

# **Animal feeding stuffs — Determination of cadmium and lead by graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion**

The European Standard EN 15550:2007 has the status of a  
British Standard

ICS 65.120

## National foreword

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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.

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atomique à four graphite (GF-AAS) après digestion sous  
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Futtermittel - Bestimmung von Cadmium und Blei mittels  
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**Management Centre: rue de Stassart, 36 B-1050 Brussels**

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## Foreword

This document (EN 15550:2007) 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of an identical text or by endorsement, at the latest by April 2008, and conflicting national standards shall be withdrawn at the latest by April 2008.

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

**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

**3.4  
animal feeding stuffs**  
substances that comply with the definition of animal feeding stuffs given in the Regulation (EC) No 178/2002

## 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 µl to 20 µl is used.

**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

### 5.1 General

Use only reagents of recognized analytical grade, and water conforming to grade 2 of 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 Hydrogen peroxide**, mass fraction not less than 30 %.

### 5.4 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.

### 5.5 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 (5.4). 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.

### 5.6 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. 10 µl of the mixed solution is equal to 15 µg Pd and 10 µg  $\text{Mg}(\text{NO}_3)_2$ . It is advisable to use this solution for not longer than one week.

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The combination of Pd and Mg(NO<sub>3</sub>) is regarded as a "universal" modifier that could be used for a lot of elements. Other matrix modifiers may be used as well, e.g. palladium nitrate modifier and ammonium dihydrogen phosphate modifier.

### 5.7 Purge and protective gas

Argon, Ar purity not less than 99,99% by volume.

## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

### 6.1 Laboratory grinder

**6.1.1** Use laboratory grinders that are 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 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 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 perfluoro alkoxy (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 (5.7) 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.

## 7 Sampling

Sampling is not part of the method specified in this International 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 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 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  $70\text{ °C} \pm 5\text{ °C}$  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.

#### 8.3.3 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 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 (6.1.3) or mortar (6.1.4), 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 General

Use pressure digestion. Proceed in accordance with 9.1.2.

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### 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 (see EN 13805, 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 (5.2) and 0,5 ml of hydrogen peroxide (5.3), 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 and proceed in accordance with 9.2.

#### 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 (5.2), 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 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 9.2.

## 9.2 Calibration

Calibration shall be performed by means of external calibration, preferably with a blank calibration solution and five equidistant calibration solutions (5.5). 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.

## 9.3 Determination

### 9.3.1 General

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

### 9.3.2 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 optimise 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 (5.7) 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.

For evaluation the integrated absorbance (peak area) is recommended.

Program the autosampler to deliver sample volumes to the graphite furnace. Measure the calibration solutions (5.5.5) and the test solutions.

## 10 Calculation and expression of the result

The analyte concentrations of the test sample solutions and blank test solution are read off the calibration curve or calculated from the calibration function. The analyte concentrations of the test sample solutions are corrected by subtracting the analyte concentrations of the blank test solution.

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:

$$w_{elem} = \frac{(c_f - c_{bl})}{m} \times V_t \quad (1)$$

where

$c_f$  is the concentration, in mg per l, of the test solution;

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

$m$  is the mass of sample, in kg, taken for the extraction by digestion;

$V_t$  is the total volume, in l, of the test solution.

If the test solution has been diluted further, take into account the dilution factor.

If  $(c_f - c_{bl})$  is lower than the sample solution detection limit, substitute  $(c_f - c_{bl})$  with the value of the detection limit in the sample solution for calculation of the result in the sample.

If the sample has been pre-dried (8.3.2) or freeze-dried (8.3.3), recalculate the result to the fresh weight of the sample taking into account the loss of moisture during pre-drying or freeze-drying.

## 11 Precision

### 11.1 Interlaboratory test

Two interlaboratory tests were carried out in 2004 (ring test 1) and 2005 (ring test 2). 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 other 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.

### 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.

**Table 2 — Precision data**

<b>Cd</b>			
<b>Samples</b>	Mean, $\bar{x}$ (mg/kg)	$r$ (mg/kg)	$R$ (mg/kg)
Pig feed <sup>(1)</sup>	0,058	0,021	0,041
Sheep feed <sup>(1)</sup>	0,091	0,026	0,041
Phosphate <sup>(1)</sup>	4,88	0,88	1,91
Phosphate <sup>(2)</sup>	5,65	0,87	2,90
Mineral premixture <sup>(2)</sup>	0,15	0,045	0,18
Mineral mixture <sup>(1)</sup>	0,13	0,036	0,069
Mineral mixture <sup>(2)</sup>	0,21	0,050	0,21
CuSO <sub>4</sub> <sup>(2)</sup>	0,13	0,029	0,052
MgO <sup>(2)</sup>	0,015	0,006	0,017
CaCO <sub>3</sub> <sup>(2)</sup>	0,052	0,015	0,055
Bentonite <sup>(2)</sup>	0,24	0,056	0,14
<b>Pb</b>			
<b>Samples</b>	Mean, $\bar{x}$ (mg/kg)	$r$ (mg/kg)	$R$ (mg/kg)
Pig feed <sup>(1)</sup>	0,18	0,067	0,11
Sheep feed <sup>(1)</sup>	0,55	0,17	0,27
Phosphate <sup>(2)</sup>	5,20	1,33	2,32
Mineral premixture <sup>(2)</sup>	1,70	0,51	1,54
Mineral mixture <sup>(2)</sup>	1,69	0,30	0,98
CuSO <sub>4</sub> <sup>(2)</sup>	6,20	1,08	4,32
MgO <sup>(2)</sup>	0,71	0,25	0,36
CaCO <sub>3</sub> <sup>(2)</sup>	5,27	1,14	2,05
Bentonite <sup>(2)</sup>	40,28	8,8	18,8
<sup>(1)</sup> ringtest 1			
<sup>(2)</sup> ringtest 2			

