

**SLOVENSKI STANDARD  
SIST EN 15550:2017****01-oktober-2017****Nadomešča:  
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**Krma: metode vzorčenja in analize - Določevanje kadmija in svinca z atomsko absorpcijsko spektrometrijo (GF-AAS) z grafitno kiveto po razklopu pod tlakom**

Animal feeding stuffs: Methods of sampling and analysis - Determination of cadmium and lead by graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion

**iTeh STANDARD PREVIEW**

Futtermittel - Probenahme und Untersuchungsverfahren - Bestimmung von Cadmium und Blei mittels Graphitrohrfurnen-Atomabsorptionsspektrometrie (GF-AAS) nach Druckaufschluss

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Aliments des animaux - Méthodes d'échantillonnage et d'analyse - Dosage du cadmium et du plomb par spectrométrie d'absorption atomique à l'aide d'un four graphite après minéralisation sous pression

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EUROPEAN STANDARD

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## Animal feeding stuffs: Methods of sampling and analysis - Determination of cadmium and lead by graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion

Aliments des animaux - Méthodes d'échantillonnage et  
d'analyse - Dosage du cadmium et du plomb par  
spectrométrie d'absorption atomique à l'aide d'un four  
graphite après minéralisation sous pression

Futtermittel - Probenahme und  
Untersuchungsverfahren - Bestimmung von Cadmium  
und Blei mittels Graphitrohrofen-  
Atomabsorptionsspektrometrie (GF-AAS) nach  
Druckaufschluss

This European Standard was approved by CEN on 6 February 2017.

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**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

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## European foreword

This document (EN 15550:2017) 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of an identical text or by endorsement, at the latest by February 2018, and conflicting national standards shall be withdrawn at the latest by February 2018.

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

This document supersedes EN 15550:2007.

**WARNING** — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

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

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**EN 15550:2017 (E)****1 Scope**

This European Standard specifies a method for the determination of the elements cadmium and lead in animal feeding stuffs by graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion.

The method was successfully tested in the range of 0,015 mg/kg to 5,65 mg/kg for Cd and 0,18 mg/kg to 40,3 mg/kg for lead in 11 animal feeds: 2 complete feeds (pig feed, sheep feed), 2 complementary feeds (2 mineral feeds), 1 mineral premixture, 4 feed materials (MgO, 2 phosphates, CaCO<sub>3</sub>) and 2 feed additives (CuSO<sub>4</sub>, bentonite).

For the determination of extractable lead in minerals and feeds, containing phyllosilicates (e.g. kaolinite clay) wet digestion with diluted nitric acid should be used.

The method limit of quantification for each element is dependent on the sample matrix as well as the instrument. For cadmium a limit of quantification of 0,05 mg/kg should normally be obtained while for lead, a limit of quantification of 0,5 mg/kg should be obtained.

**2 Normative references**

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

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

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

**3 Terms and definitions**

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

**3.1**  
**limit of detection**  
**(LOD)**  
concentration (mass fraction) derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure

Note 1 to entry:  $x_{LOD}$  denotes the limit of detection expressed as concentration (mass fraction).

[SOURCE: ISO 11843-1, see [1]]

Note 2 to entry: For analytical systems where the validation range does not include or approach it, the detection limit does not need to be part of a validation.

[SOURCE: IUPAC 2002, see [2]]

Note 3 to entry: The various conceptual approaches to the subject depend on the estimate of precision at or near zero concentration (mass fraction) under repeatability or reproducibility conditions.

### 3.2

#### limit of quantification (LOQ)

lowest concentration (mass fraction) determined with satisfactory measurement uncertainty

Note 1 to entry:  $x_{LOQ}$  denotes the limit of quantification expressed as concentration (mass fraction)

[SOURCE: ISO 11843-1, see [1]]

Note 2 to entry: It is useful to state a concentration (mass fraction) below which the analytical method cannot operate with an acceptable precision. Such precision is arbitrarily defined as relative standard deviation of 10 %.

