2) into a 100 ml volumetric flask (6.7) and make up to the mark with hydrochloric acid solution (5.8).

9.2.5.2 Preparation of the blank solution

Pipette 50,0 ml of the filtrate of the blank test (9.1.2) into a 100 ml volumetric flask (6.7) and make up to the mark with hydrochloric acid solution (5.8).

9.2.5.3 Preparation of the addition

Pipette 50,0 ml of the filtrate of the test portion (9.1.2) into a 100 ml volumetric flask (6.7), add 10 ml of the multi-element standard solution — minerals in HCl (5.8) (see 5.10.2) — and make up to the mark with hydrochloric acid solution (5.8).

9.2.6 Example of calibration with one addition after wet digestion — HNO_3 (5.4)

EXAMPLE Determination of arsenic in a mineral compound with expected concentration 20 mg/kg As.

9.2.6.1 Preparation of the test solution

Pipette 10,0 ml of the filtrate of the test portion (9.1.4) into a test tube for ICP.

9.2.6.2 Preparation of the blank solution

Pipette 10,0 ml of the filtrate of the blank test (9.1.4) into a test tube for ICP.

9.2.6.3 Preparation of the addition

Pipette 10,0 ml of the filtrate of the test portion (9.1.4) into a test tube for ICP. Pipette 40 µl of the multi-element standard solution — Cd, Pb, As in HNO_3 (5.4) (see 5.10.4).

9.3 Determination

9.3.1 General

Analytical lines, selectivity, limits of determination and quantification, precision, linear working area, and interferences have to be established before operating the ICP-AES system.

9.3.2 Determination by ICP-AES

Table 1 gives relevant analytical lines and possible interferences for determination by ICP-AES. Other wavelengths than those specified in Table 1 can also be used (see Annex B).

Table 1 — Selected emission wavelengths and interferences for determination by ICP-AES

Element	Wavelength of emission (nm)	Interference
As	188,979	
	189,042	
	193,696	
	197,197	
Ca	315,887	Co
	317,933	Fe, V
	393,366	
Cd	214,438	
	226,502	
	228,802	
Co	228,616	Ti
Cu	324,754	Ti, Fe
	327,396	
Fe	238,200	Co
	259,940	
K	766,490	Mg, Ar
	769,900	
Mg	279,079	
	279,553	
	285,213	Fe
Mn	257,610	Fe, Mo, Cr
	293,306	Al, Fe
Mo	202,030	Al, Fe
	204,598	
Na	330,237	
	588,995	
	589,592	Ar
P	178,287	I
	213,618	Cu, Fe, Mo, Zn
	214,914	Cu, Al, Mg
	177,428	Cu
Pb	216,999	
	220,353	
	261,418	
Zn	206,200	
	213,856	P

9.3.2.1 External calibration method

Aspirate the blank test solution (9.1), the calibration solutions (9.2.1), and the test solution (9.1) in ascending order separately into the plasma and measure the emission of the element to be determined. Each value should be determined from at least three individual measurements. Average the values if the values fall within an accepted range. After each measurement, aspirate nitric acid solution (5.5).

9.3.2.2 Standard addition technique

Aspirate the blank test solution (9.2.4.2, 9.2.5.2 or 9.2.6.2), the test solution (9.2.4.1, 9.2.5.1 or 9.2.6.1), and the addition (9.2.4.3, 9.2.5.3 or 9.2.6.3) in ascending order separately into the plasma, and measure the emission of the element to be determined. Perform at least two replicates. Average the values if the values fall within an accepted range. After each measurement, aspirate nitric acid solution (5.5).

10 Calculation and expression of the result

NOTE Net signal is defined as the number of counts at the selected wavelength, corrected for background contributions.

10.1 External calibration

For a linear calibration curve constructed with one blank calibration solution and one calibration solution, the calibration function can be described as follows:

$$S_{\mathrm{st}} = \rho_{\mathrm{st}}\, b + a \tag{1}$$

where

S_{st} is the net signal of the calibration solution;

ρ_{st} is the concentration, in milligrams per litre, of the calibration solution.

Calculate the element concentration, ρ_{f}, in milligrams per litre, in the filtrate of the test portion using the slope b and the intersection a found in Equation (1) as follows:

$$\rho_{\mathrm{f}} = \frac{S_{\mathrm{f}} - a}{b} \tag{2}$$

where S_{f} is the net signal of the test solution.