## 12 Test report

The test report shall contain at least the following information:

- a) test method used, with reference to this European Standard (EN 15550:2007);
- b) information necessary for the complete identification of the sample;
- c) any particular points observed in the course of the test;
- d) operating 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 per kg of animal feeding stuff.

## Annex A (informative)

### Results of the interlaboratory tests

Two interlaboratory tests were carried out in 2004 (ring test 1 = <sup>(1)</sup>) and 2005 (ring test 2 = <sup>(2)</sup>) with 15 participating laboratories and 11 different animal feeding stuffs, including a complete feed for pigs, a complete feed for sheep, two different rock phosphates (<sup>(1)</sup> and <sup>(2)</sup>), two different mineral mixtures (<sup>(1)</sup> and <sup>(2)</sup>), two different mineral premixtures (<sup>(1)</sup> and <sup>(2)</sup>), CaCO<sub>3</sub>, CuSO<sub>4</sub>, MgO and bentonite. The samples were homogenized centrally and distributed to the participants. The tests yielded the data given in Annex A. Repeatability and reproducibility were calculated according to ISO 5725-1.

**Table A.1 — Statistical results of interlaboratory tests – Cd**

Cd					
Parameter	Pig feed ( <sup>1</sup> )	Sheep feed ( <sup>1</sup> )	Phosphate ( <sup>1</sup> )	Phosphate ( <sup>2</sup> )	Mineral premix ( <sup>2</sup> )
Number of laboratories	11	11	10	13	13
Number of laboratories after elimination of outliers	10	9	10	13	13
Number of outliers	1	2	0	0	0
Mean value, $\bar{x}$ (mg/kg)	0,058	0,091	4,88	5,65	0,15
Repeatability standard deviation $s_r$ (mg/kg)	0,007	0,009	0,31	0,31	0,016
Repeatability limit $r$ (mg/kg)	0,021	0,026	0,88	0,87	0,045
Reproducibility standard deviation $s_R$ (mg/kg)	0,014	0,015	0,68	1,03	0,063
Reproducibility limit $R$ (mg/kg)	0,041	0,041	1,91	2,90	0,18
Horrat $R$ index	1,0	0,7	1,1	1,5	2,0

  

Cd						
Parameter	Mineral mix ( <sup>1</sup> )	Mineral mix ( <sup>2</sup> )	CuSO <sub>4</sub> ( <sup>2</sup> )	MgO ( <sup>2</sup> )	CaCO <sub>3</sub> ( <sup>2</sup> )	Bentonite ( <sup>2</sup> )
Number of laboratories	10	12	12	10	12	11
Number of laboratories after elimination of outliers	9	11	12	10	11	11
Number of outliers	1	1	0	0	1	0
Mean value, $\bar{x}$ (mg/kg)	0,13	0,21	0,13	0,015	0,052	0,24
Repeatability standard deviation $s_r$ (mg/kg)	0,013	0,018	0,010	0,002	0,005	0,020
Repeatability limit $r$ (mg/kg)	0,036	0,050	0,029	0,006	0,015	0,056
Reproducibility standard deviation $s_R$ (mg/kg)	0,025	0,075	0,019	0,006	0,020	0,049
Reproducibility limit $R$ (mg/kg)	0,069	0,21	0,052	0,017	0,055	0,14
HorRat $R$ index	0,8	1,8	0,7	1,4	1,5	1,0

Table A.2 — Statistical results of interlaboratory tests – Pb

Pb				
Parameter	Pig feed ( <sup>1</sup> )	Sheep feed ( <sup>1</sup> )	Phosphate ( <sup>2</sup> )	Mineral premix ( <sup>2</sup> )
Number of laboratories	9	11	11	11
Number of laboratories after elimination of outliers	9	9	9	11
Number of outliers	0	2	2	0
Mean value, $\bar{x}$ (mg/kg)	0,18	0,55	5,20	1,70
Repeatability standard deviation $s_r$ (mg/kg)	0,024	0,060	0,48	0,18
Repeatability limit $r$ (mg/kg)	0,067	0,17	1,33	0,51
Reproducibility standard deviation $s_R$ (mg/kg)	0,039	0,095	0,83	0,55
Reproducibility limit $R$ (mg/kg)	0,11	0,27	2,32	1,54
Horrat $R$ index	1,0	1,0	1,3	2,2

