BS EN 15510:2007

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

The European Standard EN 15510:2007 has the status of a British Standard

ICS 65.120

British Standards

National foreword

This British Standard is the UK implementation of EN 15510:2007.

The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2007

ISBN 978 0 580 56881 7

Amendments issued since publication

Amd. No.	Date	Comments

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15510

August 2007

ICS 65.120

English Version

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

Futtermittel - Bestimmung von Calcium, Natrium, Phosphor, Magnesium, Kalium, Eisen, Zink, Kupfer, Mangan, Cobalt, Molybdän, Arsen, Blei und Cadmium mittels ICP-AES

This European Standard was approved by CEN on 30 June 2007.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 15510:2007: E

Contents

Page

Foreword 3
1 Scope 4
2 Normative references 4
3 Terms and definitions 4
4 Principle 5
5 Reagents 5
6 Apparatus, usual laboratory apparatus and, in particular, the following. 7
7 Sampling 7
8 Preparation of the test sample 8
9 Procedure 9
10 Calculation and expression of the result 13
11 Precision 15
12 Test report 20
Annex A (informative) Results of the interlaboratory test 21
Annex B (informative) Notes on the detection technique, interferences and quantification 26
Bibliography 29

Foreword

This document (EN 15510:2007) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs – Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2008, and conflicting national standards shall be withdrawn at the latest by February 2008.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This European Standard specifies inductively coupled plasma atomic emission spectroscopy (ICP-AES) method for the determination of:

- minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, molybdenum in animal feeding stuffs,
- elements arsenic, lead and cadmium in minerals on their own, in pre-mixtures or mixtures for use in animal nutrition.

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

NOTE This method can also be used for the determination of minerals in products with high mineral content (> 5%), 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.

EN ISO 3696, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

ISO 6498, *Animal feeding stuffs – Preparation of test samples*

3 Terms and definitions

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

3.1
limit of detection (LOD)
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 ($n \geq 10$, were n = number of measures) performed under reproducibility conditions.

3.2
limit of quantification (LOQ)
lowest content of the analyte that 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 ten times the standard deviation of the mean of blank determinations ($n \geq 10$, were n = number of measures) performed under reproducibility conditions.

3.3
feed additives
substances are feed additives when they comply with the definition of feed additives given in regulation EU 1831/2003'

3.4
animal feeding stuffs
substances that comply with the definition of animal feeding stuffs given in regulation EU 178/2002'

4 Principle

For the determination of the minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, 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 the elements calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, cadmium and lead is determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using external calibration or standard addition technique.

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

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Water, complying with grade 2 as defined in EN ISO 3696.

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

5.3 Dilute nitric acid, to be prepared by mixing 1 volume of nitric acid (5.2) with 1 volume of water.

5.4 Nitric acid solution of 5 % (m/v), to be prepared: pipette 160 ml of dilute nitric acid (5.3) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

5.5 Nitric acid solution of 2 % (v/v), to be prepared: pipette 20 ml of nitric acid (5.2) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

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

5.7 Dilute hydrochloric acid, to be prepared by mixing 1 volume of hydrochloric acid (5.6) with 1 volume of water.

5.8 Hydrochloric acid solution of 1 % (m/v), to be prepared: pipette 60 ml of dilute hydrochloric acid (5.7) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

5.9 Element stock solutions

Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo, Cd, Pb, As

c = 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 are not to be used after the expiry date.

NOTE 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 regarded. Spectral interferences from other elements in multi-element standard solutions also need to be considered (Annex B.2.2). The examples given below also consider the measuring range of various inductively coupled plasma atomic emission spectrometers 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.

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

5.10.2 Multi-element standard solution – Minerals in 1 % HCl

c (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 of dilute hydrochloric acid (5.7). Fill 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 1 % HCl

c (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 of dilute hydrochloric acid (5.7). Fill to the mark with water and transfer to a suitable storage bottle.

5.10.4 Multi-element standard solution – Cd, Pb, As in 5 % HNO_3

c (Cd, Pb, As) = 100 mg/l

Pipette 100,0 ml of each element stock solution (Cd, Pb, As) (5.9) into a 1000 ml volumetric flask (6.7). Add 160 ml dilute nitric acid (5.3). Fill 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 grinder

6.1.1 Use laboratory grinders that are equipped such that they do not lead to contamination of the samples.

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

6.1.3 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.