10.2 Standard addition method with only one addition

In the most simple case of standard addition, where only one addition is made, the element concentration, ρ_{f}, in milligrams per litre, in the filtrate of the test portion is determined as follows:

$$\rho_{\mathrm{f}} = \frac{S_0\, V_{\mathrm{s}}\, \rho_{\mathrm{s}}}{\left(S_1 - S_0\right) V_{\mathrm{f}}} \tag{3}$$

where

ρ_{s} is the concentration, in milligrams per litre, of the standard solution;

V_{s} is the volume, in litres, of the standard solution added;

V_{f} is the volume, in litres, of the filtrate of the test portion used to prepare the test solution;

S_0 is the net signal of the test solution;

S_1 is the net signal after addition.

10.3 Standard addition method with several additions

In case of several additions, regression techniques on the linear model of variable y as a function of variable x, have to be used to determine the element concentration of the test solution. Generally, this model can be written as:

$$y_i = a + b\, x_i \tag{4}$$

In this particular case of three standard additions,

$$y_i = S_i \quad \text{(for } i = 0, 1, 2, 3\text{)} \tag{5}$$

$$x_i = \rho_\text{s} V_i \quad \text{(for } i = 0, 1, 2, 3\text{)} \tag{6}$$

where

ρ_s is the concentration, in milligrams per litre, of the standard solution;

V_i are the various volumes, in litres, of the standard solution added;

S_i are the net signals after the various additions.

The values of a and b can then be calculated as follows:

$$b = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{n \sum x_i^2 - \left(\sum x_i\right)^2} \tag{7}$$

$$a = \frac{\sum y_i - b \sum x_i}{n} \tag{8}$$

where n is the number of solutions measured (n = 4 in the case of three additions).

The element concentration, ρ_f, in milligrams per litre, of the filtrate of the test portion can then be found using Equation (9):

$$\rho_\text{f} = \frac{a}{b\, V_\text{f}} \tag{9}$$

where V_f is the volume, in litres, of the filtrate of the test portion used to prepare the test solution.

10.4 Calculation of the element content in the sample

The element content in the sample or mass fraction of element w_elem, expressed in milligram of element per kilogram of animal feeding stuff, is determined using Equation (10):

$$w_\text{elem} = \frac{\left(\rho_\text{f} - \rho_\text{bl}\right)}{m} V_\text{t} \tag{10}$$

where

ρ_f is the concentration, in milligrams per litre, of the filtrate of the test portion, as determined using Equations (2), (3) or (9);

ρ_bl is the concentration, in milligrams per litre, of the blank solution;

m is the mass of sample, in kilograms, taken for the extraction, and corrected for water content;

V_t is the total volume, in litres, of the extract (filtrate of the test portion).

If the sample has been diluted, take into account the dilution factor.

If the sample has been pre-dried or freeze dried (8.3), recalculate the result to the fresh weight of the sample taking into account the loss of moisture during pre-drying or freeze-drying.

The result of the determination is expressed as a percentage mass fraction for the minerals Ca, Na, P, Mg and K, and in milligrams per kilogram for the elements Fe, Zn, Cu, Mn, Co, Mo and for the elements As, Cd, Pb.

10.5 Example of calculation after standard addition technique with one addition

Applying standard addition, with one addition, to determine the copper content (see 9.2.4), resulted in the values 76 057 counts, 152 440 counts, 0,050 l, 0,002 l and 50,00 mg/l for S_0, S_1, V_f, V_s, and ρ_s, respectively. As a result, the concentration ρ_f from Equation (3) equals 1,99 mg/l.

The mass of sample, m, taken for the extraction being 0,005 kg, and the total volume, V_t, of extract being 0,500 l, the copper content in the sample or mass fraction of copper w_{Cu}, can be calculated using Equation (10) as 199 mg/kg. In this case the concentration of the blank solution is considered to be zero.

11 Precision

11.1 Interlaboratory test

Two interlaboratory tests were carried out in 2004 and 2005. Details of interlaboratory tests on the precision of the method are summarized in Annex A. The values derived from these tests may not be applicable to concentration ranges and matrices others than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using 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, shall in not more than 5 % of cases be greater than the repeatability limits, r, given in Table 2 (minerals Ca, Na, Mg, P, K), Table 3 (elements Fe, Mn, Cu, Zn, Co, Mo) and Table 4 (As, Pb, Cd).

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in the same laboratory with different operators using different equipment, shall in not more than 5 % of cases be greater than the reproducibility limits, R, given in Table 2 (minerals Ca, Na, Mg, P, K), Table 3 (elements Fe, Mn, Cu, Zn, Co, Mo) and Table 4 (As, Pb, Cd).

