



SLOVENSKI STANDARD

SIST EN 15621:2012

01-april-2012

Nadomešča:

SIST-TS CEN/TS 15621:2007

Krma - Določevanje kalcija, natrija, fosforja, magnezija, kalija, žvepla, železa, cinka, bakra, mangana in kobalta po razklopu pod tlakom z ICP-AES

Animal feeding stuffs - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES

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Futtermittel - Bestimmung von Calcium, Natrium, Phosphor, Magnesium, Kalium, Schwefel, Eisen, Zink, Kupfer, Mangan und Kobalt nach Druckaufschluss mittels ICP-AES

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Aliments pour animaux - Dosage du calcium, du sodium, du phosphore, du magnésium, du potassium, du soufre, du fer, du zinc, du cuivre, du manganèse et du cobalt après digestion sous pression par ICP-AES

Ta slovenski standard je istoveten z: EN 15621:2012

ICS:

65.120

Krmila

Animal feeding stuffs

SIST EN 15621:2012

en,fr,de

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EUROPEAN STANDARD

EN 15621

NORME EUROPÉENNE

EUROPÄISCHE NORM

February 2012

ICS 65.120

Supersedes CEN/TS 15621:2007

English Version

Animal feeding stuffs - Determination of calcium, sodium,
phosphorus, magnesium, potassium, sulphur, iron, zinc, copper,
manganese and cobalt after pressure digestion by ICP-AES

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Phosphor, Magnesium, Kalium, Schwefel, Eisen, Zink,
Kupfer, Mangan und Kobalt nach Druckaufschluss mittels
ICP-AES

This European Standard was approved by CEN on 30 December 2011.

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Foreword

This document (EN 15621:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

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

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1 Scope

This European Standard specifies a method for the determination of the minerals calcium, sodium, phosphorus, magnesium, potassium and sulphur and the elements iron, zinc, copper, manganese, cobalt in animal feeding stuffs by inductively coupled plasma atomic emission spectrometry (ICP-AES) after pressure digestion.

The method was fully statistically tested and evaluated within 11 animal feeding stuff samples for the minerals calcium, sodium, phosphorus, magnesium, potassium and sulphur and the elements iron, zinc, copper, manganese and cobalt.

For potassium and sulphur the HORRAT values were mostly higher than 2. Therefore, for these elements the method is more applicable as a screening method and not for confirmatory purposes.

Other elements like molybdenum, lead, cadmium, arsenic were not fully statistically tested and evaluated within 11 animal feeding stuff samples because these elements did not occur in concentrations higher than the limit of quantification in most of these samples. A single laboratory validation is therefore necessary for the use of this multi element method for these elements.

The method limit of quantification 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. A limit of quantification of 1 mg/kg should normally be obtained.

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.

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2 Normative references

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

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

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

3 Principle

For the determination of calcium, sodium, phosphorus, magnesium, potassium and sulphur, iron, zinc, copper, manganese, cobalt, molybdenum, lead, cadmium and arsenic a test portion of the sample is digested under pressure.

The concentration of the elements is determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using external calibration or standard addition technique.

WARNING — The 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.

4 Reagents

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

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4.1 Nitric acid concentrated, not less than 65 % (mass fraction), $c(\text{HNO}_3) = 14,4 \text{ mol/l}$, having a density of approximately $\rho(\text{HNO}_3) 1,42 \text{ g/ml}$.

4.2 Nitric acid solution, with a volume fraction of 2 %.

To be prepared: pipette 20 ml nitric acid concentrated (4.1) in a 1 000 ml volumetric flask (5.4) and fill to the mark with water.

4.3 Hydrogen peroxide, not less than 30 % (mass fraction).

4.4 Element stock solutions.

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

$c = 1\ 000 \text{ mg/l}$.

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.

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

4.5 Standard solutions.

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 present in multi-element standards also need to be considered.

Various combinations of elements at different concentrations can be used, provided that the stock standard solutions (4.4) 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.

The multi-element standard solutions are considered to be stable for several months, if stored in the dark.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

NOTE For the determination of sodium in low concentrations it is advisable not to use glassware since glassware can be a source of sodium contamination.

5.1 Laboratory grinder.

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

5.1.2 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.

NOTE It should be checked that the mill used does not influence the concentration of elements in the sample (e.g. adsorption, contamination) which have to be analysed.

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

5.3 Pressure digestion apparatus, commercially available.

The apparatus shall be tested for safety pressure vessels made of acid-resistant materials and having holders for the sample of acid-resistant material with low level of contamination by elements to be determined. Apparatus is available which uses a high-pressure incinerator with or without ambient autoclave pressure.

Instead of polytetrafluoroethylene (PTFE) holders, it is better to use graduated quartz holders, fluorinated ethylene propylene (FEP) holders or perfluoroalkoxy (PFA) holders. Quartz is advisable to be used for decomposition temperatures above 230 °C.

5.4 One-mark volumetric flasks, of capacity 1 000 ml.

5.5 Inductively coupled plasma – Atomic Emission Spectrometer.

The instrument shall be equipped with radial plasma as a minimum requirement; 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, and number of replicates) shall be optimised according the manufacturer's instructions.

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

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in EN 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.

7 Preparation of the test sample

7.1 General

Prepare the test sample in accordance with prEN ISO 6498:

- The grinding must be done in conditions such that the substance is not appreciably heated and that no contamination takes place by the grinding tools;
- The operation is to be repeated as many times as is necessary and it must 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 must be thoroughly mixed for reasons of homogeneity. Since a maximum of 0,5 g of sample is used for the digestion it is of the utmost importance to have a homogeneous sample in order to take a representative sub sample.