6.1.4 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 Beaker, of capacities 100 ml, 250 ml.

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

6.8 Inductively coupled plasma – Atomic Emission Spectrometer

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 optimised according 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 Standard. A recommended sampling method is given in EN ISO 6497.

It is important that the laboratory receives a sample that 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.

- Grinding must be carried out in conditions such that the substance is not appreciably heated.
- Operation is to be repeated as many times as is necessary and it must be affected as quickly as possible in order to prevent any gain or loss of constituents (water).
- 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 must 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 (6.1.2) or mortar (6.1.4), until a particle size of 1 mm or less has been reached.

8.3 Liquid animal feeding stuffs

8.3.1 General

Liquid feeding stuffs shall be pre-dried according to the procedure described in 8.3.2 or freeze-dried according to the procedure described in 8.3.3.

8.3.2 Pre-drying

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

8.3.3 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 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. $MgCl_2$ $6H_2O$, shall be ground using a grinder (6.1.3) 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 Extraction with 1% HCl

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

Add 30 ml dilute hydrochloric acid (5.7). Add about 100 ml of 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, dilute to the mark with water.

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

Carry out a blank determination 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 9.1.2, but use 12 ml dilute hydrochloric acid (5.7) and 70 ml of water, and transfer the liquid into a 100 ml volumetric flask (6.7).

9.1.3 Dry ashing – 1% HCl

Weigh 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 of dilute hydrochloric acid (5.7). Add 100 ml of 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, dilute to the mark with water.

After homogenising, filter through a dry folded filter paper into a dry conical 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 conical flask with the filtrate shall be stoppered.

Carry out a blank determination 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 9.1.3, but use 12 ml dilute hydrochloric acid (5.7) and about 70 ml of water, and transfer the liquid into a 100 ml volumetric flask (6.7).

9.1.4 Extraction with 5% HNO_3

Weigh about 2 g of the prepared test sample to the nearest 1 mg into a beaker of 100 ml (6.6).

Add 16 ml dilute nitric acid (5.3). Add about 70 ml of 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, dilute to the mark with water.

After homogenising, filter through a dry folded filter paper into a dry conical 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 conical flask with the filtrate shall be stoppered.

Carry out a blank determination 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. It is important that the measurements are 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 regular basis. If linearity is guaranteed, calibrate with at least two calibration solutions, if linearity is not guaranteed, calibrate with at least three equidistant calibration solutions (B.3.2).

9.2.3 Standard addition technique

The standard addition curve should consist of at least two points one of which is 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 are additions. If three additions are used, the concentration of the highest standard should be three to five times the concentration in the sample solution.

9.2.4 Example of calibration with one addition after dry ashing – 1% HCl

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 fill to the mark with 1 % hydrochloric acid solution (5.8).

9.2.4.2 Preparation of the blank solution

Pipette 50,0 ml of the filtrate of the blank (9.1.3) into a 100 ml volumetric flask (6.7) and fill to the mark with 1 % 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 1 % HCl (5.10.3) and fill to the mark with 1 % hydrochloric acid solution (5.8).

9.2.5 Example of calibration with one addition after wet digestion – 1 % HCl

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 fill to the mark with 1 % hydrochloric acid solution (5.8).

9.2.5.2 Preparation of the blank solution

Pipette 50,0 ml of the filtrate of the blank (9.1.2) into a 100 ml volumetric flask (6.7) and fill to the mark with 1 % 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 1 % HCl (5.10.2) and fill to the mark with 1 % hydrochloric acid solution (5.8).

9.2.6 Example of calibration with one addition after wet digestion – 5 % HNO_3

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 (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 5 % HNO_3 (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 inductively coupled plasma – atomic emission spectrometry

9.3.2.1 General

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

Table 1 — Selected emission wavelengths and interferences for determination with 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	Al, Co, Ti
	261,418	
Zn	206,200	Cr
	213,856	P, Cu, Ni, Fe

9.3.2.2 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 2 % nitric acid solution (5.5).