Table 2 — Precision data — Ca, Na, Mg, P, K

	Ca		
Samples	**Mean**, $\bar{w}_{\mathrm{Ca}}$ (%)	r (%)	R (%)
Pig feed 1)	1,09	0,07	0,15
Sheep feed 1)	1,00	0,05	0,16
Phosphate 1)	10,78	0,47	1,34
Mineral premixture 1)	21,78	1,25	2,58
Mineral mixture 1)	2,43	0,17	0,56
Mineral mixture 2)	14,6	0,7	2,9
	Na		
Samples	**Mean**, $\bar{w}_{\mathrm{Na}}$ (%)	r (%)	R (%)
Pig feed 1)	0,17	0,02	0,04
Sheep feed 1)	0,40	0,04	0,08
Phosphate 1)	0,11	0,02	0,04
Mineral premixture 1)	6,56	0,42	0,75
Mineral mixture 2)	11,5	0,9	2,9
	Mg		
Samples	**Mean**, $\bar{w}_{\mathrm{Mg}}$ (%)	r (%)	R (%)
Pig feed 1)	0,21	0,02	0,05
Sheep feed 1)	0,38	0,02	0,07
Phosphate 1)	11,12	0,66	1,73
Mineral premixture 1)	0,36	0,03	0,06
Mineral mixture 1)	10,31	0,50	1,03
	P		
Samples	**Mean**, $\bar{w}_{\mathrm{P}}$ (%)	r (%)	R (%)
Pig feed 1)	0,49	0,03	0,09
Sheep feed 1)	0,50	0,03	0,08
Phosphate 1)	19,48	0,84	1,67
Mineral mixture 1)	0,023	0,01	0,01
Mineral mixture 2)	4,07	0,17	0,60
	K		
Samples	**Mean**, $\bar{w}_{\mathrm{K}}$ (%)	r (%)	R (%)
Pig feed 1)	0,93	0,08	0,26
Sheep feed 1)	1,18	0,06	0,27
Phosphate 1)	0,076	0,01	0,02
Mineral premixture 1)	0,13	0,02	0,06
Mineral mixture 2)	0,04	0,01	0,03

1) Ring test 1.

2) Ring test 2.

Table 3 — Precision data — Fe, Mn, Cu, Zn, Co, Mo

	Fe		
Samples	**Mean**, $\bar{w}_{\mathrm{Fe}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	293	26	81
Sheep feed [1)]	407	36	95
Phosphate [1)]	2 629	194	380
Mineral premixture [1)]	5 561	752	1 601
Mineral mixture [1)]	8 182	544	1 241
Mineral mixture [2)]	3 215	240	837
	Mn		
Samples	**Mean**, $\bar{w}_{\mathrm{Mn}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	127	15	25
Sheep feed [1)]	92,8	12	16
Phosphate [1)]	135	11	19
Mineral premixture [1)]	3 527	620	952
Mineral mixture [1)]	215	34	94
Mineral mixture [2)]	2 188	117	490
	Cu		
Samples	**Mean**, $\bar{w}_{\mathrm{Cu}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	166	18	41
Sheep feed [1)]	13,8	2,4	3,1
Phosphate [1)]	11,1	1,3	3,9
Mineral premixture [1)]	514	41	124
Mineral mixture [1)]	6,8	1,5	4,3
Mineral mixture [2)]	775	252	304
	Zn		
Samples	**Mean**, $\bar{w}_{\mathrm{Zn}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	169	16	34
Sheep feed [1)]	119	17	29
Phosphate [1)]	181	11	25
Mineral premixture [2)]	3 574	334	735
Mineral mixture [1)]	27,4	6,6	15
Mineral mixture [2)]	3 626	183	827
	Co		
Samples	Mean, $\bar{w}_{\mathrm{Co}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	0,75	0,25	0,52
Sheep feed [1)]	1,13	0,27	0,57
Phosphate [1)]	1,07	0,14	0,80
Mineral premixture [1)]	35,0	6,3	23,9
Mineral premixture [2)]	19 942	1 661	6 849
Mineral mixture [1)]	3,34	0,80	1,43

Table 3 (*continued*)

	Mo		
Samples	**Mean**, $\bar{w}_{\mathrm{Mo}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Pig feed [1)]	1,09	0,40	0,75
Sheep feed [1)]	1,21	0,18	1,09
Phosphate [1)]	2,30	0,54	1,33
Mineral premixture [1)]	1,06	0,46	0,75
Mineral premixture [2)]	16 672	1 448	5 283

[1)] Ring test 1.