Note 3 to entry: Usually the limit is set as a fixed multiple (e.g. 3.3) of the detection limit.

Note 4 to entry: Measurements below LOQ are not devoid of information content and are fit for purpose.

[SOURCE: IUPAC 2002, see [2]]

### 3.3

#### feed additives

substances that comply with the definition of feed additives given in the Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition [3]

### 3.4

#### animal feeding stuffs

substances that comply with the definition of animal feeding stuffs given in the Regulation (EC) No 178/2002 [4]

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## 4 Principle

For the determination of the elements cadmium and lead, a test portion of the sample is digested under pressure.

The concentration of the elements is determined by graphite furnace atomic absorption spectrometry (GF-AAS) using external calibration.

The method detection limit for each element is dependent on the sample matrix as well as the instrument, the type of atomizer and the use of chemical modifiers. A typical sample volume of 10  $\mu$ l to 20  $\mu$ l is used.

## 5 Reagents

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

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.

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

**5.2 Hydrogen peroxide**, mass fraction not less than 30 %.

**EN 15550:2017 (E)****5.3 Element stock solutions**

Cd, Pb

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

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

Stock solutions should not be used after expiration dates.

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

**5.4 Calibration solutions**

Prepare a range of standards including a blank calibration solution, which covers the linear range of the element to be determined by diluting the element stock solutions (5.3). Appropriate matrix matching of the calibration solutions shall be performed (see Annex B), e.g. adjust the acid concentration of the standards to the acid concentration of the samples. Dilute to volume with water.

**5.5 Matrix modifier (e.g. Palladium nitrate/magnesium nitrate modifier)**

$\text{Pd}(\text{NO}_3)_2$  solution (Pd-nitrate solution) is commercially available (mass concentration 10 g/l). Dissolve 0,259 g of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (Mg-nitrate solution) in 100 ml of water. Mix the Pd-nitrate solution with twice as much Mg-nitrate solution. Ten  $\mu\text{l}$  of the mixed solution is equal to 15  $\mu\text{g}$  Pd and 10  $\mu\text{g}$   $\text{Mg}(\text{NO}_3)_2$ . It is advisable to use this solution for not longer than one week.

NOTE The combination of Pd and  $\text{Mg}(\text{NO}_3)_2$  is regarded as a "universal" modifier that is used for many elements. Other possible matrix modifiers are e.g. palladium nitrate modifier and ammonium dihydrogen phosphate modifier.

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**5.6 Purge and protective gas, Argon, Ar** purity not less than 99,99 % by volume.

**6 Apparatus**

The usual laboratory apparatus and, in particular, the following should be used:

**6.1 Laboratory Grinder**

**6.1.1 laboratory grinder** that is equipped so that samples cannot be contaminated

**6.1.2 laboratory grinder** capable of grinding to a particle size of less than or equal to 0,5 mm, e.g. a knife mill or equivalent

**6.1.3 laboratory grinder** capable of grinding to a particle size of less than 0,5 mm or less than 0,1 mm for mineral matrices, e.g. a ball mill or equivalent

**6.1.4 mortar with pestle**, free of contamination

**6.2 Analytical balance**, capable of weighing with 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 a low level of contamination. Apparatus are available that 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 perfluoroalkoxy (PFA) holders. Quartz is advisable to be used for decomposition temperatures above 230 °C.

**6.4 Graphite furnace atomic absorption spectrometer**, with background correction, e.g. Zeeman, supplied with auto sampler, an appropriate gas (5.6) supply and hollow cathode lamps or EDL-lamps for lead and cadmium

NOTE It is necessary to place an exhaust venting system over the furnace to remove any smoke and vapours that might be harmful.

**6.5 Graphite tubes**, pyrolytically coated and preferably with platforms

**6.6 Freeze drying equipment**, capable of freeze-drying liquid animal feeding stuffs

**6.7 Oven for pre-drying**, capable to hold a temperature of  $(70 \pm 5)$  °C

**6.8 One mark volumetric flasks**, of capacities 100, 1 000 ml

**6.9 Breaker**, of capacity 250 ml

**6.10 Electric hot plate**, with temperature control

## 7 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in EN ISO 6497 [5].

NOTE 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 EN ISO 6498.