9.3.2.3 Standard addition technique

Aspirate the blank test solution (9.2.4.2 or 9.2.5.2 or 9.2.6.2), the test solution (9.2.4.1 or 9.2.5.1 or 9.2.6.1), and the addition (9.2.4.3 or 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 2 % 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

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

$$S_{st} = c_{st} \times b + a \tag{1}$$

where

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

c_{st} is the concentration, in mg/l, of the calibration solution;

b is the slope;

a is the intersection.

Calculate the element concentration c_f, in mg/l, in the filtrate of the test portion using the slope b and the intersection a found in (1) as follows:

$$c_f = \frac{S_f - a}{b} \tag{2}$$

where

S_f is the net signal of the test solution.

10.2 Standard addition method with only one addition

In the simplest case of standard addition, where only one addition is made, the element concentration c_f, in mg/l, in the filtrate of the test portion is determined as follows:

$$c_f = \frac{S_0 \times V_s \times c_s}{(S_1 - S_0) \times V_f} \tag{3}$$

where

c_s is the concentration, in mg/l, of the standard solution;

V_s is the volume, in l, of the standard solution added;

V_f is the volume, in l, 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:

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

In this particular case of three standard additions,

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

$$x_i = c_s \times V_i \qquad \text{(for i = 0, 1, 2, 3)} \tag{6}$$

where

c_s is the concentration, in mg/l, of the standard solution;

V_i are the various volumes, in l, 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:

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

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

where

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

The element concentration c_f, in mg/l, of the filtrate of the test portion can then be found using the following equation:

$$c_f = \frac{a/b}{V_f} \tag{9}$$

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 mg of element per kg of animal feeding stuff, is determined using the following equation:

$$w_{elem} = \frac{(c_f - c_{bl})}{m} \times V_t \tag{10}$$

where

c_f is the concentration, in mg/l, of the filtrate of the test portion, as determined using equation (2) or (3) or (9);

c_{bl} is the concentration, in mg/l, of the blank solution;

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

V_t is the total volume, in l, of 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 in percentage or in g/kg for the minerals Ca, Na, P, Mg and K and in mg/kg for the elements Fe, Zn, Cu, Mn, Co, Mo and for the elements As, Cd and 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 c_s respectively. As a result the concentration c_f (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 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, will in not more than 5 % of the cases be greater than the repeatability limit *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, will in not more than 5 % of the cases be greater than the reproducibility limit *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, $\bar{x}$ (%)	*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 pre-mixture (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, $\bar{x}$ (%)	*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, $\bar{x}$ (%)	*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, $\bar{x}$ (%)	*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, $\bar{x}$ (%)	*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, $\bar{x}$ (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, $\bar{x}$ (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, $\bar{x}$ (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, $\bar{x}$ (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

(continued)

Table 3 *(concluded)*

Co			
Samples	Mean, $\bar{x}$ (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)	19942	1661	6849
Mineral mixture (1)	3,34	0,80	1,43
Mo			
Samples	Mean, $\bar{x}$ (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)	16672	1448	5283
(1) ring test 1 (2) ring test 2			

Table 4 — Precision data – As, Pb, Cd

As			
Samples	Mean, $\bar{x}$ (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, $\bar{x}$ (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

(continued)

Table 4 *(concluded)*

Cd			
Samples	Mean, $\bar{x}$ (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) test method used, with reference to this European Standard;

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

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

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

e) results obtained of the determination, expressed as mass fraction w_{elem}, in mg/kg of animal feeding stuff or in percentage or g/kg for the minerals.

Annex A
(informative)

Results of the interlaboratory test

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 Table A.1, Table A.2 and Table 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 in phosphates.

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

Ca						
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	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, $\bar{x}$ (%)	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
Horrat *R* index	1,3	1,5	1,6	1,7	2,3	2,7

Na					
Parameter	**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, $\bar{x}$ (%)	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
Horrat *R* index	1,5	1,6	2,0	1,4	2,8

(Continued)

Mg					
Parameter	**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, $\bar{x}$ (%)	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
Horrat *R* index	1,5	1,4	2,0	1,2	1,3

P					
Parameter	**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, $\bar{x}$ (%)	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
Horrat *R* index	1,5	1,2	1,2	2,5	1,6