7.2 Animal feeding stuffs which can be ground as such

Grind the laboratory sample (usually 500 g), using a laboratory grinder (0) or mortar, until a particle size of 1 mm or less has been reached.

EN 15621:2012 (E)**7.3 Liquid animal feeding stuffs****7.3.1 General**

Liquid feeding stuffs shall be pre-dried according to the procedure described in 7.3.2 or freeze-dried according to the procedure described in 7.3.3.

7.3.2 Pre-drying

Pre-dry the laboratory sample at a temperature of $70\text{ °C} \pm 5\text{ °C}$ 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 (5.2). Grind the pre-dried sample in accordance with 7.2.

7.3.3 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (5.6). The mass of the sample before and after the freeze-drying is to be determined using an analytical balance (5.2). Grind the freeze-dried sample in accordance with 7.2.

7.4 Mineral animal feeding stuffs

Mineral compounds, except mineral products containing crystalline water, e.g. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, shall be ground using a laboratory grinder (5.1.2) or mortar until a particle size of 0,1 mm or less has been reached. Mineral products containing crystalline water should not be ground.

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8 Procedure**8.1 Pressure digestion – Preparation of the blank test solution and the test solution**

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8.1.1 General

Match the initial sample mass to the capacity of the digestion vessel, with the manufacturer's instructions being strictly observed for safety reasons. Determine the necessary digestion temperature and digestion time (see EN 13805 [1], see Annex B).

8.1.2 Example of microwave digestion

When using 100 ml vessels, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (4.1) and 0,5 ml of hydrogen peroxide (4.3), seal the digestion vessel and the pressure holders in the correct manner. Leave to pre-digest outside the microwave for about 30 min. Apply low microwave energy at the beginning of the digestion and slowly raise the energy to the maximum power, e.g. start with 100 W, raise up to 600 W within 5 min, hold for 5 min, raise to 1 000 W, hold for 10 min, cool down for minimum 20 min to 25 min. Treat a blank in the same way.

Dilute the digestion solution accordingly with water. The solution obtained after dilution is called the test solution. Proceed in accordance with 8.2.

8.1.3 Example of a high pressure digestion

When using a 100 ml vessel, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (4.1), seal the digestion vessel and the pressure vessel in the correct manner and heat from room temperature to 150 °C in 60 min, then to 300 °C in 40 min and keep 300 °C for 90 min before cooling down. Treat a blank in the same way.

Dilute the digestion solution accordingly with water. The solution obtained after dilution is called the test solution. Proceed in accordance with 8.2.

8.2 Calibration

8.2.1 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).

8.2.2 External calibration

The calibration is performed with at least two calibration solutions, of which, one is a blank calibration solution. If the working range is not linear, the calibration should be performed with a blank calibration solution and at least three equidistant calibration solutions.

8.2.3 Standard addition technique

The standard addition curve should consist of at least two points, of which, one is an addition. 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 3 to 5 times the concentration in the sample solution.

8.3 Determination

8.3.1 General

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

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8.3.2 Determination by inductively coupled plasma – atomic emission spectrometry

8.3.2.1 General

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

Element	Wavelength of emission (nm)	Interference	Element	Wavelength of emission (nm)	Interference
Ca	315,887	Co	Mn	257,610	Fe, Mo, Cr
	317,933	Fe, V		293,306	Al, Fe
	393,366		Mo	202,030	Al, Fe
Co	228,616	Ti		204,598	
Cu	324,754	Ti, Fe	Na	330,237	
	327,396			588,995	
Fe	238,200	Co		589,592	Ar
	259,940		P	178,287	I
K	766,490	Mg, Ar		213,618	Cu, Fe, Mo, Zn
	769,900			214,914	Cu, Al, Mg
Mg	279,079			177,428	Cu
	279,553		S	181,972	
	285,213	Fe		182,036	
As	188,979		Zn	206,200	
	189,042			213,856	P
	193,696		Cd	214,438	
	197,197			226,502	
Pb	216,999			228,802	
	220,353				
	261,418	Al, Co, Ti			

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

8.3.2.2 External calibration method

Aspirate the blank test solution (8.1), the calibration solutions prepared from the standard solutions (4.5), and the test solution (8.1) in ascending order separately into the plasma and measure the emission of the element to be determined using an external calibration (8.2.2).

NOTE If the test solution contains residues, decant the solution into the test tube from which the test solution is aspirated into the ICP. Use the first decanted portion to rinse the test tube.

Perform at least two replicates. Average the values if the values fall within an accepted range. After each measurement, aspirate water or nitric acid solution (4.2).

8.3.2.3 Standard addition technique

Aspirate the blank test solution (8.1), the test solution (8.1), and the test solution prepared by standard addition technique (8.2.3) in ascending order separately into the plasma, and measure the emission of the element to be determined.

NOTE If the test solution (8.1) contains residues, decant the solution into the test tube from which the test solution is aspirated into the ICP. Use the first decanted portion to rinse the test tube.

Perform at least two replicates. Average the values if the values fall within an accepted range. After each measurement, aspirate water or nitric acid solution (4.2).

9 Calculation and expression of the result

9.1 General

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

9.2 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:

$$S_{st} = c_{st} \times b + a \quad (1)$$

where

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

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

a is the intersection;

b is the slope.

Calculate the element concentration c_f , in mg/l, in the test solution (8.1) using the slope b and the intersection a found in (1) as follows:

$$c_f = \frac{S_f - a}{b} \quad (2)$$

where

S_f is the net signal of the test solution.

9.3 Standard addition method with only one addition

In the most simple case of standard addition, where only one addition is made, the element concentration c_f , in mg per l, in the test solution (8.1) is determined as follows:

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

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;