

# SLOVENSKI STANDARD

## SIST-TS CEN/TS 15621:2007

01-november-2007

Živali za prehrano - Določitev vsebnosti kalcija, natrija, fosforja, magnezija, kalija, žvepla, železa, cinka, bakra, mangana, kobalta in molibdena po tlačni prebavi s pomočjo ICP-AES

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

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

Aliments pour animaux - Détermination du calcium, du sodium, du phosphore, du magnésium, du potassium, du soufre, du fer, du zinc, du cuivre, du manganèse, du cobalt et du molybdène après digestion sous pression par spectrométrie d'émission atomique à plasma couplage inductif (spectrométrie ICP-AES)

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### **ICS:**

65.120          Krmila          Animal feeding stuffs

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ICS 65.120

English Version

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

Aliments pour animaux - Détermination du calcium, du sodium, du phosphore, du magnésium, du potassium, du soufre, du fer, du zinc, du cuivre, du manganèse, du cobalt et du molybdène après digestion sous pression par spectrométrie d'émission atomique à plasma couplage inductif (spectrométrie ICP-AES)

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This Technical Specification (CEN/TS) was approved by CEN on 9 June 2007 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

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## Foreword

This document (CEN/TS 15621:2007) 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.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: 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.

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## 1 Scope

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

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

## 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 13805: 2002, *Foodstuffs – Determination of trace elements – Pressure digestion*.

EN ISO 3696:1995, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*.

ISO 6498:1998, *Animal feeding stuffs – Preparation of test samples*.

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## 3 Terms and definitions

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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 ( $n \geq 10$ , where  $n$  = number of measures) performed under reproducibility conditions.

**3.2 limit of quantification (LOQ)**  
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 ten times the standard deviation of the mean of blank determinations ( $n \geq 10$ , where  $n$  = 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 are animal feeding stuffs when they comply with the definition of animal feeding stuffs given in regulation EU 178/2002

## 4 Principle

For the determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum, 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 Technical Specification can involve hazardous materials, operations and equipment. This Specification does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this Technical Specification 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),  $c(\text{HNO}_3) = 14,4 \text{ mol/l}$ , having a density of approximately  $\rho(\text{HNO}_3) 1,42 \text{ g/ml}$ .

**5.3 Nitric acid solution of 2 % (v/v)**, to be prepared: pipette 20 ml nitric acid (5.2) in a 1000 ml volumetric flask (6.4) and fill to the mark with water.

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

**5.5 Element stock solutions**

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

$c = 1000 \text{ mg/l}$ .

The user shall 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 1000 mg/l may be used as well.

## 5.6 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 element stock solutions (5.5) 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.

## 6 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.

### 6.1 Laboratory grinder

**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.

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.

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

**6.3 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, perfluoro ethylene propylene (FEP) holders or perfluoro alkoxy (PFA) holders. Quartz is advisable to be used for decomposition temperatures above 230 °C.

**6.4 One-mark volumetric flasks**, of capacity 1000 ml.

### 6.5 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, number of replicates, ...) shall be optimised according the manufacturer's instructions.

**6.6 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 EN-ISO 6497[1].

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 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 affected 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.

### 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.

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#### 8.3.1 Pre-drying <https://standards.iteh.ai/catalog/standards/sist/eb2176d3-2036-4514-97fd-4a362ca4e340/sist-ts-cen-ts-15621-2007>

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 (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 (6.6). 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.  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 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

Use pressure digestion. Proceed in accordance with 9.1.1.

### **9.1.1 Pressure digestion**

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 in accordance with EN 13805 (see Annex B).

#### **9.1.1.1 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 (5.2) and 0,5 ml of hydrogen peroxide (5.4), 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 1000 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 9.2.

#### **9.1.1.2 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 (5.2), 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 9.2.

## **9.2 Calibration**

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).

### **9.2.1 External calibration**

The calibration is performed with at least two calibration solutions of which one blank calibration solution. If the working range is not linear, the calibration should be performed with a blank calibration solution and at least 3 equidistant calibration solutions.

### **9.2.2 Standard addition technique**

The standard addition curve should consist of at least two points of which one 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 additions. If three additions are used, the concentration of the highest standard should be 3 to 5 times the concentration in the sample solution.

## **9.3 Determination**

### **9.3.1 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.

### 9.3.2 Determination by inductively coupled plasma – atomic emission spectrometry

**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	
Zn			Zn	206,200	
				213,856	P

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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).

#### 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.

NOTE If the test solution contains residues, transfer the solution into the test tube from which the test solution is aspirated into the ICP. Use the first transferred 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 2 % nitric acid solution (5.3).

#### 9.3.2.2 Standard addition technique

Aspirate the blank test solution (9.1), the test solution (9.1), and the standard addition technique (9.2.2) in ascending order separately into the plasma, and measure the emission of the element to be determined.

NOTE If the test solution contains residues, transfer the solution into the test tube from which the test solution is aspirated into the ICP. Use the first transferred 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 2 % nitric acid solution (5.3).

## 10 Calculation and expression of the result

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