[2)] Ring test 2.

Table 4 — Precision data — As, Pb, Cd

	As		
Samples	**Mean**, $\bar{w}_{\mathrm{As}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Phosphate [2)]	4,56	0,58	1,54
MgO [2)]	6,04	1,23	3,18
$CaCO_3$ [2)]	7,92	2,04	4,88
Bentonite [2)]	10,3	1,03	3,76
Mineral mixture [2)]	3,44	0,41	1,36
	Pb		
Samples	**Mean**, $\bar{w}_{\mathrm{Pb}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Phosphate [2)]	4,93	0,76	2,61
$CaCO_3$ [2)]	4,88	1,27	2,93
Bentonite [2)]	38,7	2,03	6,34
$CuSO_4$ [2)]	6,26	1,41	3,55
Mineral mixture [2)]	1,86	0,36	0,72
	Cd		
Samples	Mean, $\bar{w}_{\mathrm{Cd}}$ (mg/kg)	r (mg/kg)	R (mg/kg)
Phosphate [1)]	4,78	0,32	2,35
Phosphate [2)]	5,15	0,44	1,83

[1)] Ring test 1.

[2)] Ring test 2.

12 Test report

The test report shall contain at least the following information:

a) the test method used, with reference to this International Standard;

b) all information necessary for the complete identification of the sample;

c) any particular points observed in the course of the test;

d) all operation details not specified in this document, or regarded as optional, together with details of any incidents which might have affected the results;

e) the results obtained of the determination, expressed as mass fraction w_{elem}, in milligrams per kilogram of animal feeding stuff or as a percentage mass fraction for the minerals.

Annex A
(informative)

Results of the interlaboratory tests

Two interlaboratory tests were carried out in 2004 (ring test 1 = $^{(1)}$) and 2005 (ring test 2 = $^{(2)}$) with 30 participating laboratories and 11 different animal feeding stuffs, including a complete feed for pigs, a complete feed for sheep, two different rock phosphates ($^{(1)}$ and $^{(2)}$), two different mineral mixtures ($^{(1)}$ and $^{(2)}$), two different mineral premixtures ($^{(1)}$ and $^{(2)}$), $CaCO_3$, $CuSO_4$, MgO, and bentonite. The samples were homogenized centrally and distributed to the participants. The tests yielded the data given in Tables A.1, A.2, and A.3. Repeatability and reproducibility were calculated according to ISO 5725-1[1]. The element cadmium was analysed in all samples. Yet, the cadmium content of all the samples except that of the phosphates ($^{(1)}$ and $^{(2)}$) was lower than the limit of quantification of the method. Consequently, statistical data on cadmium are only available for phosphates.

Table A.1 — Statistical results of interlaboratory tests — Ca, Na, Mg, P, K

Parameter	Ca					
	Pig feed (1)	Sheep feed (1)	Phosphate (1)	Mineral premix (1)	Mineral mix (1)	Mineral mix (2)
Number of laboratories	12	12	12	12	12	12
Number of laboratories after elimination of outliers	10	12	11	12	12	12
Number of outliers	2	0	1	0	0	0
Mean value, $\bar{w}_{\text{Ca}}$ (%)	1,09	1,00	10,78	21,78	2,43	14,6
Repeatability standard deviation, s_r (%)	0,026	0,017	0,17	0,45	0,062	0,25
Repeatability limit, r (%)	0,07	0,05	0,47	1,25	0,17	0,7
Reproducibility standard deviation, s_R (%)	0,055	0,058	0,48	0,92	0,20	1,03
Reproducibility limit, R (%)	0,15	0,16	1,34	2,58	0,56	2,9
Horwitz ratio, HorRat R	1,3	1,5	1,6	1,7	2,3	2,7

Parameter	Na				
	Pig feed (1)	Sheep feed (1)	Phosphate (1)	Mineral premix (1)	Mineral mix (2)
Number of laboratories	12	12	13	12	12
Number of laboratories after elimination of outliers	12	12	12	11	10
Number of outliers	0	0	1	1	2
Mean value, $\bar{w}_{\text{Na}}$ (%)	0,17	0,40	0,11	6,56	11,5
Repeatability standard deviation, s_r (%)	0,008	0,013	0,008	0,15	0,34
Repeatability limit, r (%)	0,02	0,04	0,02	0,42	0,9
Reproducibility standard deviation, s_R (%)	0,013	0,029	0,013	0,27	0,88
Reproducibility limit, R (%)	0,04	0,08	0,04	0,75	2,5
Horwitz ratio, HorRat R	1,5	1,6	2,0	1,4	2,8

