SLOVENSKI STANDARD
SIST EN 16159:2012

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Krma - Določevanje selena s hidridno atomsko absorpcijsko spektrometrijo (HGAAS) po mikrovalovnem razklopu (razklop s 65 % dušikovo kislino in 30 % vodikovim peroksidom)

Animal feeding stuffs - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65 % nitric acid and 30 % hydrogen peroxide)

Futtermittel - Bestimmung von Selen mit Atomabsorptionsspektrometrie-Hydridtechnik (HD-AAS) nach Mikrowellen-Druckaufschluss (Aufschluss mit 65 % Salpetersäure und 30 % Wasserstoffperoxid)

Aliments pour animaux - Dosage du sélénium par spectrométrie d'absorption atomique par génération d'hydrures (SAAGH) après digestion par micro-ondes (extraction avec de l'acide nitrique à 65 % et du peroxyde d'hydrogène à 30 %)

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 16159:2012) 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of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

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1 Scope

This European Standard specifies a method for the determination of selenium in animal feeding stuffs by hydride generation atomic absorption spectrometry (HGAAS) after microwave pressure digestion.

The method was successfully tested by an inter-laboratory study of CEN/TC 327/WG 4 in the range of 0,25 mg/kg to 74 mg/kg.

The limit of quantification is 0,5 µg/l of the test solution which corresponds to the calibration standard 2. Using a test portion of 0,5 g and a volume of the test solution of 25 ml after pressure digestion the limit of quantification is calculated as 0,125 mg/kg in the feed material.

NOTE A lower limit of quantification could be achieved – each laboratory has to prove it.

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 6497, *Animal feeding stuffs — Sampling* (ISO 6497)

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

3 Principle

Selenium is determined in the test solution by hydride generation atomic absorption spectrometry (fluorescence hydride generation atomic absorption) after microwave pressure digestion and a pre-reduction step.

The homogenised feeding stuff test sample is digested by nitric acid and hydrogen peroxide under pressure and high temperatures in a microwave-heated pressure digestion system.

Selenium ions of the test solution are reduced with hydrochloric acid to selenium (IV) and converted to selenium hydride (SeH_2) by sodium borohydride. This selenium hydride is transferred by a gas stream to a heated measurement cell and decomposed. The absorption at the selenium line at 196,0 nm corresponds to the amount of selenium.

NOTE Selenium (VI) is not determined by the hydridisation as described here. It is therefore necessary to adjust the digestion conditions and to exercise a pre-reduction step with hydrochloric acid to yield only selenium (IV).

Other digestion procedures with the same digestion efficiency or other measurement systems like FI-HGAAS or hydride generation inductively coupled plasma optical emission spectrometry are possible (see Annex C).

WARNING — The use of this 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 standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

4 Reagents

The concentration of the trace elements in the reagents and water used shall be low enough not to affect the results of the determination. A blank should be measured simultaneously with the test samples on each day of the analysis to control contamination and carry over with selenium in the reagents and apparatus used.

Use only water of quality 2 as described in EN ISO 3696.

NOTE High purity is essential to avoid potential contamination. Therefore, only use reagents available with high purity or perform an extraction by a sub-boiling distillation for nitric acid (4.1).

4.1 Nitric acid, not less than 65 % (mass fraction), of approximately ρ (HNO_3) = 1,4 g/ml.

4.2 Diluted nitric acid, mix 100 ml nitric acid (4.1) with water to 1 l.

4.3 Hydrogen peroxide, not less than 30 % (mass fraction), of approximately ρ (H_2O_2) ≥ 1,1 g/ml.

4.4 Hydrochloric acid, 30 %, mass concentration of approximately ρ (HCl) = 1,15 g/ml.

4.5 Diluted hydrochloric acid, e.g. about 3 % (mass fraction), as carrier solution for the use in the flow-injection-procedure.

EXAMPLE Dilute approximately 90 ml of hydrochloric acid (4.4) to 1 l with water.

4.6 Sodium borohydride solution, e.g. c = 2 g/l.

Dissolve 2 g of sodium hydroxide pellets in water, add 2 g of sodium borohydride and dilute to 1 000 ml with water into 1 000 ml flask (5.3). Prepare a fresh solution daily and, when necessary, filter before use. When the analysis procedure takes longer, it is recommended to cool the sodium borohydride solution, i.e. with ice around the flask, during its use in the HGAAS measurement.

NOTE 1 The concentration by mass of the sodium borohydride solution may vary with the system and the instructions of the relevant manufacturer shall therefore be observed.

NOTE 2 Sodium borohydride, stable aq. solution, 4,4 mol/l in 14 mol/l NaOH is also commercially available.

WARNING – It is essential to observe the safety instructions for working with sodium borohydride. Sodium borohydride forms hydrogen with acids and this can result in an explosive air/hydrogen mixture. A permanent extraction system shall be provided at the point where measurements are carried out.

4.7 Selenium stock solution, c (Se) = 1 000 mg/l.

The stock solution is commercially available. It is advisable to use certified stock solutions.

Otherwise, dissolve 1,4053 g of selenium dioxide (SeO_2) and 2 g sodium hydroxide in approximately 50 ml water, and dilute to 1 000 ml with water.