- Grinding must be done 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 effected 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 0,5 mm or less has been reached.

**EN 15550:2017 (E)****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  $70\text{ °C} \pm 5\text{ °C}$  during at least 16 h to reduce the moisture content using an oven (6.7). 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.

**8.3.3 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 matrices**

Mineral matrices, except mineral products containing crystalline water, e.g.  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , shall be ground using a grinder (6.1.3) or mortar (6.1.4), until a particle size of 0,5 mm or less has been reached. Mineral products containing crystalline water should not be ground.

**9 Procedure**

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**9.1 Digestion****9.1.1 General**

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Use pressure digestion. Proceed in accordance with 9.1.2.

**9.1.2 Pressure digestion****9.1.2.1 General**

Match the initial sample mass to the capacity of the digestion vessel, strictly observing the manufacturer's instructions for safety reasons. Determine the necessary digestion temperature and digestion time (EN 13805 [6], see Annex B).

**9.1.2.2 Example of microwave digestion**

When using 70 ml to 100 ml vessels, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (5.1) and 0,5 ml of hydrogen peroxide (5.2), 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 and proceed in accordance with 9.3.

**9.1.2.3 Example of a high pressure digestion**

When using a 70 ml vessel, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (5.1), 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 at 300 °C for 90 min before cooling down. Treat a blank in the same way.

Dilute the digestion solution accordingly and proceed in accordance with 9.3.

## 9.2 Extractable lead in minerals and feeds containing phyllosilicates (e.g. kaolinite clay) – extraction with diluted nitric acid

Weigh about 2 g of the prepared test sample to the nearest 1 mg into a beaker of 250 ml. Add 16 ml dilute nitric acid (1 volume of concentrated nitric acid, not less than 65 % mass fraction with 1 volume of water). Add about 70 ml of water. Cover the beaker with a watch-glass and boil for 30 min on an electric hot plate with temperature control. Allow to cool. Transfer the liquid into a 100 ml volumetric flask, rinse the beaker and the watch-glass several times with water. Dilute to the mark with water. After homogenizing, 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. Treat a blank in the same way.

## 9.3 Calibration

Calibration shall be performed by means of external calibration, preferably with a blank calibration solution and five equidistant calibration solutions (5.4). Appropriate matrix matching of the calibration solutions shall be performed (see Annex B). For unknown matrix effects use the standard addition procedure.

It is important that the measurements are made in the linear range of the calibration function for each element.

## 9.4 Determination

### 9.4.1 General

Analytical lines, selectivity, limits of detection and quantification, precision, linear working area, and interference shall be established before operating the AAS system.

### 9.4.2 Determination by AAS graphite furnace – atomic absorption spectrometry

A temperature program for the graphite furnace (6.4) consists usually of four steps: drying, pyrolysis, atomisation and cleaning. Table 1 gives guidelines for instrumental parameter settings. These guidelines are only general. There may be great differences between instruments from different manufacturers, and between old and new models. It is recommended to use the temperatures proposed by the manufacturer as a start. Evaluate new matrices by means of ash/atomise curves to optimize parameters of the graphite furnace technique.

**Table 1 — General guidelines for instrumental parameter settings for determination of lead and cadmium by AAS graphite furnace**

Element	Wavelength (nm)	Slit width (nm)	Pyrolysis temperature (°C)		Atomisation temperature (°C)	
			Without modifier	With modifier	Without modifier	With modifier
Cd	228,8	0,7	300	900	1 250	1 600
Pb	283,3	0,7	600	1 200	1 500	2 000

During the atomisation step the argon (5.6) flow should be interrupted.

Background correction should always be used. Alternative wavelengths (with different sensitivities) may be used. E.g. for lead, one may use the wavelength 217,0 nm, where the sensitivity is about twice of that at 283,3 nm. However, the noise is higher and the risk from interferences is greater.