Table A.1 (*continued*)

Parameter	Mg				
	Pig feed (1)	**Sheep feed** (1)	**Phosphate** (1)	**Mineral premix** (1)	**Mineral mix** (1)
Number of laboratories	12	12	13	11	13
Number of laboratories after elimination of outliers	11	12	13	9	11
Number of outliers	1	0	0	2	2
Mean value, $\bar{w}_{\text{Mg}}$ (%)	0,21	0,38	11,1	0,36	10,31
Repeatability standard deviation, s_r (%)	0,008	0,009	0,23	0,012	0,18
Repeatability limit, r (%)	0,02	0,02	0,66	0,03	0,50
Reproducibility standard deviation, s_R (%)	0,016	0,024	0,62	0,021	0,37
Reproducibility limit, R (%)	0,05	0,07	1,73	0,06	1,03
Horwitz ratio, HorRat R	1,5	1,4	2,0	1,2	1,3

Parameter	P				
	Pig feed (1)	**Sheep feed** (1)	**Phosphate** (1)	**Mineral mix** (1)	**Mineral mix** (2)
Number of laboratories	12	12	12	12	12
Number of laboratories after elimination of outliers	11	12	12	10	12
Number of outliers	1	0	0	2	0
Mean value, $\bar{w}_{\text{P}}$ (%)	0,49	0,50	19,48	0,023	4,07
Repeatability standard deviation, s_r (%)	0,009	0,010	0,30	0,003	0,06
Repeatability limit, r (%)	0,03	0,03	0,84	0,01	0,17
Reproducibility standard deviation, s_R (%)	0,032	0,027	0,60	0,004	0,21
Reproducibility limit, R (%)	0,09	0,08	1,67	0,01	0,60
Horwitz ratio, HorRat R	1,5	1,2	1,2	2,5	1,6

Parameter	K				
	Pig feed (1)	**Sheep feed** (1)	**Phosphate** (1)	**Mineral premix** (1)	**Mineral mix** (2)
Number of laboratories	12	12	12	12	12
Number of laboratories after elimination of outliers	12	12	11	12	11
Number of outliers	0	0	1	0	1
Mean value, $\bar{w}_{\text{K}}$ (%)	0,93	1,18	0,076	0,13	0,04
Repeatability standard deviation, s_r (%)	0,027	0,02	0,003	0,009	0,004
Repeatability limit, r (%)	0,08	0,06	0,01	0,02	0,01
Reproducibility standard deviation, s_R (%)	0,092	0,10	0,009	0,02	0,01
Reproducibility limit, R (%)	0,26	0,27	0,02	0,06	0,03
Horwitz ratio, HorRat R	2,4	2,1	1,9	2,9	3,6

Table A.2 — Statistical results of interlaboratory tests — Fe, Mn, Cu, Zn, Co, Mo