K					
Parameter	**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, $\bar{x}$ (%)	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
Horrat *R* index	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, $\bar{x}$ (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	1601	1241	837
Horrat *R* index	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, $\bar{x}$ (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
Horrat *R* index	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, $\bar{x}$ (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
Horrat *R* index	1,2	0,7	1,1	1,4	1,9	2,4

(Continued)

Zn						
Parameters	**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, $\bar{x}$ (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
Horrat *R* index	1,0	1,1	0,7	1,6	2,0	1,7

Co						
Parameters	**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, $\bar{x}$ (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	6849	1,43
Horrat *R* index	1,5	1,1	1,7	2,6	3,4	1,1

Mo					
Parameters	**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, $\bar{x}$ (mg/kg)	1,09	1,21	2,30	1,06	16672
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	1448
Reproducibility standard deviation s_R (mg/kg)	0,27	0,39	0,47	0,27	1887
Reproducibility limit *R* (mg/kg)	0,75	1,09	1,33	0,75	5283
Horrat *R* index	1,5	2,1	1,5	1,6	3,1

Table A.3 — Statistical results of interlaboratory tests – As, Pb, Cd

As					
Parameter	**Phosphate (2)**	**MgO (2)**	**$CaCO_3$ (2)**	**Bentonite (2)**	**Mineral mix (2)**
Number of laboratories	11	11	10	11	8
Number of laboratories after elimination of outliers	10	9	10	10	7
Number of outliers	1	2	0	1	1
Mean value, $\bar{x}$ (mg/kg)	4,56	6,04	7,92	10,3	3,44
Repeatability standard deviation s_r (mg/kg)	0,21	0,44	0,73	0,37	0,15
Repeatability limit *r* (mg/kg)	0,58	1,23	2,04	1,03	0,41
Reproducibility standard deviation s_R (mg/kg)	0,55	1,14	1,74	1,34	0,49
Reproducibility limit *R* (mg/kg)	1,54	3,18	4,88	3,76	1,36
Horrat *R* index	0,9	1,5	1,9	1,2	1,1

Pb					
Parameter	**Phosphate (2)**	**$CaCO_3$ (2)**	**Bentonite (2)**	**$CuSO_4$ (2)**	**Mineral mix (2)**
Number of laboratories	9	10	10	9	6
Number of laboratories after elimination of outliers	9	9	9	8	6
Number of outliers	0	1	1	1	0
Mean value, $\bar{x}$ (mg/kg)	4,93	4,88	38,7	6,26	1,86
Repeatability standard deviation s_r (mg/kg)	0,27	0,45	0,73	0,50	0,1
Repeatability limit *r* (mg/kg)	0,76	1,27	2,03	1,41	0,36
Reproducibility standard deviation s_R (mg/kg)	0,93	1,05	2,3	1,3	0,3
Reproducibility limit *R* (mg/kg)	2,61	2,93	6,34	3,55	0,72
Horrat *R* index	1,5	1,7	0,6	1,7	0,9

Cd		
Parameter	**Phosphate (1)**	**Phosphate (2)**
Number of laboratories	9	10
Number of laboratories after elimination of outliers	8	10
Number of outliers	1	0
Mean value, $\bar{x}$ (mg/kg)	4,78	5,15
Repeatability standard deviation s_r (mg/kg)	0,12	0,16
Repeatability limit *r* (mg/kg)	0,32	0,44
Reproducibility standard deviation s_R (mg/kg)	0,84	0,65
Reproducibility limit *R* (mg/kg)	2,35	1,83
Horrat *R* index	1,4	1,0

Annex B
(informative)

Notes on the detection technique, interferences and quantification

B.1 General

Atomic emission spectroscopic techniques are widely used for qualitative and quantitative analysis. This annex describes some phenomena that can be of importance for the interpretation of the procedures of this standard. Although some theoretical considerations will be made, this annex has not the intention of being a handbook of spectroscopic techniques.

B.2 Interferences

B.2.1 General

For the determination of a specific analyte in a sample, usually the most sensitive lines are preferred. In case of interferences, especially spectral interferences, another line has to be selected, even when it is a less sensitive one. It is known that the ICP-AES technique suffers from a variety of interferences that are shortly described hereafter.