4.8 Selenium standard solution, c (Se) = 1 mg/l.

Dilute e.g. 100 µl of the stock solution (4.7) in a 100 ml flask (5.3) to give a concentration of 1 mg/l. The selenium standard solution shall contain an adequate amount of hydrochloric acid, e.g. 2 ml of hydrochloric acid (4.4) per 100 ml.

NOTE The standard solution is stable for at least three months.

4.9 Selenium calibration solutions.

For the preparation of five calibration solutions, the following procedure is recommended: Take aliquots of 0 µl, 50 µl, 250 µl, 500 µl and 1 000 µl of the selenium standard solution (4.8) into 100 ml flasks (5.3). After the addition of 20 ml of nitric acid (4.2) and 10 ml of hydrochloric acid (4.4) the calibration solutions are heated for 20 min in a water bath at 80 °C (see pre-reduction step 6.3.2). After cooling down to room temperature, the flasks (5.3) are made up to the mark with water and the calibration solutions are measured.

The selenium concentrations of the calibration solutions are: 0 µg/l; 0,5 µg/l; 2,5 µg/l; 5 µg/l and 10 µg/l (see Table 1).

Table 1 — Recommended calibration solutions (4.9) for the determination of selenium

Selenium (Se)	**Concentration of calibration solution (4.9) after pre-reduction procedure** µg/l	**Aliquot of selenium standard solution (4.8) transferred in 100 ml flasks (5.3) (pre-reduction step)** µl
Calibration standard 1	0	0
Calibration standard 2	0,5	50
Calibration standard 3	2,5	250
Calibration standard 4	5	500
Calibration standard 5	10	1 000

Choose the concentrations of the calibration solutions so as not to exceed the linear range of the calibration function. It is recommended to use a minimum of five calibration solutions with different concentrations. The calibration solutions are measured from the lowest to the highest concentration. In general, the calibration curve should be linear. Using a non-linear calibration function is possible if it is well described.

NOTE Prepare fresh calibration solutions (inclusive pre-reduction step) on the day of the analysis.

5 Apparatus and equipment

To minimise the contamination, all apparatus which come into direct contact with the sample and the solutions should be carefully pre-treated according to EN 13804.

5.1 Microwave-heated pressure digestion apparatus with inert reaction vessels, i.e. made of polytetrafluoroethylene (PTFE), perfluoroalkoxy (PFA), fluorinated ethylene propylene (FEP) or quartz, suitable for digestion temperatures of more than 200 °C.

NOTE 1 The microwave oven should be generally resistant to corrosion and especially the electronics should be protected against corrosion to ensure safe operation. The ventilation should transfer the acid vapours to an extractor hood or a fume cupboard.

NOTE 2 The reaction vessels should have a safety valve designed for a pressure of 1 000 kPa.

5.2 Pipettes, volumetric and/or graduated, 100 µl, 250 µl, 600 µl, 1 000 µl, 1 500 µl, 2 ml, 2,5 ml and 10 ml.

5.3 Volumetric flasks, 25 ml, 50 ml, 100 ml, 500 ml and 1 000 ml.

5.4 Flow-injection hydride system, with sample loop, e.g. 500 µl.

5.5 Atomic absorption spectrometer (AAS), with measurement recording system, background correction, heated quartz cell and accessories for the hydride procedure.

5.6 Element-specific lamp for selenium.

NOTE An electrodeless discharge lamp would provide a higher sensitivity compared to a hollow-cathode lamp

5.7 Ultrasonic bath and/or water bath.

5.8 Analytical balance, accurate to 0,1 mg.

6 Procedure

6.1 General

Sampling and preparation of a test sample is not part of the method. A recommended sampling method and method for sample preparation is given in EN ISO 6497 and prEN ISO 6498.

NOTE The use of a stationary or especially for mineral feeds of a rotary riffler for mass reduction and the use of a sieve size of 0,5 mm or lower for particle size reduction is recommended because of low weights of ≤ 0,5 g of the test portions to ensure homogeneity.

6.2 Preparation of the test solution

NOTE 1 The following digestion procedure leads in most cases to results for selenium and for other minerals and trace elements which correspond to the total contents of these elements. For some specific problems check whether modifications of the digestion program or other acid mixtures are necessary.

The weight of a test sample depends on the organic percentage of the sample material and on the size of the reaction vessels of the microwave digestion system.

Using reaction vessels of 20 ml to 100 ml sizes respectively a test portion of 0,2 g to 0,5 g of the homogenised and ground (to a particle size of ≤ 0,5 mm or lower) test sample is weighed to exactly 1 mg for digestion.

Add e.g. 5 ml nitric acid (4.1) and 2,5 ml hydrogen peroxide (4.3) using reaction vessels of 100 ml size, the reaction vessels are locked and fixed in the microwave digestion system (5.1).

NOTE 2 For the pre-reaction, let the reaction vessels bleed before the pressure digestion is started.

WARNING 1 — For some samples heavy reactions may result after addition of nitric acid and hydrogen peroxide. Therefore, let the reactions fade off at room temperature, i.e. overnight.

To avoid contamination and/or carry over, steam stripping of the reaction vessels with nitric acid before use is recommended. To check for potential contamination and/or carry over, digest a control blank in parallel with the test samples.

The digestion with the microwave system is performed with a temperature program adapted to the matrices considering the operating manual of the manufacturer.