	Fe					
Parameter	**Pig feed** (1)	**Sheep feed** (1)	**Phosphate** (1)	**Mineral premix** (1)	**Mineral mix** (1)	**Mineral mix** (2)
Number of laboratories	11	12	12	12	13	12
Number of laboratories after elimination of outliers	11	12	11	11	13	12
Number of outliers	0	0	1	1	0	0
Mean value, $\bar{w}_{\mathrm{Fe}}$ (mg/kg)	293	407	2 629	5 561	8 182	3 215
Repeatability standard deviation, s_r (mg/kg)	9	13	69	269	194	86
Repeatability limit, r (mg/kg)	26	36	194	752	544	240
Reproducibility standard deviation, s_R (mg/kg)	29	34	136	572	443	299
Reproducibility limit, R (mg/kg)	81	95	380	1 601	1 241	837
Horwitz ratio, HorRat R	1,5	1,3	1,1	2,4	1,3	2,0
	Mn					
Parameter	**Pig feed** (1)	**Sheep feed** (1)	**Phosphate** (1)	**Mineral premix** (1)	**Mineral mix** (1)	**Mineral mix** (2)
Number of laboratories	12	12	12	12	13	12
Number of laboratories after elimination of outliers	12	11	11	11	13	12
Number of outliers	0	1	1	1	0	0
Mean value, $\bar{w}_{\mathrm{Mn}}$ (mg/kg)	127	92,8	135	3 527	215	2 188
Repeatability standard deviation, s_r (mg/kg)	5	4	4	221	12	42
Repeatability limit, r (mg/kg)	15	12	11	620	34	117
Reproducibility standard deviation, s_R (mg/kg)	9	6	7	340	34	175
Reproducibility limit, R (mg/kg)	25	16	19	952	94	490
Horwitz ratio, HorRat R	0,9	0,8	0,7	2,1	2,2	1,6
	Cu					
Parameter	**Pig feed** (1)	**Sheep feed** 1)	**Phosphate** (1)	**Mineral premix** (1)	**Mineral mix** (1)	**Mineral mix** (2)
Number of laboratories	11	11	11	12	11	12
Number of laboratories after elimination of outliers	11	10	10	11	11	11
Number of outliers	0	1	1	1	0	1
Mean value, $\bar{w}_{\mathrm{Cu}}$ (mg/kg)	166	13,8	11,1	514	6,83	775
Repeatability standard deviation, s_r (mg/kg)	6,4	0,85	0,48	15	0,55	90
Repeatability limit, r (mg/kg)	18	2,4	1,3	41	2,5	252
Reproducibility standard deviation, s_R (mg/kg)	15	1,1	1,4	44	1,5	109
Reproducibility limit, R (mg/kg)	41	3,1	3,9	124	4,3	304
Horwitz ratio, HorRat R	1,2	0,7	1,1	1,4	1,9	2,4

Table A.2 (*continued*)

Parameters	Zn					
	Pig feed (1)	Sheep feed (1)	Phosphate (1)	Mineral premix (1)	Mineral mix (1)	Mineral mix (2)
Number of laboratories	12	12	12	12	13	12
Number of laboratories after elimination of outliers	11	12	11	12	13	12
Number of outliers	1	0	1	0	0	0
Mean value, $\bar{w}_{\text{Zn}}$ (mg/kg)	169	119	181	3 574	27,4	3 826
Repeatability standard deviation, s_r (mg/kg)	5,9	6,2	3,9	119	2,4	65
Repeatability limit, r (mg/kg)	16	17	11	334	6,6	183
Reproducibility standard deviation, s_R (mg/kg)	12	10	9,1	263	5,3	295
Reproducibility limit, R (mg/kg)	34	29	25	735	15	827
Horwitz ratio, HorRat R	1,0	1,1	0,7	1,6	2,0	1,7

Parameters	Co					
	Pig feed (1)	Sheep feed (1)	Phosphate (1)	Mineral premix (1)	Mineral premix (2)	Mineral mix (1)
Number of laboratories	8	10	6	10	12	10
Number of laboratories after elimination of outliers	8	10	6	10	12	10
Number of outliers	0	0	0	0	0	0
Mean value, $\bar{w}_{\text{Co}}$ (mg/kg)	0,75	1,13	1,07	35,0	19 942	3,34
Repeatability standard deviation, s_r (mg/kg)	0,09	0,10	0,05	2,3	593	0,28
Repeatability limit, r (mg/kg)	0,25	0,27	0,14	6,3	1 661	0,80
Reproducibility standard deviation, s_R (mg/kg)	0,19	0,20	0,28	8,5	2 446	0,51
Reproducibility limit, R (mg/kg)	0,52	0,57	0,80	23,9	6 849	1,43
Horwitz ratio, HorRat R	1,5	1,1	1,7	2,6	3,4	1,1

Parameters	Mo				
	Pig feed (1)	Sheep feed (1)	Phosphate (1)	Mineral premix (1)	Mineral premix (2)
Number of laboratories	7	8	6	8	11
Number of laboratories after elimination of outliers	7	8	6	6	11
Number of outliers	0	0	0	2	0
Mean value, $\bar{w}_{\text{Mo}}$ (mg/kg)	1,09	1,21	2,30	1,06	16 672
Repeatability standard deviation, s_r (mg/kg)	0,14	0,07	0,19	0,17	517
Repeatability limit, r (mg/kg)	0,40	0,18	0,54	0,46	1 448
Reproducibility standard deviation, s_R (mg/kg)	0,27	0,39	0,47	0,27	1 887
Reproducibility limit, R (mg/kg)	0,75	1,09	1,33	0,75	5 283
Horwitz ratio, HorRat R	1,5	2,1	1,5	1,6	3,1