B.2.2 Spectral interferences

Spectral line interference, where atomic lines overlap or are unresolved, is often encountered in atomic emission where light is emitted not only by the element of interest but also from all other elements present in the sample. Very often this kind of interference can be eliminated by the proper choice of emission line.

A kind of spectral interference encountered in emission techniques is the occurrence of band-emission spectra due to the presence of molecular species.

B.2.3 Ionisation interferences

Ionisation interferences are caused by the presence of easily ionisable elements in the matrix of the sample, resulting in a change of the ionisation equilibrium of the analyte due to an increase in the electron number density.

Adding larger amounts of an easily ionisable element to sample and calibration solutions can be used to overcome this kind of interference.

B.2.4 Physical interferences

Physical interferences are caused by differences in some physical properties of the solutions (sample and calibration standards) such as viscosity, surface tension and vapour pressure. These differences can then cause changes in aspiration, nebulization, or atomisation efficiency.

They can be overcome to some extent by applying matrix matching of the calibration solutions, by dilution or adding relatively high acid concentrations, or by means of the standard addition technique.

B.3 Quantification and matrix matching

B.3.1 General

As spectroscopic techniques are not able to measure concentrations directly, but by means of a conversion of the emission signal into concentration, calibration is inevitable. Calibration can be performed by means of a calibration curve or by means of standard addition.

Given the various kinds of interferences, and the fact that most animal feeding stuffs have complex matrices, some kind of matrix matching between calibration solutions and sample solution has to be performed in order to eliminate matrix effects.

If an unknown sample is to be handled, it seems more appropriate to determine the concentration of the analyte by means of standard addition.

B.3.2 Calibration curve

A calibration curve is constructed by adding increasing amounts of the substance to be studied to a solution of a supporting matrix. The most difficult condition to meet is making the solutions used for the calibration curve exactly identical to those for the sample analysis. However, calibration curves are frequently recorded in solutions containing only the studied compound, whereas the sample itself introduces various other substances.

Insufficient knowledge about the sample composition may create serious difficulties for matrix matching. However, in case the composition of the samples in very well known, and doesn’t vary too much from sample to sample, matrix matching is preferred over standard addition, especially in case of multi-element determinations.

Often the ratio of the analyte intensity to the intensity of a second element added to the sample (internal standard) is used to improve the precision of the analysis. In this way also some of the variables in the excitation and processing of spectra can be minimized or eliminated by adopting the internal standard technique.

If the linearity is guaranteed, two calibration solutions should be enough to set up the calibration curve. Nevertheless, three to five calibration solutions are recommended. In all cases the linearity should be checked on a regular basis, e.g. by measuring two standard solutions, whose concentration range falls within the calibration range, as samples. Thus the calibration and the linearity are examined at the same time. The working area should be chosen in such a way that the concentration of the sample solution is situated in the middle of the calibration curve.

B.3.3 Matrix matching

In case of known matrices the technique of matrix matching between calibration solutions and sample solutions is carried out by adding the appropriate amounts of analytical grade reagents to the calibration solutions to imitate the matrix of the sample solution.

B.3.4 Standard addition

Standard addition is a way of measuring concentrations suited particularly well for samples with high but unknown total ionic strength (matrix) or for samples with highly variable solution components. This approach does not require the preparation of a calibration curve. The standard addition method is also used in this way to compensate for chemical and other matrix interferences and effects.

Usually the standard addition is a small volume of a concentrated solution so that the total solution volume and ionic strength are not changed appreciably. The most accurate determinations are made when the change in concentration of the element in study is such that the total concentration is approximately doubled.

Standard addition is particularly affected by non-linearity's, and a minimum number of additions of three to five is therefore recommended. The best precision can be achieved by adding several small increments, rather than one single standard addition measurement.

When this method is used, the condition of identical compositions of compared solutions is most closely fulfilled.

Bibliography

[1] ISO 5725-1:1994, Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions

[2] EN ISO 6497, *Animal feeding stuffs – Sampling (ISO 6497:2002)*

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001. Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at http://www.bsi-global.com.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111. Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002. Fax: +44 (0)20 8996 7001. Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsi-global.com/bsonline.

Further information about BSI is available on the BSI website at http://www.bsi-global.com.

Copyright

BSI
389 Chiswick High Road
London
W4 4AL