  

Pb					
Parameter	Mineral mix ( <sup>2</sup> )	CuSO <sub>4</sub> ( <sup>2</sup> )	MgO ( <sup>2</sup> )	CaCO <sub>3</sub> ( <sup>2</sup> )	Bentonite ( <sup>2</sup> )
Number of laboratories	10	12	11	12	11
Number of laboratories after elimination of outliers	9	11	9	11	10
Number of outliers	1	1	2	1	1
Mean value, $\bar{x}$ (mg/kg)	1,69	6,20	0,71	5,27	40,3
Repeatability standard deviation $s_r$ (mg/kg)	0,11	0,38	0,089	0,41	3,14
Repeatability limit $r$ (mg/kg)	0,30	1,08	0,25	1,14	8,8
Reproducibility standard deviation $s_R$ (mg/kg)	0,35	1,54	0,13	0,73	6,70
Reproducibility limit $R$ (mg/kg)	0,98	4,32	0,36	2,05	18,8
HorRat $R$ index	1,4	2,0	1,1	1,1	1,8

## **Annex B** (informative)

### **Notes on the detection technique, interferences and quantification, and pressure digestion**

#### **B.1 General**

Atomic absorption spectroscopic (AAS) 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 AAS technique is susceptible to a variety of interferences as described below.

##### **B.2.2 Spectral interferences**

Band-emission spectra due to the presence of molecular species, is often encountered in atomic absorption technique. Light scattering from solid particles on the other hand can cause false absorption signals.

Continuous and structured background contributions, for instance the molecular band structures, can generally be eliminated by the proper background correction procedure.

##### **B.2.3 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 by adding relatively high acid concentrations, or by means of the standard addition technique.

##### **B.2.4 Chemical interferences**

Chemical combinations of the analyte with other elements in the sample can lead to so-called chemical interferences.

#### **B.3 Matrix matching**

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



## **B.4 Pressure digestion conditions**

### **B.4.1 General**

As an alternative for dry ashing or wet digestion, pressure digestion can be used. Before the pressure digestion apparatus is used, read the operating manual and observe safety instructions. Pay particular attention to the risk posed to the laboratory staff by nitrous gases (see EN 13805).

### **B.4.2 Initial sample mass and acid volumes**

Match the initial sample mass to the capacity of the digestion vessel, with the manufacturer's instructions being strictly observed for safety reasons. When digesting unknown samples, observe caution since a too large amount of sample may lead to explosions.

If the capacity is e.g. 70 ml, up to 400 mg (to the nearest milligram) of dry matter equivalent to a carbon content of 200 mg, can as a rule be digested. If the carbon content is lower, the test portion may be increased. The volume of acid necessary for the digestion will depend on the nature of the sample material. Usually 3 ml of concentrated nitric acid will be sufficient to digest the amounts mentioned above.

### **B.4.3 Digestion temperature**

Determine the necessary digestion temperature and consequently the completeness of the digestion, by the measuring method subsequently used (e.g. higher temperatures result in lower values of residual carbon in the digestion solutions. Thereby the background in AAS measurements is reduced.)

A smooth rise in temperature at the beginning of the digestion is advantageous.

In general, it applies that the quality of the digestion will become better with increasing digestion temperature.

### **B.4.4 Digestion time**

A suggested digestion time for homogenised sample material is about 3 h. In case of microwave systems digestion time it is typically 15 min to 30 min. For some samples the digestion will be gentler if a preliminary reaction is allowed to take place at room temperature, e.g. overnight, after adding the acid.

### **B.4.5 Digestion solution**

To reduce the pressure inside the digestion vessel, cool the still sealed pressure vessel to near the ambient temperature.

After the digestion vessel has been cooled and opened, initially place it under a fume hood until fumes are no longer visible. It is highly recommended to degas the digestion solution in an ultrasonic bath. The digestion solution shall be clear and its volume roughly the same as before digestion. A marked reduction in volume indicates that the pressure vessel was not leak-tight and in such cases, the digestion shall be repeated.

Transfer the digestion solutions to vessels made of quartz, FEP, PFA or another suitable container of appropriate purity, and fill up to a specified volume with water (diluted digestion solution).

### **B.4.6 Blank solution**

To check for contamination, prepare a reagent blank containing the same amount of acids as in the sample and up to 4 ml of water (depending on the initial sample weight), then carry out all the steps described in the method.

## Bibliography

- [1] EN 13805: 2002, Foodstuffs – Determination of trace elements – Pressure digestion
- [2] EN ISO 6497, Animal feeding stuffs – Sampling (ISO 6497:2002)
- [3] ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions



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